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ORIGINAL RESEARCH

Diagnostic Utility of Conventional Smear versus Cell Block in Fluid Cytology

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ABSTRACT

Background: Cytological examination of body fluids is one of the commonly performed investigations.[1]. It helps to diagnose benign and malignant lesions of effusion.[2] The cell block particularly helps when cytological abnormalities are misleading in reactive mesothelial cells or adenocarcinoma.[3] In this study, we will be assessing and comparing the utility of cell block and conventional smear in fluid cytology. **Methods:** The present study was conducted in 55 samples of all body fluids (pleural, ascitic, synovial) obtained from patients, sent to the Cytopathology Section of the Department of Pathology, People's college of medical science & Research Centre, Bhopal.All body fluid were analysed by both conventional smear and cell block methods. **Result:** Amongs 55 fluids, 37 cases of pleural fluid (67.3%), Ascitic fluid 15 cases (27.3%) and synovial fluid was noted in 3 cases (5.5%). Most common complains of pleural effusion (21.8%), followed by Tuberculosis with pleural effusion (14.5), Tuberculosis (9.1%), Pneumonia (9.1%) and Chronic liver disease (5.5%). Other causes were ascites with chronic liver disease (5.5%), synovitis were 3.6% and carcinoma of lung, colorectal carcinoma were (1.8%). The maximum number of samples were in the age group of more than 60 years (25.5%), Only 14.5% of patients were in the age group of 41-50 years. Male predominance 61% and 38.2% females in case of body fluids. **Conclusion:** Cell block technique helps to better appreciated on cellular morphology, Nuclear and cytoplasmic details. Sensitivity of cell block in malignant.

Key words: Cell block, Conventional smear.

INTRODUCTION

The introduction of the cell block technique was given by Bahrenburg nearly a century ago, since then it has been used routinely for processing the fluids. Cytological examination of body fluids is one of the commonly performed investigations.[1] It helps to diagnose benign and malignant lesions of effusion.[2] Cytological examination of body fluids gives information about the inflammatory condition of serous membranes, bacteria, fungi, or viruses.[4]

It is a simple, minimally invasive technique for the etiology of effusion. It has been studied that most often, it becomes a diagnostic problem to rule out accurate identification of cells either as malignant or reactive mesothelial cells in conventional smear.[5] Cellblock preparation is useful in the examination of effusion. Cellblock when combined with smears may improve the accuracy of the test by demonstrating the architectural pattern of cell aggregates and by facilitating the performance of histochemical and immunocytochemical stain.[6] Advantage of cell block-Preservation of architectural patterns like cell balls, papillae, and three-dimensional clusters. Cellblock sections are suitable for histochemical stains and IHC. In this study, we will be assessing and comparing the utility of cell block and conventional smear in fluid cytology.

MATERIALS AND METHODS

It is Cross-sectional and observational study which was conducted in cytopathology section of the Department of Pathology and Medicine between 1st December 2018 to 30th June 2020. A total of 55 sample of body fluids (pleural, ascitic, synovial)specimen were collected . After receiving the samples, physical examination of all fluids will be done and the following parameters will be noted - quantity, colour, appearance, presence of blood/clot. Approximately 10 ml of the sample will be taken, then it will be divided into 2 parts:5 ml is used for the conventional smear method and the next 5 ml is kept for cell block technique

Conventional method –

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5 ml sample is centrifuged at 3000 rpm for 10 mins and supernatant fluid is discarded. A minimum of 3 smears is prepared from the sediment. One smear prepared after air drying is stained with May-Grunwald -Giemsa stain and the other two smears are fixed in 95% alcohol and stained with Papanicolaou stain

Cellblock Technique

The other portion of the fluid specimen was processed by Fixed Sediment Method 2 of Cellblock according to Nathan et al.[7] The 5 ml fluid specimens were fixed in alcohol formalin fixative (9 parts absolute alcohol & 1 part 10% formalin) in the ratio of 1:1 for one hour. After fixation, the sample is centrifuged at 3000rpm for 10 mins. The supernatant fluid is discarded and cell sediment is formed and further 3 ml alcohol formalin is once again added to the sediment and kept for overnight fixation.

Sediment is drained off by inverting the tube over filter paper, then sediment is wrapped in filter paper and processed in the histopathology department as part of routine processing for making cell block and Haematoxyline & eosin stain was used .

Cytological and cell block diagnosis will be given for each case and individual slide will be analysed for cellularity, arrangement, cytoplasmic and nuclear details. All data pertaining to cases will be collected and analysed using statistical tool.

RESULT

Out of 55 fluid samples, the age of patients range from 30-60 yrs.34 cases (61.8%) were males and 21 cases (38.2%) were females. Among 55 cases, pleural fluid samples(37 cases),15 cases ascitic fluid and 3 case of synovial fluid. Number of samples received is clear (43.6%).cellularity in conventional smear was mild to moderate .cellularity in cell block was moderate to mild. Distribution of cell in conventional smear was lymphocytes (30.9%) Predominantely followed by atypicalcells(12.7%).In cell block lymphocytes (38.2%)followed by pleomorphic cells(18.2%). Pleomorphic cells suggest malignant cells. By CS Method benign (80%),suspicious(10.9%) and malignant (9.1%) respectively. By CB benign(80%),suspicious (0%) and malignant lesions (20%) respectively. Thus, diagnosing 11% additional malignancies by cell block method.

Table 1: Comparison of cellularity in conventional smear versus cell block

Cellularity	CS		CB	
	Frequency (n=55)	Percentage	Frequency (n=55)	Percentage
Mild 1+	23	41.8	19	34.5
Moderate 2+	19	34.5	26	47.3
Marked 3+	12	21.8	11	20.0

Table 2: Analysis of body fluid in CS and CB in final diagnosis

Diagnosis	CS		CB	
	Frequency (n=55)	Percentage	Frequency (n=55)	Percentage
Benign	44	80.0%	44	80.0%
Malignant	5	9.1%	11	20.0%
Suspicious	6	10.9%	0	0%

Table 3: Statical significance of the Present study in cellularity

CB	CS			
	1+	2+	3+	Total
Mild 1+	16 (69.6)	1 (5.3)	1(0)	17 (32)
Moderate2+	7 (30.4)	18 (94.7)	1 (8.3)	26 (47.3)
Marked3+	0 (0)	0 (0)	11 (91.7)	11 (20)
Chi sq =73.4; p=0.001(HS)				

The cellularity between CS and CB method is highly significant in our study.

P-value =0.001(Highly significant)

Table 4: Statical significance of the Present study in diagnosis

CB	CS		
	M	B	T
Malignant(M)	11	0	11
Benign(B)	0	44	44
Total(T)	11	44	55
Chi squ χ^2=55; κ=1, p=0.01(HS)			

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K=1 very Good, P value =0.01(Highly significant)
Sensitivity: 100%, Accuracy-100%
The utility of cell block is highly significant in malignant effusion as compared to conventional smear.

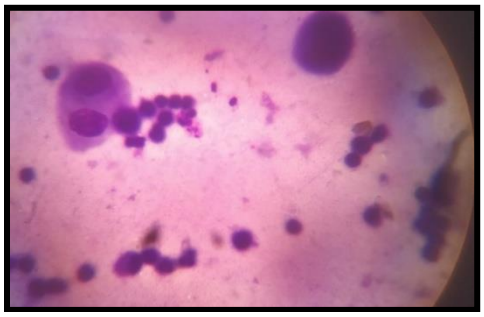

Fig 1: Photomicrograph showing a reactive mesothelial cell in window space in reactive effusion, showing binucleation. CS. (Giemsa stain 400X)

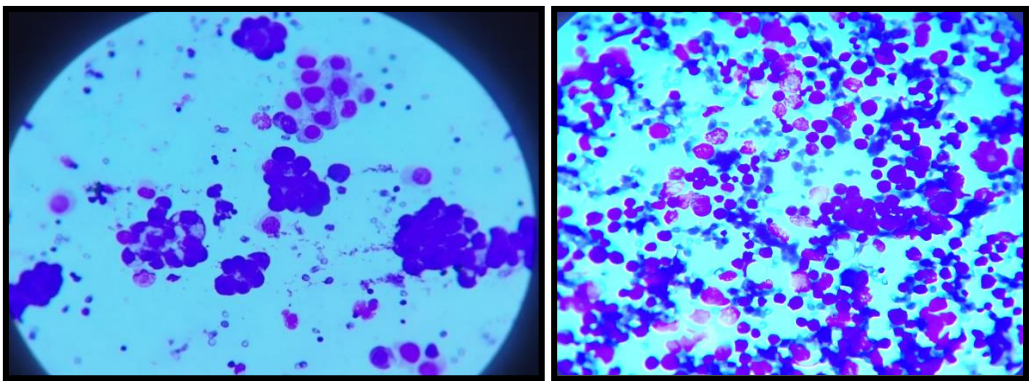

Fig 2: Photomicrograph showing malignant cells in conventional smear (Giemsa stain 400X).

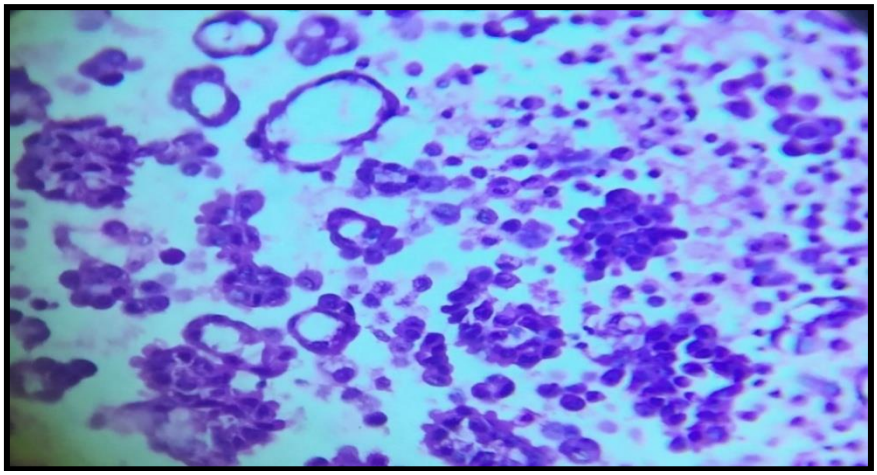

Fig 3: Photomicrograph showing malignant cells arranged in an acinar pattern in adenocarcinoma. CB (H & Estain 400X)

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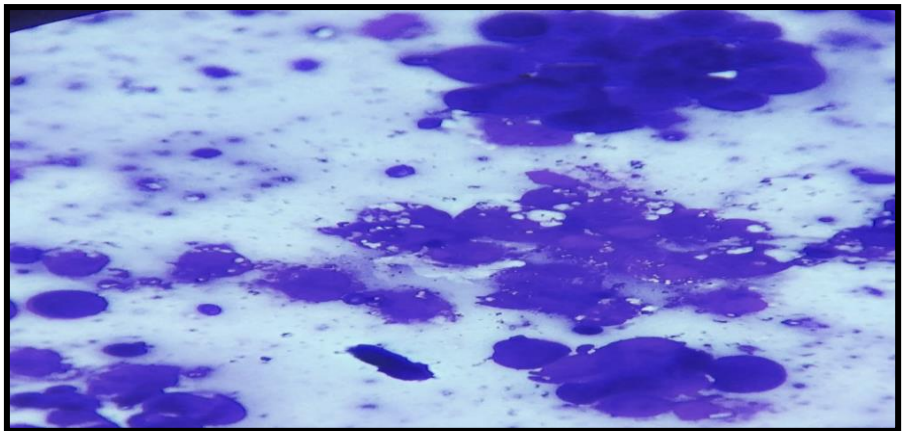

Fig 4: Photomicrograph showing malignant cell arrange in cluster acinar pattern with metastatic deposit CS. (Giemsa stain 400X)

DISCUSSION

Cytological examination of body fluids is a routinely done procedure in cytology. It helps in staging with primary malignancy known. The presence of malignant cells in body fluids indicates metastasis or malignancy. Reactive mesothelial cells, an abundance of inflammatory cells, and paucity of representative cells contribute to considerable difficulties in making a conclusive diagnosis on conventional smear.[1]Lack of morphological details of representative cells contributes to difficulties in the conventional smear. To overcome these difficulties a study was done.In this study, an attempt was made to prepare and analyse conventional smear and cell block from the same body fluids. In this study, 10%alcohol- formalin has been used as a fixative for cell block preparation.

The advantage of formalin is that it preserved better morphology of cells when compared in the conventional smear, its principle is that formalin cause proteins to cross-linked, and gel is formed which cannot be dissolved in any material used for processing so it prevents cell loss. Therefore it is better preservation of antigenicity and cytomorphological features.[8]

We received 55 samples of body fluids, the maximum common site of effusion in the present study was pleural, followed by peritoneal and synovial fluid. Similar findings were noted by Bhanvadia VM et al[8], Dr. Tanu Agrawal et al[9], Falguni Goswami et al.[2], Meenu Thapar et al.[5] The overall age-wise distribution of patients in the present study ranged from 30 years to >-60 years, with a majority of cases of effusion being in the age group of 41-50 yrs . In our study males compromised 34 cases and females 21cases. In the present study the most common presentation was pleural effusion (21.8%) with tuberculosis (14.5%) and CLD (5.5%).similar findings were noted in a study by Thapar et al[5], Bhanvadia et al[8],Geethu G nair et al[10] In the present study out of 55 cases mild cellularity found in 41% by CS method, moderate cellularity showed in 34% cases by CS and marked cellularity showed 21% by CS method. Most common cellularity seen by moderate that is 47% by CB method. A similar study done by Anjali Sonkar et al.[11] on 85 cases found mild cellularity 62%, moderate 35%, marked 2.4% on CS method. We got increased cellularity by the cell block method. In the present study, out of 55 cases, 44 benign, 6 suspicious, and 5 malignant by a conventional method. In a study done by Raghuwanshi Priyanka et al[12], out of 55 cases ,Benign were 42 cases on CS and 40 cases on CB, Suspicious cases were on CS and 0 on CB ,Malignant cases were 7 on CS and 12 were detected on CB .Our study is similar with this study. By using the cell block method, we diagnosed 5 extra malignantlesions in our study. So a total of 11 cases was diagnosed as a malignant lesion.

Table 5: The additional yield of malignancy in various studies by cell block

S. No	Study	%
1	Takaji F et al[13]	18%
2.	Shivkumar Swamyetal.[1]	15%
3	Richardson et al.[14]	5%
4	Khan et al.[5]	16%
5	Dekkar and Bupp.[15]	38%
6	Present study	20%

Inthepresentstudydiagnosticyieldformalignancywassignificantlyincreased by the cellblock method. The present study identified an additional 20% (11 cases) malignant lesions by cellblock method when compared to conventional smear study. Besides this, the suspicious cases in conventional smear were diagnosed as a malignant lesion by the cell block method.

The reason for suspicious cases would be an atypical cell, reactive mesothelial cell. At a time, the reactive mesothelial cell was difficult to differentiate from malignant cells as the nucleus appeared much darker due to binucleation, a larger size, and cytoplasmic vacuoles giving the appearance of pseudo signet ring cell simulating adenocarcinoma. The background of a smear of malignant effusion was hemorrhagic as compared to reactive cell effusion. This correlating with clinical history and correlation enabled an interpretation of reactive mesothelial cells. None of the cases on follow-up appeared to represent a false

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negative diagnosis.The most common malignancy we found in our study is adenocarcinoma of the lung followed by GIT and 1 cases were of metastatic adenocarcinoma of the ovary and lung.

Cytomorphology of adenocarcinoma in conventional smear showed 3D clusters /aggregates of a cell arranged in either an acinar pattern. Cells with high nuclear-cytoplasmic ratio, large pleomorphic nucleoli, hyperchromatic nuclei, nuclear pleomorphism, cytoplasm with indefinite cytoplasmic borders enable diagnosis of malignancy. To differentiate the glandular pattern we used cell block in our study by using H and E stain we differentiate the better architectural pattern (glands, papillae, cell balls) to confirm our diagnosis. In malignant cases, adenocarcinoma of GIT shows malignant cells. Individual malignant cell round to oval with High N: C ratio, vesicular chromatin, cytoplasm shows large single to multiple vacuoles. The vacuoles often displace the nucleus towards the periphery giving rise to signet ring appearance, margins are irregular with nuclear pleomorphism.

The various disadvantage faced in CS were CB technique: -

Improper fixation, smear, and staining errors in CS cause overlapping, cell loss, and artifacts. While in CB there is the preservation of morphology, minimum background obscuring material, and raised cellularity. Due to less availability of samples, we could not get proper findings of various sites for further studies to rule out the various malignant diagnosis.

In our study Cellblock is superior to the conventional smear method in the final diagnosis. It helps in the recognition of histological patterns that rule out in smear preparation like glandular structures, papillary structures, and mucin in the cytoplasm which form a signet ring that can be seen in a cell block in cases of adenocarcinomas. It helps in the diagnosis of metastasis of adenocarcinoma and mesothelioma. CB shows sensitivity 100% but conventional smear shows only 40-70% of sensitivity

CONCLUSION

Cell block technique helps in better appreciated on cellular morphology, Nuclear and cytoplasmic details. Multiple sections can be taken for further studies like IHC or special stains. Sensitivity of cell block in malignant lesion had significantly increased as compared to conventional smear. Statistical analysis shows cell block has 100 %sensitivity to a conventional smear. The level of agreement between a conventional smear and cell block was 1.0 which shows a very good degree of agreement whose p-value is <0.01.The utility of cell block and conventional smear is highly significant.

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