ORIGINAL ARTICLE

Utility of Cell Block in Diagnosis of Serous Effusions in Comparison with Conventional Cytological Smears

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ABSTRACT

Background and Objectives: Diagnostic issues arise in everyday practice to reach a conclusive diagnosis after cytological evaluation of smears. This study was designed to determine the diagnostic accuracy of cell block technique by comparing with conventional cytological smears in cytopathological diagnosis of serous effusions.

Methods: This study of diagnostic accuracy comprising of one hundred clinically and radiologically proven cases of pleural and peritoneal effusions was conducted in the Department of Pathology, Rashid Latif Medical College Lahore over a period of one year (January 2018 to January 2019). Non-probability, purposive sampling technique was used. Diagnostic accuracy of conventional cytological smear and cell block was compared using histopathology as gold standard.

Results: The sensitivity, specificity, positive predictive value and negative predictive value of conventional cytological smear and cell block method was 78.40%, 69.20, 87.90%, 52.90%, and 94.6%, 88.5%, 95.90%, 85.20% respectively. Diagnostic accuracy of cytological smears was 76% as compared to 93% of cell block method.

Conclusion: To reach a conclusive diagnosis for cytological evaluation of effusion, cell block analysis is mandatory step in addition to conventional cytological methods especially in smears that remain suspicious or inconclusive on routine cytology.

KEYWORDS: Serous effusion, Conventional smear, Cell block, Reactive mesothelial cells.

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INTRODUCTION

Serous effusions are formed due to collection of fluid in pleural and peritoneal cavities that are lined by mesothelium. All effusions are considered pathological, either benign or malignant. Benign effusions can be either exudate or transudate.[1]

Involvement of the mesothelium by primary malignant tumor is uncommon as compared to secondary metastatic tumor deposits admixed with reactive mesothelial and inflammatory cells.[2] Among all malignant tumors, adenocarcinomas are the commonest which involve serous membranes with resultant malignant or reactive effusions.[3]

Cytological examination of aspirated body cavity fluids for diagnosis of malignant cells is a mandatory diagnostic procedure for correct tumor staging and management of the patient.[4]

Sometimes there may be diagnostic problems in accurate identification of reactive mesothelial cells from malignant cells by use of conventional smears only. These conventional methods are also

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reported to have lower sensitivity.[5] Reactive mesothelial cells are invariably present in serous effusion.[6] These cells have variable cytological appearance and may resemble neoplastic cells phenotypically.[7] The use of cell block technique along with conventional cytological smear examination has shown an added advantage in such cases. With the help of cell block formation, tissue architecture is better preserved and multiple sections can be made from the same material for further use of special stains and immunohistochemistry in diagnostically difficult cases.[5]

This study was therefore designed to determine the diagnostic accuracy of cell block technique by comparing with conventional cytological smears for cytopathological diagnosis of serous effusion.

METHODS

A total of hundred pleural and peritoneal effusion cases, with provisional clinical diagnosis of benign or suspected malignant effusions, were collected from the outpatient and indoor departments of Surgery and Gynecology of Arif Memorial Hospital and Hameed Latif Hospital, Lahore. Fluids from cases taking chemo-radiotherapy, recurrence of previous tumour and inadequate specimens were excluded from the study. The samples of pleural and peritoneal fluids and washings were received fresh in the Pathology laboratory. The samples were examined and findings regarding volume, color and appearance were noted. Each sample was divided into two equal parts and transferred into two separate tubes. Test tube number 1 was processed for cytological examination. It was centrifuged at 2000 revolutions per minutes (rpm) for 5 minutes. The supernatant was discarded. Smears were prepared on glass slides from the deposit obtained after centrifugation. Minimum of two slides were prepared from each sample. These slides were air dried followed by Giemsa staining.[8] Test tubes number 2 was processed for cell block preparation.[9] For hemorrhagic effusions 1 to 2 drops of 1% glacial acetic acid was added for lysis of red blood cells. The sample was centrifuged for 5 minutes at 1500 rpm. Supernatant was discarded. The deposit was then fixed in 1:1 solution of 10% formalin and centrifuged for 10 minutes again at 2500 rpm. The sediment was left in test tube overnight. Further processing of cell block was carried out in automated tissue processor by fixation, dehydration, clearing, embedding. Later the slides were stained with Hematoxylin and Eosin.[10]

The cell block slides were examined using the Olympus binocular microscope, CX-21. The scanner lens was used to examine the cellularity, architecture and pattern of the cells. Then low and high-power objective lenses were used to examine the cytologic details to categorize cell block as benign (inflammatory/reactive) or positive for malignant cells.

Biopsy samples from surgically excised specimens of the suspected malignant cases were also received and processed for histological examination. The cytological diagnosis of malignant cells, blinded of histological diagnosis, was carried out the next day and compared with the histopathological diagnosis on biopsy tissue completed on fourth day after processing and staining. For cases with provisional benign diagnosis, clinical and radiological (Ultrasonography and X-ray) correlation was used for confirmation till final diagnosis.

STATISTICAL ANALYSIS

Data was entered and analysed using Statistical Package for Social Sciences (SPSS) version 25. Descriptive statistics were presented. Results of conventional cytological smears and cell block technique on serous effusion fluids were cross tabulated. Diagnostic accuracy was determined by calculating sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV) on SPSS. Histopathology was taken as gold standard.

RESULTS

Total 100 pleural and peritoneal fluids/washings were processed. Out of these, 66 serous fluids were from females and 34 were from male patients with a F:M ratio 2:1. A total of 35% fluids were aspirated from pleural cavities and 65% were from peritoneal cavities.

On cytological smear examination, 66% effusions were reported as positive for malignant cells whereas 34% were reported as benign (Fig:1). Taking histopathological diagnosis on tissue biopsy

samples as gold standard, n=58(87.8%) cases were true positive and n=08(12.1%) cases were false positive.

For benign cases, clinico-radiological correlation was done, which revealed n=18(53%) cases were true negative for malignant cells while n=16(47%) were false negative. Out of these false negative cases, n=10(62.5%) showed very low cellularity of malignant cells on cytological smear while in rest of n=6(37.5%) smears, malignant cell features were masked by coexisting inflammatory cells.

With cell block examination, total 73% cases were reported as positive while 27% cases were negative for malignant cells (Fig:2). Histopathological diagnosis revealed that n = 70 (95.8%) cases were true positive while n = 3 (4.1%) were false positive. A total of n = 23 (85.2%) cases were true negative and n = 4 (14.8%) were false negative.

Sensitivity of conventional cytological smear was 78.40% and specificity was 69.20%, PPV was 87.90% whereas NPV was 52.90%. Sensitivity of cell block method was 94.6%, specificity was 88.5%, PPV was 95.90% whereas NPV was 85.2% (Table-1).

The results of the study indicate that diagnostic accuracy of cell block technique is 93% while conventional cytological smear examination is found to have a diagnostic accuracy of 76%.

Table-1 Cross tabulation of conventional cytological smear and cell block methods.

		Histological Diagnosis	
		Malignant n (%)	*Benign n (%)*
Conventional Cytological Smear	Malignant	58 (87.90)	8 (12.10)
	Benign	16 (47.10)	18 (52.90)
Cell Block Method	Malignant	70 (95.9)	3 (4.1)
	Benign	4 (14.8)	23 (85.2)
Total		74 (74.0)	26 (26.0)

Table-2 Etiological causes of pleural effusions and peritoneal effusions.

	Positive for Malignant Cells (n%)	**Negative for Malignant Cells (n%)**
Pleural	Lung Carcinoma = 18(85.7) Breast Carcinoma = 2 (9.5) Ovarian Carcinoma = 1 (4.7) *Total = 21*	Pulmonary Tuberculosis = 11 (78.6) Infection = 2 (14.2) Heart Disease = 1 (7.14) *Total = 14*
Peritoneal	Ovarian Carcinoma = 35 (66) Adenocarcinoma of Intestine = 6 (11.3) Hepatocellular Carcinoma = 5 (9.4) Endometrial Carcinoma = 3 (5.6) Carcinoma head of Pancreas = 2 (3.7) Cholangiocarcinoma = 2 (3.7) *Total = 53*	Tuberculosis = 4 (33.3) Infectious diseases of intestine = 3 (25) congestive heart failure =2 (16.66) Obstructive jaundice = 2 (16.66) Renal failure =1 (8.3) *Total = 12*

In this study commonest etiological factor for malignant pleural effusions was lung carcinoma followed by breast carcinoma while ovarian carcinoma was the main underlying pathology in malignant peritoneal effusions followed by adenocarcinoma of intestine (Table-2).

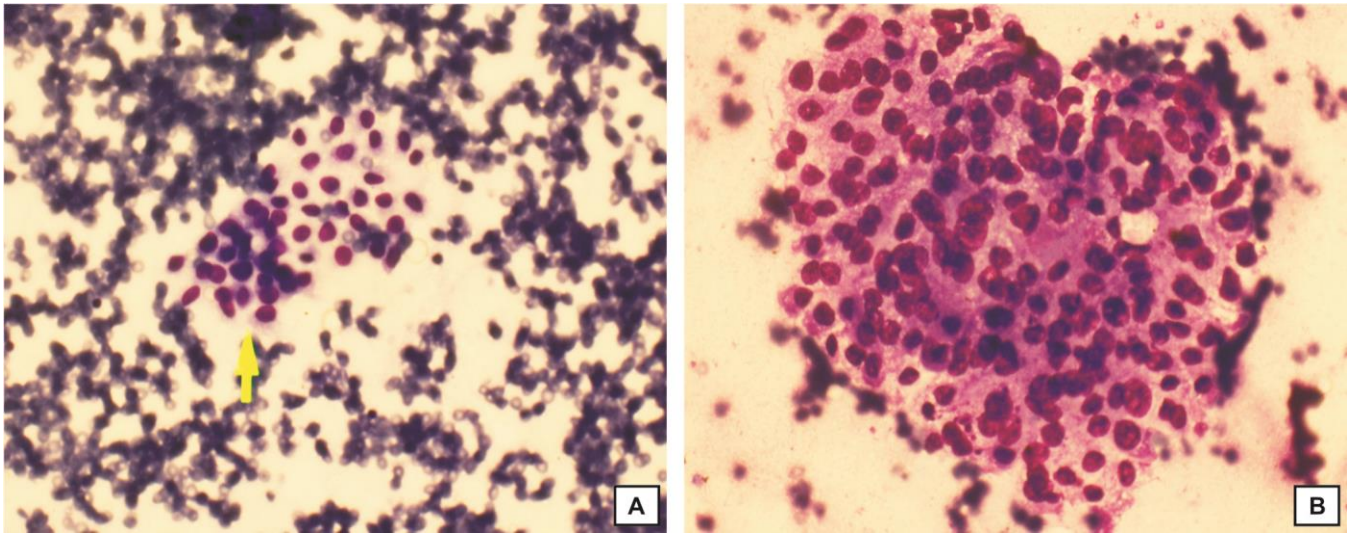

Fig: 1. Reactive mesothelial cells against background of inflammatory cells (A) and malignant cells (B) in cytological smears (Giemsa stain, 40X).

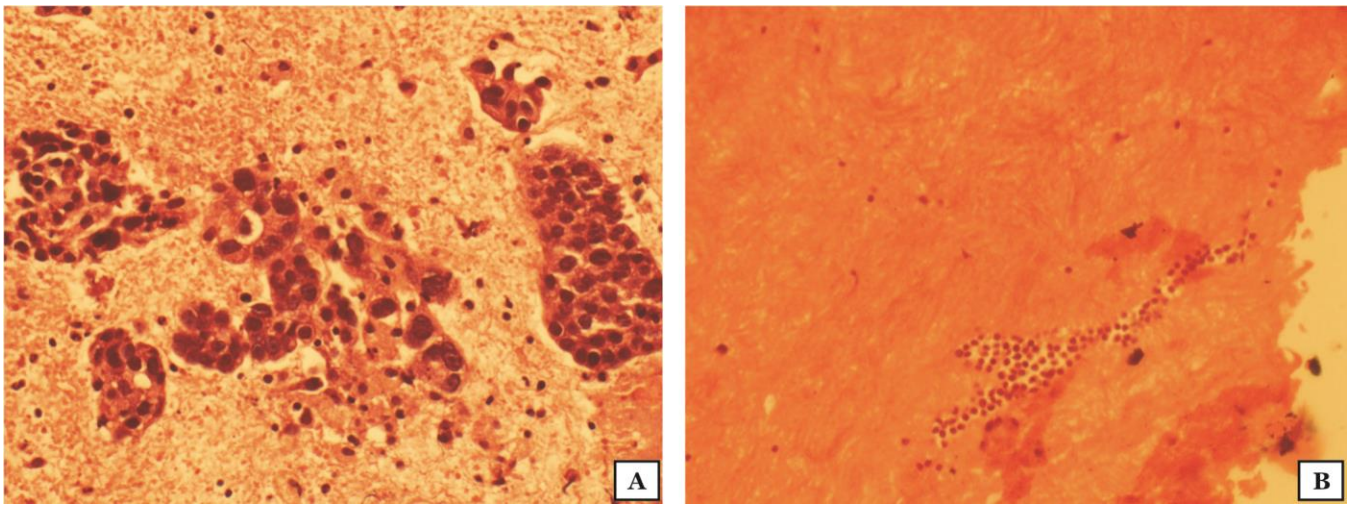

Fig: 2. Cell blocks preparation showing malignant cells (A) and reactive mesothelial cells along with inflammatory cells (B) - (H&E, 20×).

According to present study, commonest cause of benign effusions (pleural and peritoneal) was tuberculosis followed by infectious diseases of lung and intestines.

DISCUSSION

Distinction of benign reactive mesothelial cells from malignant cells is critical in cytological diagnosis of body cavity effusions. The overlapping phenotypic features between these two types of cells pose a major diagnostic challenge in routine cytology practice.[11] The workup of body cavity effusion includes examination of the cytological smears as a basic step, however the diagnostic accuracy of serous effusion cytology using routine smear is low.[12]

In this study, sensitivity, specificity, PPV and NPV of cytological smears for diagnosis of malignancy were 78.40%, 69.20%, 87.90%, 52.90% whereas Matreja et al.[13] reported these values as 69.2%, 95%, 56.25% and 97.08%, respectively thus depicting higher sensitivity and PPV while lower specificity in the present study. In another study a range of sensitivity, specificity, PPV and NPV of cytological smears for diagnosing malignancy is reported as 61 – 79%, 87 – 100%, 67 – 100% and 89 – 96%, respectively.[14] As regards cell block technique, the sensitivity, specificity, NPV and PPV of 91.3%, 100%, 100% and 98.3% respectively have been reported in an Indian study that concludes that an additional 10% of the diagnostic utility may be increased if both methods are used side by side in routine cytopathology.[15]

In the present study, cell block sensitivity, specificity, PPV and NPV were 94.6%, 88.5%, 95.90% and 85.2% respectively which were greater than that of conventional smears. On the other hand these values were quite higher, 92.3%, 99.2%, 92.3% and 99.28% respectively, in the report published by Matreja et al.[13] while taking histology as gold standard. Banosde et al.[14] have reported sensitivity, specificity, PPV and NPV of cell blocks as 88%, 100%, 100% and 96%, respectively by using clinico-radiological and histological investigation as gold standard which is also higher than the values reported in the present study. These observations are in confirmation with Nair and Manjula[16] who have reported that cell block show double the sensitivity (67.14%) as compared to conventional smear (32.3%) and is very useful adjunct to routine cytological smear method.

Carcinoma of the lung is considered as the most frequent etiological factor for pleural effusions followed by carcinoma of the breast and lymphoreticular neoplasms while malignant ascitic effusions are most commonly caused by underlying adenocarcinoma of gastrointestinal tract (GIT) followed by carcinoma of the ovary and colonic cancer.[16-18] These findings are in concordance with present study (Table-2). However, in 15% of cases, primary site remains unknown.[17]

In the present study, diagnostic accuracy of conventional cytological smear was 76% while that

of cell block was 93%. Many authors previously have also noted increased diagnostic accuracy of cell block method. According to Matreja et al.[13] accuracy of cytological smears for diagnosing malignancy was 92.8% while that of cell block was 98.6%.[13] Similar report is published by Banosde et al.[14] where accuracy of cytological smears was 85% and that of cell blocks was 97%.

The main difficulties faced by cytopathologists in making definitive diagnosis with cytological smear method are scantiness of representative cells, abundance of inflammatory cells disguising the morphology of atypical cells and presence of reactive mesothelial cells which may show reactive changes such as cytomegaly, high nucleocytoplasmic ratio, multinucleation and mitotic figures.[13] Similarly, false positive results were reported in present study that might be due to overlapping of morphological features of malignant cells and reactive mesothelial cells.

The cell block technique improves diagnosis by revealing better architectural pattern. It is simple, safe and reproducible technique and should be used for processing of all residual material after completion of cytological preparations. Cell block is essentially a mini biopsy and the effort and time taken is about the same as that of biopsy processing but there is also a risk of loss of material during processing. Compact arrangement of cells in cell block along with least amount of background staining helps easy interpretation as compared to traditional smear. In addition, various sections can be obtained from single sample that can be used for ancillary studies.

CONCLUSION

The cell block method provides high cellularity, better architectural patterns, improved morphological features, additional yield of malignant cells and increased cytodiagnostic accuracy of malignant lesions as compared to the conventional smear method. To complement the fluid cytology, cell block must be employed in addition to routine smear preparations.

LIMITATIONS OF STUDY

Primary site and nature of malignant cells cannot be determined with help of conventional smear method or cell block method without application of immunohistochemical markers.

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CONFLICT OF INTEREST

None to declare.

FINANCIAL DISCLOSURE

None to disclose.

REFERENCES

1. Geisinger KR, Raab SS, Stanley MW, Silverman JF, Abati A, editors. Effusions. In: Modern Cytopathology. Philadelphia: Churchill Livingstone; 2003.
2. Subbarayan D, Bhattacharya J, Rani P, Khuraijam B, Jain S. Use of panel of markers in serous effusion to distinguish reactive mesothelial cells from adenocarcinoma. J Cytol. 2019; 36 (1): 28-31.
3. Shameem M, Akhtar J, Baneen U, Bhargava R, Ahmed Z, Sharma P, et al. Primary peritoneal adenocarcinoma causes pleural effusion. N Am J Med Sci. 2010; 2 (6): 281-4.
4. Nam HS. Malignant pleural effusion: medical approaches for diagnosis and management. Tuberc Respir Dis. 2014; 76 (5): 211-7.
5. Thapar M, Mishra RK, Sharma A, Goyal V. Critical analysis of cell block versus smear examination in effusions. J Cytol. 2009; 26 (2): 60–4.
6. Shidham VB, Atkinson BF. Cytopathologic diagnosis of serous fluids e-book. Elsevier Health Sciences; 2007; 26. Available online at: https://www.elsevier.com/books/cytopathologic-diagnosis-of-serous-fluids/shidham/978-1-4160-0145-4. (Last accessed in January, 2020)
7. Santwani PM, Vachhani JH. Analysis of diagnostic value of cytological smear method versus cell blocks method in body fluid cytology: study of 150 cases. Ethiop J Health Sci. 2014; 24 (2): 125-30.
8. Barcia JJ. The Giemsa stain: its history and applications. Int J Surg Pathol. 2007; 15 (3): 292-6.
9. Bista P. Comparison of the diagnostic accuracy of cell block with cytology smears in serous effusions. J Pathol Nepal. 2013; 3 (6): 482-6.

10. Suvarna KS, Layton C, Bancroft JD. Bancroft's theory and practice of histological techniques E-Book. Elsevier Health Sciences; 2018. Available online at: https://www.academia.edu/40252505/Bancrofts_Theory_and_Practice_of_Histological_Techniques. (Last accessed in January, 2020)
11. Arora R, Agarwal S, Mathur SR, Verma K, Iyer VK, Aron M, et al. Utility of a limited panel of calretinin and Ber-EP4 immunocytochemistry on cytospin preparation of serous effusions: a cost-effective measure in resource-limited settings. Cytojournal. 2011; 8: 14-32.
12. Shivakumarswamy U, Arakeri SU, Karigowdar MH, Yelikar B. Diagnostic utility of the cell block method versus the conventional smear study in pleural fluid cytology. J Cytol. 2012; 29 (1): 11-5.
13. Matreja SS, Malukani K, Nandedkar SS, Varma AV, Saxena A, Ajmera A, et al. Comparison of efficacy of cell block versus conventional smear study in exudative fluids. Niger Postgrad Med J. 2017; 24 (4): 245-9.
14. Bansode S, Kumbalkar D, Nayak S. Evaluation of cell block technique in the cytodiagnosis of body fluids. Int J Sci Res. 2015; 4: 87-94.
15. Padmavathi A, Prasad BV, Anuradha B. A comparative study of fluid cytology with smear and cell block preparation. J Evid Based Med Health. 2016; 3: 3532-5.
16. Nair GG, Manjula AA. Comparative study of cell-blocks & routine cytological smears of pleural & peritoneal fluids in suspected cases of malignancy. Indian J Pathol Oncol. 2015; 2 (2): 61-8.
17. Sears D, Hajdu SI. The cytologic diagnosis of malignant neoplasms in pleural and peritoneal effusions. Acta Cytol. 1987; 31 (2): 85-97.
18. Yahya ZM, Ali HH, Hussein HG. Evaluation of the sensitivity and specificity of immunohistochemical markers in the differential diagnosis of effusion cytology. Oman Med J. 2013; 28 (6): 410-6.
19. Fetsch PA, Simsir A, Brosky K, Abati A. Comparison of three commonly used cytologic preparations in effusion immunocytochemistry. Diagn Cytopathol. 2002; 26 (1): 61-6.
20. Bodele AK, Parate SN, Wadadekar AA, Bobhate SK, Munshi MM. Diagnostic utility of cell block preparation in reporting of fluid cytology. J Cytol. 2003; 20 (3): 133-5.

Author's Contribution

NK: Conception, acquisition, analysis and interpretation of data and drafting the article.
IY: Conception, design, analysis and interpretation of data.
NWY: Drafting the article and critical revision for important intellectual content.