



ORIGINAL RESEARCH PAPER

Pathology

CYTOPATHOLOGIC STUDY OF PERITONEAL FLUIDS IN CONTEXT OF CONVENTIONAL SMEARS VERSUS CELL BLOCKS

KEY WORDS: Conventional Smear(CS), Cell Block(CB), Peritoneal effusion

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ABSTRACT

Cytopathologic study of body cavity effusions is one of the common ways to reach a definitive diagnosis. The major drawback in conventional smear(CS) cytology is the difficulty to differentiate reactive and malignant mesothelial cells and also loss of morphologic details. Cell block(CB) technique is a very old and cost effective method, but seldom prescribed by physicians. There are few studies where both the techniques CS and CB are compared for diagnostic yield in benign as well as malignant cases simultaneously. This study was undertaken to compare the morphological details and diagnostic yield of CS and CB techniques on peritoneal effusions. A one year prospective study was conducted in Government Medical College, Datia(M.P.) on cases presenting with peritoneal effusions. The study population was 94. CS cytology were stained with Papanicolaou as well as May-Grunwald Giemsa. The CB specimens were fixed in 10% alcohol-formalin and further processed as tissue specimen. The cytopathologic study was correlated with clinical details for final diagnosis. The diagnostic yield was seen better with CB (p value-0.04) in malignant cases with no significant difference in benign cases. On comparative analysis, CB was a better technique in preservation of architecture, morphological details and recognition of malignant mesothelial cells over reactive cells, thereby increasing the sensitivity of the test. So, the use of cell blocks can easily be used as an additive test to CS cytology without any extra procedure on patient.

I. INTRODUCTION

Peritoneal cavity is the largest cavity in the body, lined by flattened polyhedral cells (mesothelial cell). Normally only a few millimetres of peritoneal fluid is found in the peritoneal cavity. Amount of fluid in the body cavities is increased in certain disease process's like inflammation and neoplasm¹.

Cytological study of serous effusions is a well known diagnostic modality. It helps the clinician in etiological diagnosis as well as further prognostication and choice of therapy in malignant cases².

At the same time, distinguishing reactive and malignant mesothelial cells is a major diagnostic problem³. Diagnostic difficulties in conventional smear(CS) cytology are- indistinct morphological details, overcrowding of cells, abundance of inflammatory cells, paucity of representative cells and cell losses in sediment resulting in lower efficacy⁴. Due to these limitations a complementary technique of cell block (CB) is applied using 10% alcohol-formalin which is simple, inexpensive and requires no extra invasive procedure. The advantages of CB technique are preservation of architecture, raised cellularity, better morphological details and also application of special stains and immunohistochemistry where needed⁵.

In our study, we have focused on comparison of diagnostic yield of both CS and CB irrespective of etiology and the utility of CB in cytodiagnosis of effusion cases.

II. MATERIALS AND METHODS

This cross-sectional study was conducted on 94 patients in department of Pathology, Cytopathology laboratory, Government medical college from December 2017 to November 2018. Patients with clinical or radiological evidence of ascitis were included in the study. The study was approved by Institutional ethics committee.

The peritoneal fluids were collected along with individual relevant clinical data of the patient. The fluids were processed immediately and were refrigerated at 4°C where delayed.

The fluids were first grossly examined for volume, colour, appearance and presence or absence of coagulum. The processing was further divided into two parts- one for conventional smear (CS) cytology and the other for cell block technique (CB). For

conventional smearing the fluid was centrifuged at 1500rpm for 15 minutes and the supernatant was decanted afterwards. The smears were prepared from the sediment and stained with MGG and Papanicolaou stain. The other part was taken to prepare CB as other routine histopathological specimen. The fluid was fixed with 10% alcohol-formalin for 1 hour, then centrifuged at 1500rpm for 15 min. The supernatant was discarded and sediment was fixed for one day and then processed as other histopathological specimen.

After studying the available clinical data, CS and CB were compared on the basis of diagnostic cellularity, amount of obscuring background, preserved architecture and cell degeneration from 0-2+ scale according to Mair et al⁶.

Table no.1-Scoring system described by Mair et al⁶

Criteria	Quantitative description	Point score
Amount of diagnostic cellular material	Minimal	0
	Sufficient	1
	Abundant	2
Volume of obscuring background blood	Large	0
	Moderate	1
	Minimal	2
Retention of appropriate architecture	Minimal	0
	Moderate	1
	Excellent	2
Degree of cellular degeneration	Marked	0
	Moderate	1
	Minimal	2

All data were analyzed with a statistical software package (SPSS for Windows version 16.0). Values were expressed as mean ± standard deviation for continuous variables, and as frequencies and percentages for categorical variables. The diagnostic yields of CS and CB were compared using McNemar's test. All statistical tests were two-sided and P<0.05 was considered to be statistically significant

III. RESULTS

All the specimens were subjected to CS and CB techniques. There were total 94 patients taken in the study presenting as ascitis, comprising of 61 males and 33 females with a mean age of

60.2±16.2years.Least no. of samples were from 11-20 yrs age group.Demographic data of study population is as follows-

Table no.2-Demographic data of study population

Age,years(mean±SD)	60.2±16.2
Gender,Males	61(64.9%)
Exudative effusion	70(74.47%)
Transudative effusion	24(25.53%)

Out of 94 fluids 70(74.47%) were exudative effusion cases.Smears consisted of neutrophils, lymphocytes, macrophages, few RBC's ,occasional necrotic material and reactive mesothelial cells(Fig b) .Based on the history various causes were acute infection,TB,trauma and malignancy.Rest were 24(25.53%) transudative cases which consisted of sparse scattered inflammatory cells and few reactive mesothelial cells(Fig a).Various causes related to it were cirrhosis ,CCF and hypoproteinemia.

On CS, 83 cases were categorized as benign , 6 as suspicious and 5 as malignant. On CB, 81 cases were benign, 13 as malignant and no suspicious cases.So, by CB, additional 8 cases were detected as malignant(Fig d) ,that is a 8% more diagnostic yield than the CS technique i.e. the cellular yield obtained by CB was more in comparison of CS method(Fig c).So the sensitivity and specificity was increased by CB technique(Table no.3).

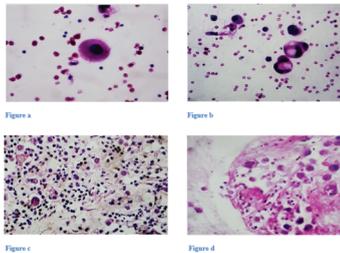


Figure a-Conventional smear showing reactive mesothelial cell along with lymphocytes(score 1 cellularity)(Pap400x)

Figure b-Conventional smear showing cells with vacuolated cytoplasm and eccentric nuclei(suspicious for malignancy) (Pap400x)

Figure c -Cell Block shows reactive effusion with increased mesothelial cells and lymphocytes(score 2 cellularity)(H&E400x)

Figure d-Cell Block shows malignant cells displaying hyperchromasia, prominent nucleoli, multinucleation(H&E400x)

Table no.3-Comparison of CS and CB in the final diagnosis of specimens

Diagnosis	CS	CB	P value
Benign	83	81	Pvalue 0.04 Which is <0.05 i.e. the value of p is significant
Suspicious/Atypical	6	0	
Malignant	5	13	
TOTAL	94	94	

Out of total malignant cases, 5 were secondaries from colon, 4 were due to liver malignancy, 3 were from ovarian carcinoma and 1 was undiagnosed primary.The malignant effusion was more common in males(M:F-1.6:1)

The quality of smears and CB were compared on the scoring system of Mair et al on 0-2+ scale .The architectural patterns such as glands ,3-D clusters, sheets, cell balls were commonly observed in CB cases whereas in CS ,there were singly scattered cells.The background obscuring material was more in the CS technique leading to misdiagnosis of few malignant cases by CS technique(Table no.4)

***Table no.4-Comparison of quality of smear by CS and CB technique/**

Technique	Conventional smear			Cell block		
	0	1	2	0	1	2
Diagnostic cellularity	18(19.14%)	72(76.6%)	4(4.3%)	16(17.02%)	47(50%)	41(43.6%)
Amount of obscuring blood	3(3.2%)	51(54.3%)	30(31.9%)	2(2.12%)	23(24.5%)	69(73.4%)
Preserved architecture	11(11.7%)	80(85.1%)	3(3.2%)	1(1.06%)	53(56.4%)	40(42.55%)
Cellular degeneration	9(9.6%)	83(88.3%)	2(2.12%)	1(1.06%)	88(93.6%)	5(5.3%)

The statistical analysis of CS and CB showed a higher cellular yield by CB method in the cytodiagnosis of malignant effusions which was highly significant as compared to CS technique.(p value-0.04)

IV.DISCUSSION

Cytopathologic study of body cavity effusions is one of the best ways to reach a definitive diagnosis⁷ .Cytopathologic examination of serous fluids supports as a confirmatory test for diagnosis and further therapeutic as well as prognostic purposes in malignant cases⁸.Hence,presently the examination of body cavity fluids has become a routine laboratory procedure for the workup of suspected malignancies and also for excluding metastasis⁹.

In 1896,Bahrenberg first introduced the cell block technique. A study by Oyafaso et al showed the sensitivity,specificity,positive predictive value and negative predictive value by CS as 44.55%,95.7%,98.7% and 20% respectively¹⁰.There are certain difficulties with the CS technique among which the major drawback in CS is to differentiate reactive and malignant mesothelial cells³ which may be due to physical,chemical or metabolic insults to serous membrane of mesothelial cells or due to poor fixation and staining technique¹¹.There is abundance of obscuring background cells and paucity of representative cells in CS¹².This obviates a need of adjuvant technique to CS.In this study,an attempt was made to prepare CB from the same specimen along with CS method.Cell Blocks were prepared by 10% alcohol-formalin as fixative which is a simple and cost effective method and further processed as routine histopathological specimen.Therefore, this study was conducted to explore the benefits of CB¹³.

A total of 94 cases were studied comprising male to female ratio of 1.6:1 with a mean age distribution of 60.2±15.2 which was similar to study of Pal et al¹⁴.Our study result correlated with the study of Sujathan et al¹⁵, Joshi et al¹⁶ and Nathani et al¹⁷.In our study,exudative effusions outnumbered transudative ones,which was contrary to study of Bhanvadia et al.

We analysed the cases simultaneously for CS and CB , which were further compared on the basis of scoring system 0-2+ given by Mair et al⁶ .For preparing CB ,we used 10% alcohol formalin as a fixative agent which is a very cost effective method.Bodele et al¹⁸ and Shivkumarswami et al¹⁹ have also used the same method as fixative for CB.

The various disadvantages faced in CS were overcome by CB technique .Improper fixation,smear and staining errors in CS causes overlapping , cell loss and artifacts⁵.While in CB, there is preservation of morphology ,minimum background obscuring material and raised cellularity²⁰.In CB, there is a possibility of multiple section cutting, special staining and IHC application afterwards on the paraffin blocks²¹ .Specimens of CB can easily be stored in paraffin blocks. By using the combination of CS and CB for reporting malignant effusions, the primary site could be detected with 81% accuracy²². In this study, the additional diagnostic yield of CB over CS was 8% in malignant cases. As far as benign etiology was considered ,the diagnostic yield was very much similar by CS and CB methods. Clinical history along with cytochemical study were enough to make a differential diagnosis . Regarding the diagnostic cellularity, CB technique was superior to CS .Shukla et al²³ showed the similar results. In case of

retention of architecture ,background obscuration and cell degeneration ,CB was a better procedure to reach a diagnosis.

Thus, the CB technique is a cost effective method in resource limited laboratories.It has a better diagnostic yield in malignant cases. So, it can be easily used as an additive test to CS.

The limitations of our study were inadequate sample with loss of cellular material in preparation of CB in few cases, the longer processing duration needed in CB technique and our study could be more informative if further IHC was performed.

V.CONCLUSION

In conclusion, we demonstrated that , CB method provided a similar diagnostic performance to CS in benign lesions but a better diagnostic method in diagnosis of malignant peritoneal effusions.Combined approach of cell block and conventional smears can easily be used as a routine procedure as it is simple,cost effective and do not require extra invasive procedure.Cell block method should be recommended as a routine procedure to decrease suspicious malignant cases and increase the sensitivity of our procedure.

Cell blocks have an advantage of more diagnostic cellularity,preserved morphologic details and less obscuring background , in addition cell blocks can also be easily further stored for longer duration and can be used for Immunohistochemistry and special staining .

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