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Original Research Article

Utility of Modified Cell Block Technique in Cases of Pleural Effusion Suspected of Malignancy

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ABSTRACT

The value of cytological examination of pleural effusions is widely recognized and well documented. Diagnostic problem arise in every day practice to differentiate reactive mesothelial cells and malignant cells by conventional smears (CS) method. Modified Cell block (MCB) method gives better architectural patterns, morphological features and helps to differentiate reactive mesothelial cells and malignant cells and thereby increases the efficacy of cytodiagnosis.

A combined approach of cytology and MCB technique in diagnosing the cause of pleural effusion suspected of malignancy was made in this study.

Pleural fluid received was subjected for gross examination, cytological evaluation by CS and MCB techniques. Results were categorized as inflammatory-acute, chronic, specific non specific and neoplastic-benign, suspicious of malignancy, malignant pleural effusion.

Additional yield for malignancy was 46.15 % more by modified cell block method when compared to conventional smears.

In conclusion a combined approach by both conventional smears and MCB helps in diagnosing the cause of pleural effusion suspected of malignancy.

Key words: Pleural effusion, Conventional smear (CS), Modified Cell Block (MCB).

INTRODUCTION

Cytological examination of pleural fluid is of paramount importance, it reveals information about inflammatory conditions of serous membrane, parasitic infestation, infection with bacteria, fungi, viruses, finding of cancer cells. Cytological examination not only helps for diagnosis of cancer but also for staging and prognosis of diseases. It is a complete diagnostic modality which aims at pointing out the etiology of effusion as well as prognosis of disease. [1]

The diagnostic performance of cytological study of pleural 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. [2]

Cell blocks (CB) prepared from residual pleural fluids can be useful adjuncts to smears. A modified cell block technique using a improvised ethanol formalin

fixative, followed by simple paraffin processing offers excellent cytomorphologic evaluation of malignant cells. [3]

MATERIALS AND METHODS

This study on pleural fluid cytology and modified cell block technique was undertaken in the department of Pathology, in Sree Siddhartha Medical college hospital Tumkur, over a period of two years from August 2008 to September 2010 and 100 cases of pleural effusion were studied

Relevant and available clinical information regarding age, sex, symptoms and accompanying clinical signs were obtained from the patient.

An effort was made in this study to immediately process the fluid, but in small number of samples, when there is a delay these specimens were stored in refrigerator at 4^{o}C. No preservatives were used.

After receiving the sample, gross examination of pleural fluid is done for – Total volume, Color, Clarity, Specific gravity. And as a preliminary step-Any clot if were there, they were removed with a spatula by pressing the clot against the side of the container and clot was cut in small fragments and was fixed in 10% buffered formalin and processing was done. Total cell count is done with neubauer counting chamber with WBC diluting fluid as a diluent. The remaining fluid is shaked and centrifuged for 5 min at 2000 rpm and was used for toluidine blue stained wet film, CS and MCB.

Wet film technique

With a wire loop top most layer of sediment is taken and was transferred on to center of slide. An appropriate equal size drop of toluidine blue was placed on that and mixed. An appropriate size cover slip was put and seen under microscope.

Conventional smears (CS) - With a wire loop one or two drops of top most layer of sediment was transferred to slide and spread it and it was fixed immediately in 95% ethanol and stained with routine haematoxylin and eosin, Pap stain. few air dried and stained with Giemsa and Leishman stain

Modified cell block technique (MCB)

The specimen which was centrifuged at 2000 rpm for 5 min was taken. The sediment with 0.5 ml of supernatant was resuspended in thrice the volume of ethanol, acetic acid and formalin fixative and centrifuged at 2000 rpm for 5 min.

The cell button was again centrifuged with fresh fixative at 3000 rpm for 10 min and tube was set aside for 4-6 hrs to allow the cell button to harden. Then the button was wrapped in lens paper and processed with routine surgical specimens and stained with haematoxylin and eosin.

Interpretation of conventional smears versus cell block

After studying all the available clinical data, based on morphology the smears were categorized as inflammatory-acute, chronic, specific, non specific and neoplastic-benign, suspicious of malignancy, malignant pleural effusion. The following morphological criteria such as cellularity, arrangement (acini, papillae, and cell balls) cytoplasmic and nuclear pattern was done. Combined evaluation of CS and MCB was done and tabulation of cytomorphological characters was studied to identify the malignancy and probable primary site.

RESULTS

A total of 100 samples of pleural fluid were received for cytological examination.

Following observations were recorded.

Maximum number of samples was in the age group of 51-60 Yrs. Least number of samples was in age group 1-10 Yrs.

Females had predominance of malignant effusions (53.84%) compared to males (46.15%) with M:F ratio of 1:1.2.

84% 0f the samples are exudative and 16% of samples are transudative effusions

Among samples received maximum number of samples had a clinical diagnosis of Tuberculosis (52%) and cytology of 4 cases of these smears showed presence of epithelioid cells in singles in CS, but showed a well formed granulomas in MCB in these 4 cases.

Other Clinical diagnoses were Pneumonia (25%), CCF (5%), Anaemia/ Hypoproteinemia (6%) and Malignant effusion (10%). The least number of samples had a clinical diagnosis of pulmonary infarction (2%).

The appearences of inflammatory effusion were same in CS and MCB.

Cellularity was more in MCB compared to CS

Compared to males (6%) females (7%) had more incidence of malignant effusions.

Of the samples received clinical diagnosis of malignant effusions was made in 10 cases and 3 cases which were diagnosed as tuberculous effusion, turned out to be malignant effusion by cytology.

7 cases of malignant effusion were diagnosed by conventional smears and 6 cases were given as suspicious of malignancy by conventional smears which were diagnosed as malignant effusions by cell block.

The different architectural pattern observed in CS and CB are Singly scattered, Cell balls, Cell clusters, Papillae, Glands, Sheets.

Commonest site of primary site for malignant effusions was the GIT in males and in females 2 cases were there each from Breast, Ovary, and GIT.1 unknown cases each from male and female.

Additional yield for malignancy was 46.15 % more by cell block method when compared to conventional smears.

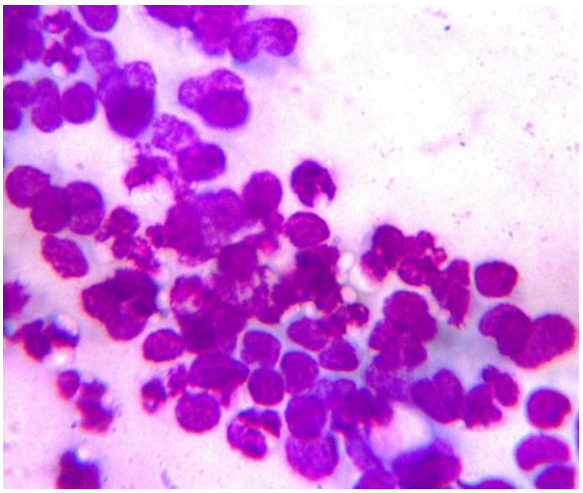

Fig.1. Photomicrograph showing epithelioid in conventional smears. (100 ×, Leishman)

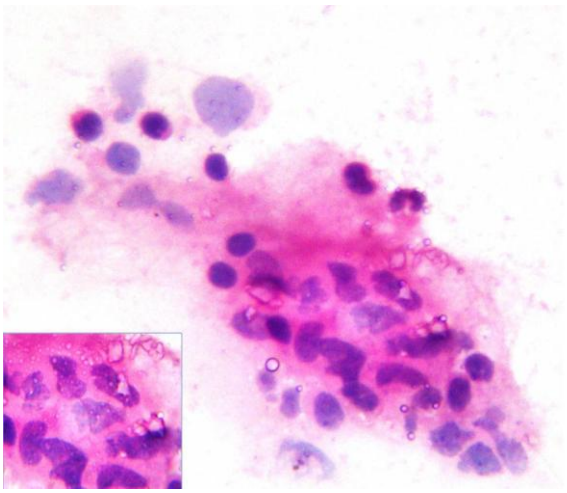

Fig.2. Photomicrograph showing epithelioid cell granuloma in cell block. (40 ×, Hematoxylin and Eosin) Inset shows similar cells in × 1000

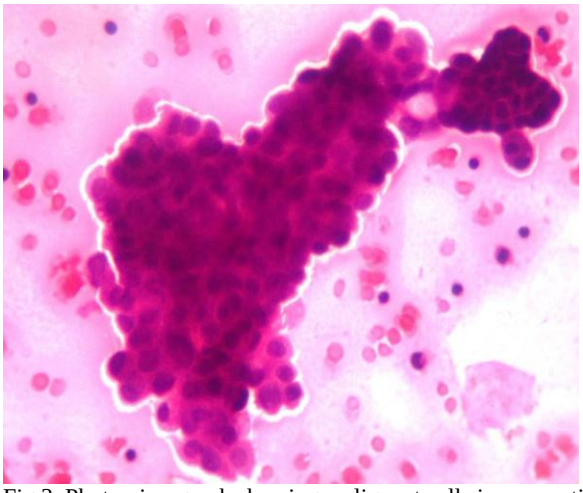

Fig.3. Photomicrograph showing malignant cells in conventional smears. (40 ×, Hematoxylin and Eosin)

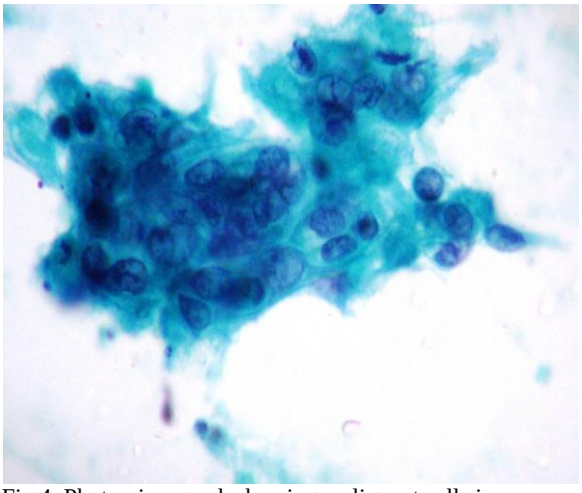

Fig.4. Photomicrograph showing malignant cells in conventional smears. (40 ×, Papanicoloau)

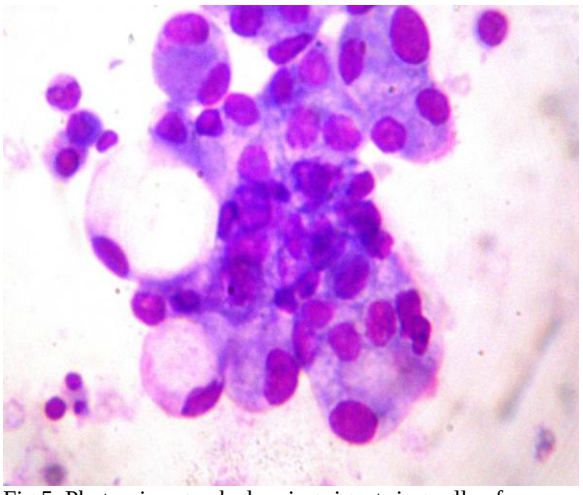

Fig.5. Photomicrograph showing signet ring cells of adenocarcinoma in conventional smears. (40 ×, Giemsa)

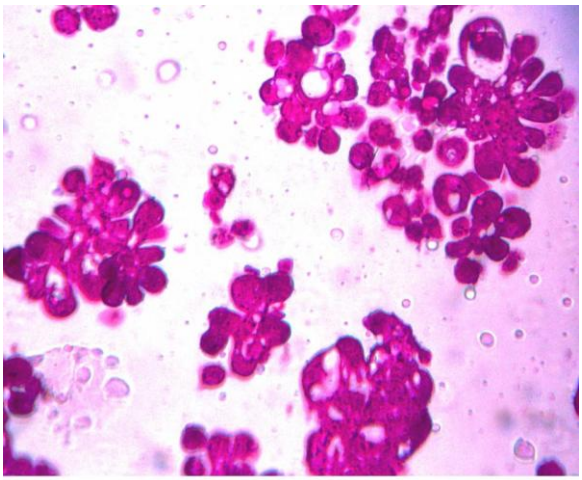

Fig.6. Photomicrograph showing signet ring cells of adenocarcinoma in Cell block. (40 ×, Periodic Acid Schiff)

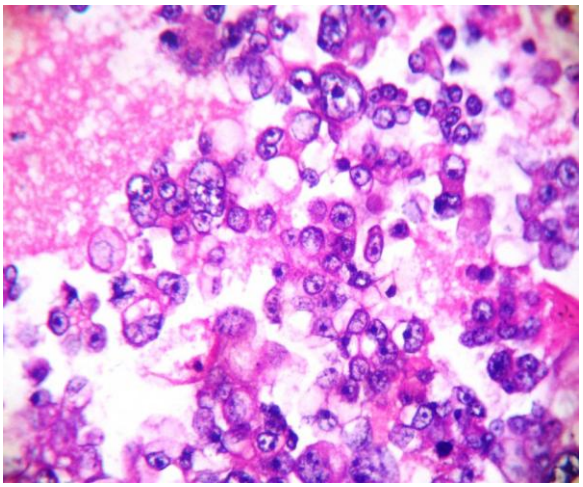

Fig.7. Photomicrograph showing deposits of breast carcinoma in cell block. (40 ×, Hematoxylin and Eosin)

DISCUSSION

Cytological examination of serous effusions is of paramount importance in diagnostic, therapeutic and prognostic implications. The performance of cytological studies of the fluid may be attributable to the fact that the cell population present in serous effusions is representative of much larger surface area than obtained by needle biopsy. [2]

Reactive mesothelial cells, abundance of inflammatory cells, and paucity of representative cells contribute to considerable difficulties in making conclusive diagnosis on conventional smear.

[4] For these reason in this study an attempt is made to prepare and analyze both smears and cellblock from the same specimen.

In this study, 10% alcohol-formalin was used as a fixative for modified cellblock preparation. By this better cellularity was obtained compared to conventional smears as formalin minimized the cell loss by forming protein cross links. Similar fixative was used in study by Bodele et al. [4] 100 samples of pleural fluid was received and effusion was found to be less common before 20 yrs of age and highest number seen in 5th decade and effusion was more common in males compared to females. We got increased cellularity by MCB with better morphological details and preservation of architectural pattern like in dimensional clusters, cell balls, acinar pattern compared to CS. But there were no helpful diagnostic yield in inflammatory effusions in MCB compared to CS, except for the presence of well formed granulomas in 4 cases of tuberculous effusion.

In the present study diagnostic yield for malignancy was increased by MCB An additional of 6 cases of malignancy was identified by modified cell block method out of 13 cases, which were given as suspicious of malignancy by CS so additional yield was 46.15% in MCB. Dekker and Bupp [5] study reported that samples obtained by cell block method and smear technique for malignant lesions were double that of conventional smear technique. By using cell block method, tumors were subsequently demonstrated in 38% of patients who had negative or atypical cytological reports. In a study by Khan et al, additional findings were diagnostic in 16% of malignant cases. Khan et al in another study titled as usefulness of cellblock preparation, was 20% greater than that obtained for specimen examined in smears only.

According to various studies additional diagnostic yield for malignancy was noted if conventional smear technique is supplemented by cell block method.

In present study among malignant effusions diagnosed majority (46.15%) were from GIT, (15.8%) each from Ovary and Breast, (7.69%) from Lung.

Primary site was determined based on age, sex, clinical features, clinical investigations, radiology, and by morphology and architectural pattern in cytology smears supplemented by MCB.

In study by Dekker and Bupp, maximum cases of malignant effusions were from Breast. In study by Johnston, [6] maximum cases of malignant effusion were from Lung.

Study by Khan et al also had maximum cases (69%) of malignant effusion from lung.

Malignant pleural effusions were found to affect females more than males (1.2:1) in present study.

Thus this study is in agreement with study done by Serar's D et al [7] in 1987. He found female to male ratio of (1.55:1) in malignant pleural effusion. This could be attributed to higher incidence of mammary and ovarian carcinoma in females. Sherwani R et al (2005) showed female to male ratio of 0.92:1, in malignant pleural effusions.

CONCLUSION

- The present study demonstrates that the pleural fluid cytology and modified cell block technique are the most useful tests in establishing the diagnosis of pleural effusion. Cytological examination of body fluids is a complete diagnostic modality which aims at pointing out the etiology and prognosis of effusion.
- 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.

- The value of cytologic examination of serous effusions is widely established. The primary role of cytology in this setting is detection of malignancy. In patients with known malignancy the presence of tumor cells in effusion may have important prognostic implication.
- MCB technique by using 10% alcohol-formalin as fixative is simple, inexpensive and does not require any special training or instrument.
- Morphological features were better identified by MCB, when compared to CS and additional yield of malignancy was 46.15% in MCB
- MCB is more useful in pleural effusion cases suspected of malignancy compared to inflammatory effusions.
- Multiple sections can be obtained if required for special stain or IHC study.
- There is adequate cellularity and delineation of nucleus and cytoplasmic details.
- Preservation of architectural pattern like cell balls, papillae and three dimensional clusters with intact cell membranes and crisp chromatin details.
- It bridges the gap between cytology and histology.

A combined approach of conventional smears and modified cell block technique helps to get an additional diagnostic yield for malignancy in pleural effusion which is 46.15% in our study.

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