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COMPARISON OF CYTOLOGY OF BODY FLUIDS USING ROUTINE SMEAR EXAMINATION AND CELL BLOCK TECHNIQUE

Pathology

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ABSTRACT

Introduction: Body fluids mainly comprises of peritoneal fluid, pleural fluid, synovial fluid, and cerebrospinal fluid (CSF). In the several inflammatory & neoplastic conditions, cells are exfoliated into these fluids and their study help in reaching definitive diagnosis. The cell block technique is one of the senescent and complementary methods in addition to the smear for examination of body cavity effusions **Aim:** To study cytology of various body fluid by using routine staining and cell block technique by specimens received in Department of Pathology G. R. Medical College & J. A. Group of Hospitals Gwalior from December 2021 to May 2023. **Material and Method:** A hundred cases of serous effusion were collected from different clinical departments. At first fluid were examined for their physical characteristics. Secondly it is divided in to two halves. One half was processed by routine technique and stained using Giemsa Stain & Papanicolaou stain. Remaining fluid was subjected to modified cell block technique using ethyl alcohol formalin combination and processed like a histopathology sample. These fluids were analysed for physical properties like the fluid volume, odour, and colour. Each individual slide was objectively analysed for cellularity, cell crowding, nuclear preservation, cytoplasmic preservation, background, and the ability to make definite diagnosis on them. Routine smears and cell block were both objectively given scores according to Mair et al criteria. **Results:** Out of total 100 cases, 49 were pleural fluid, 48 were ascitic fluid and 3 were synovial fluid. Maximum number of patient (19%) were in the age group of 41-50 years. On smear examination, 10/100 cases were reported positive for malignant cells. Cell block examination showed 12/100 positive cases for malignant cells. According to the scoring system, smears could obtain a maximum score of only '7' (n= 8) while cell block could secure '8' (n= 10). Two (2%) smears were unsuitable for diagnosis by smear examination and 24 (24%) smears were diagnostically superior. Cell block slides showed that 12 (12%) cases were unsuitable for diagnosis, but majority 46 (46%) cell blocks were diagnostically superior. **Conclusion:** Cell block increase the sensitivity of cytodiagnosis of serous fluids with main advantage of getting many sections for special stains and other ancillary techniques, especially immunohistochemical studies.

KEYWORDS

Pleural, Peritoneal, Smear, Cell block

INTRODUCTION

Body fluids like peritoneal fluid, pleural fluid, synovial fluid, and cerebrospinal fluid (CSF) are present normally with their constituents in particular proportions, within serous body cavities. In different pathological conditions, these fluids undergo abnormal qualitative and quantitative changes. In the several inflammatory & neoplastic conditions study of cells exfoliated into these fluids help to reach a diagnosis. The malignant cells in the pleural or the ascitic fluids were most commonly indicative of secondary's tumours, as primary malignancies which arose from the mesothelial cell lining were less frequent. In cancer patient, presence of malignant cells in effusion is an important prognostic indicator[1]. Material for such study can be obtained with less invasive procedures as compared to tissue biopsy and helps in treatment planning. In pathology, cytology is an integral part of diagnostic work up of nearly every body tissue including body fluids. An excessive accumulation of fluids in a body cavity is known as effusion. Effusions have a different kind of etiology such as inflammatory, benign and malignant. Cytological examination of effusions has diagnostic, therapeutic and prognostic implications[2]. The information provided by serous fluid analysis is very important. It helps the clinician in formulating and pointing out the etiology of effusion and list of differential diagnoses[3]. The cytological study of the fluid may be aided by the fact that the cells present in sediment is representative of a much larger surface area than that obtained by needle biopsy[4]. The cytological differentiation between adenocarcinoma cells and reactive mesothelial cells in effusion specimens may be difficult due to similarities in the morphologic features of adenocarcinoma cells & reactive mesothelial cells[5]. The reactive and hyperplastic mesothelial cells exhibit many cytologic alteration that can mimic malignancy like mesothelioma and adenocarcinoma. Most cases of effusion cytology can be diagnosed on routine cytological preparations. Use of cellblock can act as an adjunct to the cytodiagnosis[6, 7]. Direct smear prepared from centrifuged deposits of effusion are preferred by many laboratories for diagnosis. At times, because of lack of morphological details of the representative cells and some obscuring factors contributes to difficulties in making diagnosis on conventional smears. Studies have reported that cytological examination of serous effusions by means of smears at many times leaves behind a large residual fluid that is not further investigated but might contain valuable diagnostic material[8,9]. The accurate identification of cells as either malignant or reactive mesothelial cells is a diagnostic problem in conventional cytological smears. Distinguishing benign from malignant cellular changes may require meticulous screening, scrutiny of cellular features and an understanding of the range of reactive changes. Due to cellular overlapping, delaying artifact, suboptimal processing and leaving behind useful material causes lower diagnostic yield in cytology smears method. This residual material can be used in increasing diagnostic yield by the cell block method. Since the use of the cell block technique first by Bahrenburg nearly a century ago, it has been used routinely for evaluating fluids[10]. The technique is easy, safe, cheaper, and reproducible even in resource limited settings[11]. Cell blocks provided the best material for interpretation with less background staining and it give results that most closely approximate those seen in the histopathology[12]. They are particularly useful for differentiation of tumours when not possible from smears alone. The usefulness of the cell block depends on the availability of diagnostic material for further histopathological examination, histochemistry and immunohistochemistry for classification of tumor[13]. Utilizing all available material of the sample is importance to increase diagnostic accuracy and sensitivity[14]. Cell block method provides more cellularity, better architecture, better morphological features and an additional yield of malignant cells which increases the sensitivity of the cytodiagnosis compared to the cytological smears method. The use of cell block in routine non gynaecologic cytopathology is different in each institution. Despite less information is available to assess the contribution of cell blocks, the value of cell block has still been acknowledged[15,16]. Many techniques are available to prepare cell block and the type of fixative and embedding techniques used, making valid comparison difficult[17,18,19].

MATERIAL AND METHODS

The serous effusion samples of pleural, peritoneal and pericardial cavities and synovial fluid were obtained from both male and female patients of all age groups which come for cytological analysis in Cytology section of Department of pathology G. R. Medical College, Gwalior during the period from December 2021 to May 2023 (One and half years of prospective study). Cerebrospinal fluid, fluid aspirated from cystic lesions and intraoperative fluids were excluded from the study. Data was collected in a pretested proforma. Clinical findings and diagnosis of all cases of effusion samples sent to laboratory were noted. About half amount of the fluid will be processed by routine technique and staining was done using Giemsa Stain & Papanicolaou staining. Remaining fluid was subjected to modified cell block technique using ethyl alcohol formalin combination and processed like a histopathology sample. These fluids will be analysed for physical properties like fluid volume, odour, and colour. Other investigations like chest X-rays, ECG and USG of abdomen, CT scan and MRI were collected whenever necessary to see for primary sites and secondaries of malignancies. One half of specimen was centrifuged at 1500 rpm for 15 minutes. A drop of sediment was put on a glass slide and it spread with another glass slide to make smear. At a time 4 smears were made. Two were air dried and 2 wet fixed. Air dried smear were stained with Giemsa stain. Wet fixed smear was stained with Papanicolaou and Haematoxylin and Eosin stains. Another half of the sample was subjected to fixation for one hour by mixing with 5 mL of 10% alcohol-formalin (i.e., nine parts of 90% alcohol and one part of 7.5% formalin) solution. This was centrifuged at 2500 round per minute for 15 minutes after one hour of fixation. The supernatant was discarded and 3 mL of fresh 10% alcohol formalin was once again added to the sediment and it was kept for one day. On the next day, the sediment containing the cell button of the fluid sample was put out on to the filter paper and this cell button sediment sample was processed as routine histopathological specimens. The paraffin embedded cell button (cell block) sections of 4–6 micrometre thickness was prepared and stained with the Haematoxylin and Eosin stain.

A cytological diagnosis was rendered for each case on smears and cell block separately. Each individual slide was objectively analysed for several features such as cellularity, cell crowding or overlapping, nuclear preservation, cytoplasmic preservation, background and the ability to make definite diagnosis on them. Routine smears and cell block were both objectively given scoring according to Mair et al criteria[20](table 1).

Table 1: Scoring System Of Cytological Smears According To Mair Et Al[20]

Criterion	Description	Point score
1. Volume of obscuring background blood	Large amount: diagnosis greatly compromised or clot Moderate amount: diagnosis possible. Minimal: diagnosis easy, specimen of textbook quality	0 1 2
2. Amount of diagnostic Cellular material	Minimal or absent: diagnosis not possible Material present sufficient for cytodiagnosis Abundant: easy for diagnosis	0 1 2
3. Degree of cellular degeneration and cellular trauma	Marked: diagnosis impossible Moderate: diagnosis possible Minimal: good preservation	0 1 2
4. Retention of appropriate architecture	Absent or Minimal: diagnosis not Possible. Moderate: some cellular architecture preserve. Excellent closely reflecting histology: easy diagnosis.	0 1 2

According to the criteria mention above, comments were rendered on the quality of the slides by grouping them into three categories; Diagnostically unsuitable score :0, Diagnostically adequate score: 1-4, Diagnostically superior score: 5- 8.

RESULTS

Out of total 100 cases, 49(49%) were pleural fluid, 48 (48%) were ascitic fluid and 3 (3%) were synovial fluid. Maximum number of patients; 19 (19%) were in the age group of 41-50 years, followed by 17 patients (26%) in the age group of 51-60 years & 11-20 years respectively and least number of patients were in the age group of 81-90 years (01%) (table 2).

Table 2: Age-wise Distribution Of Cases

Age Group	Study Group	Percentage (%)
0-10	0	0%
11-20	17	17%
21-30	12	12%
31-40	14	14%
41-50	19	19%
51-60	17	17%
61-70	15	15%
71-80	5	5%
81-90	1	1%
Total	100	100%

In this study, there were 42 male and 58 female patients. On smear examination 10 (10%) cases were reported positive while 90 (90%) cases were negative for malignant cells. Cell block examination showed 12(12%) positive cases and 88 (88%) of case negative for malignant cells. There was one case of ascitic fluid and one case of pleural fluid which were reported positive for malignant cells on cell block, but was reported negative for malignant cells on smear examination (table 3).

Table 3: Cases Positive For Malignant Cells By Smear And Cell Block Examination

S. No.	Fluid	Diagnosis by smear	Diagnosis by cell block
1	Pleural	Positive	Positive
2	Pleural	Positive	Positive
3	Ascitic	Positive	Positive
4	Ascitic	Positive	Positive
5	Ascitic	Positive	Positive
6	Pleural	Positive	Positive
7	Pleural	Positive	Positive
8	Ascitic	Positive	Positive
9	Ascitic	Negative	Positive
10	Pleural	Negative	Positive
11	Pleural	Positive	Positive
12	Ascitic	Positive	Positive

Mair et al criteria[20] scoring system was used to access different parameters like volume of obscuring background blood, amount of diagnostic cellular material, degree of cellular degeneration and cellular trauma and retention of appropriate architecture. Score '0' was given to cases having maximum obscuring blood, while Score '2' to the cases with minimum obscuring blood. More number of smears (n=39) score '0', while maximum number of cell block (n= 46) scored 2 (table 4).

Table 4: Distribution Of Cases By Volume Of Obscuring Background Blood

Score	Smear (n=100)	Cell Block (n=100)
0	39	25
1	51	29
2	10	46

Taking the criteria of amount of diagnostic material, score '0' was given to cases having minimum amount of diagnostic material, while Score '2' to the cases with maximum diagnostic material. More number of cell blocks (n= 35) score '0' as compared to smears (n= 25). Score '2' was secured by approximately same number of smears (n= 24) and cell blocks (n= 22) (table 5).

Table 5: Distribution Of Cases By Amount Of Diagnostic Cellular Material

Score	Smear (n=100)	Cell Block (n=100)
0	25	35
1	51	43
2	24	22

Considering the criteria of cellular degeneration, score '0' was given to cases with maximum cellular degeneration and score '2' to cases with minimum cellular degeneration. Most cases of smears (n= 46) had scored '0', while score '2' was obtained by cell blocks in 32 cases as compared to 16 cases of smear (table 6).

Table 6: Distribution Of Cellular Degeneration Or Cellular Trauma

Score	Smear (n=100)	Cell Block (n=100)

0	46	22
1	38	46
2	16	32

Cases with poor retention of architecture were given score '0' while best architecture and arrangement of cells were graded as '2'. Maximum number of smear (n= 62) scored '0' while score '2' was obtained by approximately same number of smears (n=14) and cell block (n= 12) (table 7).

Table 7: Retention Of Appropriate Architecture And Cellular Arrangement

Score	Smear (n=100)	Cell Block (n=100)
0	62	40
1	24	48
2	14	12

The total score obtained by the smears and cell blocks with respect to various criterion set for scoring was calculated. The minimum of score '0' was secured by 2 smears and 4 cell blocks. Smears could obtain a maximum score of only '7' (n= 8) while cell block could secure '8' (n= 10) (table 8).

Table 8: Comparison Of Scores Of Smears And Cell Block (n=100)

Score	Smear	Cell Block
00	02	04
01	30	20
02	22	12
03	12	08
04	10	10
05	10	18
06	6	14
07	8	04
08	0	10

Cytological smears and cell block were studied for their quality by using the Point scoring system of Mair et al. It was noticed that by smearing technique, 2/100(2%) smears were unsuitable for diagnosis. Majority of smears 74/100 (74%) were diagnostically adequate and 24/100 (24%) smears were diagnostically superior. Cell block showed that 12/100 (12%) cases were unsuitable for diagnosis. 50/100 (50%) were diagnostically adequate and 46/100 (46%) cell blocks were diagnostically superior.

DISCUSSION

Morphological details of cells are sometimes lacking in conventional smear which leads to difficult diagnosis[8,9]. Hence, the cellblock technique is used additionally to avoid difficult diagnosis in centrifuged samples. In the present study, 10% alcohol-formalin was used as a fixative for cellblock preparation. Similar fixative was used in a study done by Bodele et al[19] and in similar studies[20,21]. In the present study, total cases were 100. Study done by Sherwani R[21] et al comprises of 209 cases, Thapar[22] et al did study of 120 cases. Jalal R[13] et al studied a very large number of cases i.e., 600 cases. In the present study, out of 100 cases, the majority 49 (49%) of the cases were of pleural effusion, followed by 48 (48%) peritoneal fluid cases. There were 3 (3%) cases were of synovial fluid. In a study by Thapar M et al, out of the total 120 cases of reactive effusions, most of the cases i.e., 58 (48.3%), were of pleural effusion, followed by 54 (45%) peritoneal fluid cases whereas the least number of 8 cases 8 (6.7%) were from pericardial effusion[22]. In a study by Sherwani R et al 209 cases, 75 (35.9%) cases were pleural fluids, 129 (61.7%) were ascitic and 3 (1.4%) were pericardial[21]. Jalal R et al studied total of 600 cases, of which 300 (50%) cases were of peritoneal fluid, 192 (32%) cases of pleural fluid and 108 (18%) cases of pericardial fluid[13]. Manisha B et al studied 38 cases of effusion. Out of these, majority of cases were pleural fluid (52.6%) followed by peritoneal fluid (44.7%), and 1 case was of pericardial fluid[14]. In present study, age of patients ranged from a minimum of 14 years to maximum of 89 years but the maximum number of cases were in the age group of 41-50 years (19%). Age range of patients with malignant effusion was 33 to 89 years. Khan N et al conducted a study of 58 patients with malignant peritoneal, pleural and pericardial effusions. They found that the age of patients ranged from 12 years to 75 years but the maximum number of cases were in the age group of 51-60 years (34.6%)[23]. In the present study, a greater number of female patients were seen (58%) as compared to male patients (42%). In a study by Jalal R[13] et al, out of 600 cases, 292 were males and 308 were females. The number of smears positive for malignancy was 52.6%, 23.0%, 36.8% and 10% respectively in the study done by Manisha B et al, Bodele et al, Thapar M et al and the present study[14,19,22]. This might be due to wide difference in sample size, prevailing diseases in particular geographical areas, awareness level in population and sampling technique. In the present study of 100 cases, cell block diagnosed 12 (12%) cases as malignant. In the study done by Manisha B[14] et al, 60.5% cases were diagnosed as malignant on cell block. Bodele AK[19] et al reported 30.9% cases as malignant on cell block. Thapar M[22] et al, diagnosed 36.8% cases as malignant on cell. Table 9 give a comparative representation of positivity on smear and cell block in present study with comparison with other studies.

Table 9: Comparison Of Positive Cases Between Cell Blocks And Smears With Other Studies

	Manisha B et al14	Bodele et al19	Thapar et al22	Present study
Positive cases on Smear	20 (52.6%)	29 (23.01%)	50 (41.6%)	10 (10%)
Positive cases in Cell Block	23 (60.52%)	39 (26%)	60 (50%)	12 (12%)

In the present study, within the group of 100 cases of effusion, there was absolute concordance between smear and cell block in 88 (88%) cases. In rest 12 (12%) cases, 10 (10%) smears were positive for malignant cells, while other were negative. But on cell block, all 12 (12%) cases were positive for malignant cells. Hence 2 (2%) more cases were found to be positive for malignant cells on cell block which were previously given negative for malignant cells on smear examination. Thapar M et al also studied the cytological smears and cell block techniques and assessed their quality by using the point scoring of Mair et al. In their study, 26% of the smears and 12% of cell blocks scored '0' and were unsuitable for diagnosis. 20% of the smears and 21% of cell blocks scored '1' and their quality was diagnostically adequate. More than half (54%) of the smears and 67% of cell blocks were diagnostically superior[20,26] (table 10). In the study by Bodele AK et al out of 150 cases, 34 cell blocks had shown no cellularity. But no other quality estimation was done in their study[19].

Table 10: Comparison Of The Diagnostically Suitable Smears And Cell Blocks In Various Studies (%)

Criteria	Thapar M et al22		Present study	
	Smear	Cell Block	Smear	Cell Block
Diagnostically Unsuitable	50 (26%)	23 (12%)	2 (2%)	4 (4%)
Diagnostically Adequate	38 (20%)	40 (21%)	74 (74%)	50 (50%)
Diagnostically Superior	102 (54%)	127 (67%)	24 (24%)	46 (46%)
Total	190	190	100	100

For the scoring according to volume of obscuring background blood, cell block can be efficient as the probable reason for this finding is that RBCs present in the fluid are distributed in a three-dimension cell block. Section is cut in one plane in cell block, thus the section has only some percentage of those RBCs hence making the section of better quality as compared to smear where a greater number of RBCs are on the slide making them poor in quality.

Smears showed slightly more percentage of cases with more diagnostic material. Score '0' was secured by a greater number of cell block (35%) as compared to smear. This probably can be attributed to three-dimensional nature of cell block where lesser amount of diagnostic cellular material was sectioned in one plane. Deeper cuts/serial sections will increase the amount of diagnostic material. According to objective scoring system, it was observed that amount of degeneration of cells was much more with routine smear technique (46%) as compared to cell block (22%). It is suggested that smearing and staining of smears produce more cellular degeneration. Cell block, showed better preservation of cellular morphology, which appears to be due to fixative and better handling of sample when processed as cell block. Present study showed no significant difference in retention of architecture between both the techniques if the diagnostic material was more. Cell block sections were diagnostically superior to smears, however if cytological material was abundant, it affected little on scores.

CONCLUSION

Cell block method of the study of body fluid is a simple, rapid and cost effective. Yet, conventional smear study is routinely practiced since it is easier to perform and useful for arriving at a diagnosis in a short

period of time. Cell block preparations can be combined with conventional smears whenever it is possible to improve the diagnostic accuracy.

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