

Is Cell Block Preparation Superior to Conventional Smear in Cytodiagnosis of Effusions; an Experience at Tertiary Care Rural Based Hospital

Kanchan Garg*, Hema Pant**, Deepti Agarwal****, Anurag Agarwal****, Smita Gupta*****

*Junior resident **HOD and Professor ***Assistant Professor, Department of Pathology ****HOD and Professor, Department of Pulmonary Medicine *****Associate Professor, Department of Medicine, Shri Ram Murti Smarak Institute of Medical Sciences, Bareilly, Uttar Pradesh 243001.

Abstract

Background: Getting adequate exfoliated cells in serous fluids is not an easy task and diagnosis is in dilemma which not only hampers patient's outcome but also confuses pathologists. Materials from patients submitted in the form of fluids for cytological examination can be evaluated in two fashions :1. Smear technique following centrifugation of the specimen 2. Cell block technique. The present study was done to 1. Evaluate the diagnostic efficacy of routine staining method and cell block technique 2. To diagnose the type of malignancy and to know primary site of malignant effusion by special stains and IHC wherever possible. **Methods:** Fluids from aspirates of body cavities i.e. pleural, pericardial, peritoneal were collected over a period of two years from September 2014 to July 2016. Fluids obtained were first examined by naked eye for physical characteristics and then processed in two halves. One portion was prepared as conventional method and the other was processed by Cell block technique. Smears obtained were studied and compared. **Result:** One hundred and four samples were subjected to diagnostic evaluation. Along with conventional smear, fluids were subjected to cell block technique. The age of the patients ranged from 5 to 92 years. Male: female ratio was 0.7:1. The samples were categorized into benign, malignant lesions and suspicious for malignant cells. Additional yield for malignancy was obtained by cell block method when compared to conventional smears. **Conclusion:** Cell block method provides an additional yield of malignant cells, and thereby, increases the sensitivity of the cytodiagnosis when compared to conventional smear method and use of IHC help to identify primary site of malignancy giving more definitive diagnosis.

Keywords: Cell Block; Conventional Smear; IHC.

Introduction

Diagnostic cytology is the science of interpretation of cells that are exfoliated from the epithelial surfaces or removed from various tissues. Getting adequate exfoliated cells in serous fluids is not an easy task and diagnosis is in dilemma which not only hampers patient's outcome but also confuses pathologists. Materials from patients submitted in the form of fluids for cytological examination can be evaluated in two fashions :-

1. Smear technique following centrifugation of the specimen.
2. Cell block technique [1,2].

Smears stained with the Papanicolaou technique generally have good definition of malignant cellular changes. However, in certain conditions, cytological findings of fluids on smear preparation can be misleading, for e.g. differentiating reactive mesothelial cells from a mesothelioma, differentiating an exuberant reactive mesothelial hyperplasia from peritoneal metastasis etc. The cell block technique of examining the fluids, along with concomitant use of smears has shown an added advantage in such cases [3].

Conventional smear got lower sensitivity because of overcrowding of cells, cell loss, lack of architecture, abundance of inflammatory cells and paucity of

Corresponding Author: Hema Pant, Professor & Head, Department of Pathology, Shri Ram Murti Smarak Institute of Medical Sciences, Bareilly, Uttar Pradesh 243001.
E-mail: Panthema18@gmail.com

(Received on 27.04.2017, Accepted on 12.05.2017)

representative cells which contribute to considerable difficulties in making conclusive diagnosis [4,5].

Cell block method offers many advantages. Multiple sections of the same material may be processed for routine stains and for special stains that may serve for immunohistochemistry. Cell block concentrates minimal amount 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, and as it uses histological techniques it gives better cellular morphology, better nuclear and cytoplasmic preservation, intact cell membrane and crisp chromatin details, preservation of architectural pattern like cell balls, papillae, acini and individual cell characteristic representing its primary site of malignancy [6,1].

The present study was done in order to :

1. Evaluate the diagnostic efficacy of routine staining method and cell block technique.
2. To diagnose the type of malignancy and to know primary site of malignant effusion by special stains and IHC wherever possible.

Materials and Methods

Fluids from aspirates of body cavities i.e. pleural, pericardial, peritoneal arriving at the Department of Pathology, Shri Ram Murti Smarak Institute of Medical Sciences (SRMS-IMS), Bareilly were collected over a period of two years from September 2014 to July 2016 and 104 cases were studied.

Inclusion Criteria

- Pleural fluid, peritoneal fluid, pericardial fluid
- Fluids with adequate volume.

Exclusion Criteria

Inadequate Quantity of Sample

An effort was made to immediately process the fluid, but in small number of samples, when there is a delay these specimens were stored in refrigerator at 4°C. Fluids obtained were examined for physical characteristics and then processed in two halves. One portion was prepared as conventional method and another as Cell block technique. Smears obtained were studied and compared. IHC and Special stains were used whenever needed.

Conventional Smear Technique

One half of the specimen was centrifuged at 1500rpm for 15 minutes. A minimum of 4 smears were prepared from the sediment. Two smears were prepared after air drying and stained with May-Grunwald-Giemsa stain. The other two smears were immediately fixed in 95% alcohol, and were stained with papanicolaou stain.

Cell Block Technique

Other half of the sample was centrifuged for 10-15 minutes. Discard the supernatant and add the 2ml of 2% agar in the sediment. Refrigerate it for one day to get solid button. Next day process it like other biopsy specimens. After paraffin embedding, sections were prepared from this cell button and were stained with hematoxylin and eosin stain.

Cytological diagnosis was rendered for each case and final diagnosis was based on clinico-radiological and cyto-histological findings. Statistical analysis was done.

Result

104 serous fluids were subjected to conventional smear and cell block techniques. The age of the patients ranged from 5 to 92 years. Female outnumbered male (63 females and 41 males). Male:female ratio was 0.7:1. Peritoneal fluids were more than pleural fluids (53 peritoneal fluids, 49 pleural fluid and 2 pericardial fluids). The samples were categorized into benign (78), malignant lesions (16) and suspicious for malignant cells (10) on conventional smear method. Diagnosis of atypical cells and no material were omitted. When the same samples were subjected to cell block, it picked up malignant cells in 23 cases. Additional 7 cases were reported as malignant after cell block technique (Table 1).

Out of 104 samples 43 cases were exudates, 25 cases were transudate and 36 were those in which protein level was not done.

Distribution of effusion according to site was done based on clinic radiological and cytohistological findings. Radiological finding was available in all cases whereas histopathology was available in 30 cases only.

Table 2,3 shows comparative diagnosis of serous effusions by conventional and cell block method with final diagnosis. Final diagnosis was based on clinico-radiological and cyto-histological findings.

To obtain sensitivity and specificity of Conventional Smear and cell block method, two scenarios were considered for the suspicious samples. In the first, all the suspicious samples were considered as positive by both CS and CB. By CS sensitivity obtained was 72.7% (95% CI: 54.4 – 86.7%), specificity of 97.18% (95% CI: 90.19 – 99.66%), PPV of 92.31 (95% CI: 74.87 – 99.05%) and NPV of 88.46 (95% CI: 79.22 – 94.59%). The accuracy of the method was obtained as 88%. By CB sensitivity obtained was 72.7% (95% CI: 54.4 – 86.7%), specificity of 98.59% (95% CI: 92.40% – 99.96%), PPV of 96% (95% CI: 79.65%– 99.9%) and NPV of 88.61 (95% CI: 79.47 – 94.66%). The accuracy of the method was obtained as 88.5%.

Alternatively, if all the suspicious samples were considered as negative by both methods. Conventional smear shows sensitivity of 48.8% (95% CI: 30.80 – 66.46%) and specificity of 1.000 (95% CI: 94.9%–100%).

The Positive Prediction Value (PPV) was obtained as 1.000 (95% CI: 79.4% – 100%) and Negative Prediction Value (NPV) was 80.68% (95% CI: 70.88% – 88.32%). The accuracy obtained was 83%. Cell block showed sensitivity of 69.70% (95% CI: 51.29% – 84.41%) and specificity of 1.000 (95% CI: 94.9%– 100%). The Positive Prediction Value (PPV) was obtained as 1.000 (95% CI: 85.18% – 100%) and Negative Prediction Value (NPV) was 87.65% (95% CI: 78.47% – 93.92%). The accuracy obtained was 90%. So, additional diagnostic accuracy of 7% was seen by cell block method (without IHC).

Analysis of CB with IHC was also carried out. Sensitivity was 70.59% (95% CI=52.5-84.9), specificity and positive predictive value was 100% respectively. Negative predictive value was 87.65% (95% CI=78.47-93.92). Thus, the accuracy of CB with IHC obtained was 91%.

Table 1: Comparison of results by conventional and cell block method (without IHC)

| | Conventional Method | Cell Block Method |
|------------|---------------------|-------------------|
| Negative | 78 (75%) | 79 (75.96%) |
| Positive | 16 (15.4%) | 23 (22.11%) |
| Suspicious | 10 (9.6%) | 02 (1.92%) |
| Total | 104 | 104 |

Table 2: Contingency table showing the comparative diagnosis of each method with the final diagnosis

| | | Final diagnosis | |
|--------------------|------------|-----------------|----------|
| | | Negative | Positive |
| Conventional smear | Negative | 69 | 09 |
| | Positive | 0 | 16 |
| | Suspicious | 02 | 08 |
| Cell block | Negative | 70 | 09 |
| | Positive | 0 | 23 |
| | Suspicious | 01 | 01 |

Table 3: Contingency table showing the comparative diagnosis of each method with the final diagnosis

| | | Final Diagnosis | |
|---------------------|------------|-----------------|----------|
| | | Negative | Positive |
| Conventional smear | Negative | 69 | 09 |
| | Positive | 0 | 16 |
| | Suspicious | 02 | 08 |
| Cell block with IHC | Negative | 71 | 09 |
| | Positive | 0 | 24 |

Table 4: Comparison of cytodiagnosis of serous effusions in present study with other studies

| S. No | Study & Year | No. of cases | Negative for malignancy | | Suspicious | | Positive for malignancy | |
|-------|--|--------------|-------------------------|-----|------------|----|-------------------------|----|
| | | | CS | CB | CS | CB | CS | CB |
| 1 | Sujathan et al ¹¹ (2000) | 85 | 61 | 63 | 5 | 1 | 19 | 21 |
| 2 | Bodele et al ¹ (2003) | 150 | 118 | 111 | 3 | 0 | 29 | 39 |
| 3 | Khan et al ¹² (2006) | 75 | 23 | 14 | 10 | 7 | 42 | 54 |
| 4 | Shivkumarswami et al ⁷ (2012) | 60 | 54 | 50 | 5 | 0 | 1 | 10 |
| 5 | Richa nathani et al ⁸ (2014) | 40 | 36 | 34 | 0 | 0 | 4 | 6 |
| 6 | Bhanvadia et al ¹³ (2014) | 150 | 116 | 117 | 16 | 0 | 18 | 33 |
| 7 | Shubhada et al ¹⁴ (2015) | 142 | 104 | 112 | 19 | 0 | 21 | 30 |
| 8 | Present study (with IHC) | 104 | 78 | 80 | 10 | 0 | 16 | 24 |

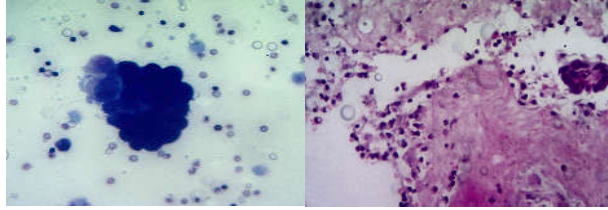


Fig. 1: Photomicrograph showing malignant cells in A) conventional smears. B. cell block (40 ×)

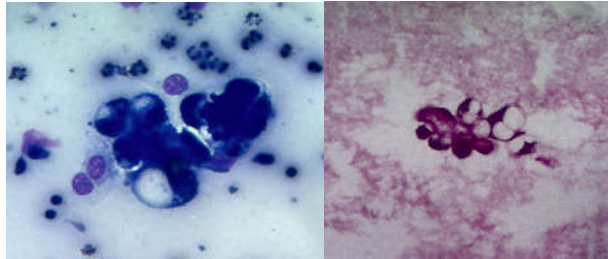


Fig. 2: Photomicrograph showing signet ring cells of adenocarcinoma in A) conventional smears B) Cell block (40 ×)

Discussion

The introduction of the CB technique was done by Bahrenburg nearly a century ago, it has been used routinely for processing fluids [7].

The minimum amount of serous fluid requested was 40 ml for our study but in most cases, the fluid submitted for examination through various wards in the pathology department was between 5 to 15 ml, so only 104 cases were taken in this study out of the total effusion samples received, after applying inclusion and exclusion criteria. In this study majority of the patients were in fifth and sixth decades of life which is consistent with the study done by Richa Nathani et al [8] and Shobha SN et al [9].

In the present study, most of the cases were in negative for malignancy category with 78 cases on CS while 80 cases on CB similar findings were seen in study done by Sujathan et al, Bodele et al, Shivkumarswami et al, Bhanvadia et al and Shubhada et al. In positive for malignancy category maximum number of cases were diagnosed on CB (n=24). Similar findings were noted by Khan et al, Shivkumarswami et al, Bodele et al (Table 4).

In the study of effusion, Luse and Reagan [10] reported that the maximum number of cases of non-malignant effusion were of pleural effusion, followed by peritoneal fluid, whereas the least number of cases were of pericardial effusion and this is consistent with our study.

Out of 10 effusion samples that were reported as suspicious for malignancy by conventional smear method of cytology, 7 cases were diagnosed as

malignant effusion, 1 case as benign effusion and the other 2 as suspicious. Thus, by using CB method, additional diagnostic yield can be obtained, which is in line with the study done by Thapar M et al [5] and Richardson et al [2].

Of the total benign effusions diagnosed on cell block, tubercular pathology was seen in 17 cases. This was based on ADA level and ZN staining. 4 cases showed bacterial pathology based on gram staining or culture. Other pathology including reactive and inflammatory pathology was seen in 57 cases. Adenocarcinoma constitutes maximum no of cases in malignant pathology i.e 83.33% (n=20). 8.33% cases (n=2) were of squamous cell carcinoma. 1(4.16%) case was of small cell carcinoma and lymphoma each. Maximum number of malignant effusion were from lung followed by ovary and least number of cases were from breast.

Even though the preparation of cell block method takes time, it got many advantages. It brings out better architectural patterns of tumor, special stains and IHC can be applied. Also the blocks prepared can be stored for future sections to be taken.

In suspicious category in CS (10 cases) diagnosis was confirmed in 8 cases on CB, 2 cases were suspicious on CB too and as IHC was an added advantage of CB. One case out of the two showed positivity and the other case was negative for malignancy. In all these malignant cases identification of primary site was also done with help of radiological findings, immunomarkers like ck7/ ck20, ER, PR and tumor specific immunomarkers.

In present study sensitivity, specificity, PPV and NPV for CS was 48.48%, 100%, 100%, 80.68% respectively and for CB was 69.70%, 100%, 100%, 87.65% and CB with IHC showed 70.59%, 100%, 100% and 87.65% respectively.

Conclusion

Cell block technique is simple, reproducible and uses routine laboratory reagents and processing. Cell block technique offers advantage like it concentrates all the cellular material, increases cellular yield and shows preservation of architectural pattern and cellular morphology can be better appreciated on cell block. Use of cell block technique eliminated the suspicious for malignancy category giving more definitive diagnosis and shows additional increase in diagnostic yield.

It is concluded from this study that for cytological examination of all serous effusions, smears should be

supplemented with cell block to increase pickup rate especially if there is suspicion of malignancy. Cell block along with routine cytology increases sensitivity to considerable extent which can be further increased if IHC is applied.

Funding: None

Competing Interests: Nil

Reference

1. Koss LG. Effusions in the absence of cancer. In: Diagnostic Cytology and its Histopathologic Bases, 5th edition. Edited by Koss LG, Melamed MR, Philadelphia: J. B. Lippincott, 2006;2:919-948.
2. Richardson HL, Koss LG, Simon TR. Evaluation of concomitant use of cytological and histological technique in recognition of cancer in exfoliated material from various sources. *Cancer* 1955;8:948-950.
3. Dekker A, Bupp PA. Cytology of serous effusion. An investigation into the usefulness of cell blocks versus smears. *Am J Clin Pathology* 1978;70:855-860.
4. Bodele AK, Parate SN, Wadadekar AA, Bobhate SK, Munshi MM. Diagnostic utility of cell block preparation in reporting of fluid cytology. *Journal of Cytology* 2003;20(3):133-135.
5. Meenu Thapar, Rajiv K Mishra, Amit Sharma, Vikas Goyal, Vibhuti Goyal. Critical analysis of cell block versus smear examination in effusions. *Journal of Cytology* 2009;26(2):60-64.
6. Sallach SM, Sallach JA, Vasquez E, Schultz L, Kvale P. Volume of pleural fluid required for diagnosis of pleural malignancy. *Chest* 2002;122:1913-7.
7. Shivakumarswamy U, Arakeri SU, Mahesh H Karigowdar MH, Yelikar BR. Diagnostic utility of the cell block method versus the conventional smear study in pleural fluid cytology. *J Cytol* 2012;29:11-15.
8. Nathani R, Hazari RS, Patle YG. Comparative analysis of cavity effusions by cell blocks and smear examination. *IJRTSAT* 2014;12(1):69-72.
9. Shobha SN, Kodandaswamy CR. Utility of modified cell block technique in cases of pleural effusion suspected of malignancy. *IJHSR* 2013;3(1):33-38.
10. Luse SA, Reagan JW. A histocytologic study of effusions: II, Effusions associated with malignant tumors. *Cancer* 1954;7:1167-81.
11. Sujathan K, Pillai KR, Chandralekha B, Kannan S, Mathew A, Nair MK. Cytodiagnosis of serous effusions: A combined approach to morphological features in Papanicolaou and May-Grunwald Giemsa stained smears and modified cell block technique. *Journal of Cytology* 2000;17(2):89-95.
12. Khan N, Sherwani KR, Afroz N, Kapoor S. Usefulness of Cell Blocks versus Smears in Malignant Effusion Cases. *Journal of Cytology* 2006;23(3):129-32.
13. Bhanvadia M, Santwani P.M, Vachhani J.H. Analysis of diagnostic value of cytological smear method versus cell block method in body fluid cytology: study of 150 cases. *Ethiop J Health Sci* 2014;24(2):125-130.
14. Shubhada B, Kumbalkar D, Nayak S. Evaluation of cell block technique in the cytodiagnosis of body fluids. *IJSR* 2013;4(7):87-94.
15. Nithyananda A, Nathan, CFIAC, Eddie Narayan, Bapp Sci, Mary M, Smith B S and Murray J Horn, Mapp Sci. Cell Block Cytology, Improved Preparation and its efficacy in Diagnostic Cytology. *Am J Clin Pathol* 2000;114:599-606.
16. Nair G, Manjula AA. Comparative study of cell blocks and routine cytological smears of pleural and peritoneal fluids in suspected cases of malignancy. *IJPO* 2015;2(2):61-68.