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COMPARISON OF DIAGNOSTIC VALUE OF CYTOLOGICAL SMEAR METHOD AND CELL BLOCK METHOD IN PLEURAL FLUID CYTOLOGY

Arwa Ahmed Ali Ahmed Al-senaeai[1]

Dr: Suman Lata Khatri[2]

[1]*MSC.MLT (Pathology), NIMS University Rajasthan, Jaipur India.*

[2]*Professor in Pathology Department, NIMS University, Rajasthan, Jaipur India*

ABSTRACT

Aspiration of serous cavities is a simple and relatively non-invasive technique to achieve diagnosis. Cytologic evaluation of pleural fluid is diagnostically challenging. The research is an experimental research that uses specimens collected b the doctors. A total 50 pleural fluid specimens will be collected and examined. Presently 15 pleural fluid specimens have been collected so far and are under examination for conventional cytological smear (CS) and cell block method (CB). Each pleural fluid specimen will be divided in two equal parts: one part was subjected to conventional smear technique, while the other part was subjected to 10% alcohol-acetic acid-formalin cell block technique. Overall morphological details, cellularity, architecture, nuclear and cytoplasmic details were studied in both CS and CB techniques.

Keywords: Cytological Smear Method, Cytology, Cell Block Method, Pleural Fluid, Cellularity

I. INTRODUCTION

In the 1830s with imprint smears and continued with sporadic microscopic examination of sediments from body fluids. And finally, in the 1920s, aspiration and exfoliative cytology were introduced. Johannes Müller (1801-1858), a pathologist in Berlin was the first, in 1838, to show cancer cells as they appeared in the microscope on scrapings from the cut surface of surgically excised tumors. He illustrated, among other things, cells of mammary carcinoma and osteosarcoma.(1)

Cytological study of body fluid is a complete diagnostic modality. The information provided by body fluid analysis serves several functions. First ,it assists the clinician in formulating and pointing out the etiology of effusion and list of differential diagnoses.(2)

The cytological examination of serous effusion 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 (4).Cytological examination of serous fluids is one of the commonly performed Investigations .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.(5)Accurately diagnosing cells as being either malignant or benign 'reactive mesothelial cells' in serous effusions is a common diagnostic problem(7).

The lower sensitivity of cyto-diagnosis 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 method.(6)

The cell block (CB) technique is one of the oldest methods for the evaluation of body cavity fluids.(5)Cell blocks prepared from residual tissue fluids and fine-needle aspirations can be useful adjuncts to smears for establishing a more definitive cyto pathologic diagnosis(7).Cell block preparation increases the sensitivity of detecting malignancies, and also has the ability to reduce false-positive interpretations. A new method of cell block preparation by using 10% alcohol-acetic acid formalin 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 immune-histochemistry.(2)The use of cell blocks in routine non-gynecologic cyto-pathology varies in each institution.(7).

Bhanvadia VM. et al said : In this study, the utility of the CB method in the cyto diagnosis of malignant effusions was found to be highly significant as compared to the CS method. The additional yield of malignancy was 10% more as was obtained by the CB method. So difference between the two techniques. In other words, CB is superior to CS method ,in Ethiop J Health Sci. in April 2014.(2)

Nathan AN et al in the Am J ClinPathol 2000 .that a new simple and reliable cell block technique that is suitable for all types of cytology specimens is presented. The contribution of cell blocks to the final cytologic diagnosis supports the view that cell blocks should be considered in all fine-needle aspiration specimens whenever possible and in selective cases of exfoliative cytology specimens after review of the smears.(7)

Sears D and Hajda SI .The development of malignant pleural effusion is common complication of cancers like pulmonary and gastric carcinomas. Which was diagnosis by cell block technique shows more sensitivity (4).

Kshatriya AS and Santwani PM. said that cytospin smear was helpful when low cellular material was obtained, and the concomitant examination of cellblocks not only confirmed the diagnosis of malignancy but also helped in classifying the obtained material and allowed further study on the same.Which was Comparison of FNAC

smears, cytospin smears, and cellblocks of transthoracic guided FNAC of suspected lung tumor at 2016 Journal of Cytology | Indian Academy of Cytologists | Published by Wolters Kluwer – Medknow.(9)

Thapar M, et al .said that The cell block technique not only increased the positive results, but also helped to demonstrate better architectural patterns, which could be of great help in making correct diagnosis of the primary site. The cell block technique was also useful for special stains and immune histochemistry and can give morphological details by preserving the architectural patterns. Which was critical analysis of cell block versus smear examination in effusions at Journal of Cytology / April 2009 / Volume 26 / Issue 2.(6)

Shivakumarswamy U, et al 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. Which was Diagnostic utility of the cell block method versus the conventional smear study in pleural fluid cytology.in the Journal of Cytology / January 2012 / Volume 29 / Issue 1.(5)

KÖKSAL1 D, et al in 2013 the cell block method combined with conventional smear increases the diagnostic yield in exudative pleural effusions accompanying lung cancer. Which was the Cell Block Method Increases the Diagnostic Yield in Exudative Pleural Effusions Accompanying Lung Cancer ,in the Türk Patoloji Dergisi/Turkish Journal of Pathology.(10)

Suri J ,et al in the months of September 28, 2015; the Cell block technique is superior to conventional smear technique, especially for malignant effusions. It gives more information about the architectural arrangement and the likely source of primary. More important is that diagnostic material in cell blocks is available for special studies for Immuno-histochemistry which can further supplement our Knowledge about the primary source of metastasis. Which was Analysis of Cell Block VS Conventional Smear in Fluid Cytology .In the Journal of Evidence based Medicine and Healthcare; Volume 2, Issue 39.(11)

Therefore, this research aimed at assessing the diagnostic value of cytological smear method and cell block method techniques of pleural fluid and can be achieved with the following objecctives:

i. To compare between cytological smear method and cell block method
ii. To assess the sensitivity of methods in diagnosis of pleural fluid.

II. METERIAL AND METHODS

This is an experimental comparative study. A total number of 50 pleural samples will be studied for the comparison between conventional smear cytology and cell block preparation .The consent of the patients will be sought about the procedure and clinical details will be obtained from the records. The pleural sample will be collected as per conventional method and send to pathology laboratory at NIMS Medical College; Jaipur where smear and cell block will be prepared. H&E and MGG stains are the two stains used in this comparative study

and special stains whenever required. 10 milliliters of pleural fluid specimen receives, divided into two equal amount of 5 milliliters for conventional smear cytology technique and 5 milliliters for cell block technique.

III. CONVENTIONAL SMEAR TECHNIQUE

In conventional smear technique, the 5 milliliter of pleural fluid specimen was centrifuged at 2500 rpm for 10 minutes; the supernatant removed and from the sediment was used to prepare smears.

1. Four smears were prepared from the sediment
2. Two smears were stained with MMG (May Grunwald-Giemsa) stain.
3. The other two smears were immediately fixed in 95% alcohol and were stained with Haematoxylin-Eosin (H&E) stain.

IV. CELL BLOCK TECHNIQUE

the basic step of cell block preparation include fixation, centrifugation and transfer of a cell pellet for paraffin embedding .the most challenging component is to harden the cell pellet so that it can be easily transferred without losing diagnostic material. For hardening the pellet ,several technical modifications have been reported and are still being improved. The most popularly used methodologies are plasma thrombin and agar techniques, Also each methods has its own advantages and disadvantages. The differences between the cell block techniques mainly affect immunocytochemistry(ICC)and molecular analysis, because of the different types of initial fixation,rather cytomorphology and cell block cellulaity depends on that of the original sample. A comparative assessment of (CB) preparation methods in terms of advantages ,limitations and utility in diagnosis and ancillary is studied.

In cell block technique, AAF fixative (95% ethyl alcohol 34ml+Glacial acetic acid 2ml+Formalin 4ml) we used.

1. Centrifuge at 2500rpm for 10 minutes.
2. Cell sediment was mixed with thrice the volume of AAF fixative.
3. One or two drops of the mixture fluid was centrifuged at 2000rpm for 10 minutes.
4. Re-suspended the cell button in AAF fixative and centrifuged again but at 3000rpm for 10 minutes.
5. The centrifuged tube was keep aside for 4-6 hours.
6. So the sediment is admixed with plasma and then fixative is used as it gets clotted comes on the top of the test tube.
7. The cell button scarped out to the filter paper and automatic tissue processor for routine histopathology section.
8. Then the cell blocks were embedded in paraffin and sectioned at 4µm.
9. Two cell block sections were stained with Hematoxylin-Eosin stain and two with the special stain or MGG stain.

V. STAINING METHODS &PROCEDURE

HEMATOXYLIN AND EOSIN STAIN

This procedure is performed with the slides in glass staining racks and the solutions in square glass staining jar.

Dip the glass rack with slides (from cryo-section or 80 ^{0}c) serially in the following jar :

1) 95% EtOH, 2 minutes.
2) 70%EtOH, 2 minutes.
3) Running water,5 minutes (note: keep regular tap water running in a big tank in the sink and place the glass rack in the tank).
4) Hematoxylin 3 minutes (note: 3 min yield optimal staining longer than that, background starts to show up.)
5) Running water for 5 minutes.
6) Scott's solutions 3 minutes.
7) Running water for 5minutes.
8) Eosin 1x, 1.5 minutes.
9) 95% EtOH, 30 seconds.
10) 100% EtOH, 30 seconds.
11) Xylene, 5 minutes
12) Mount with per-mount and cover slip.

MGG STAIN

1) Prepare and fix the smear.
2) Transfer into the staining containing MGG stain diluted in an equal quantity of water for 5 minutes.
3) Transfer without washing into staining tank containing diluted previously prepared Giemsa (1 volume of Giemsa + 9 volumes of water) for 15 minutes.
4) Rinse 2-3 times in water until complete differentiations; slides turn pinkish color.
5) Mount using DPX and cover slip.

VI. CONCLUSIONS

The research is on-going right now with targeted speciments of 50 pleural fluid to be collected and examined. However, out of the 15 samples under examination, the cell block (CB) method provides high cellularity ,better architectural patterns,morphological features and an additional yield of malignant cells. It however increases the sensitivity of the cytodiagnosis when comparing with conventional smear (CS).

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