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Cytological analysis of body fluids and comparison of precision in diagnosis between conventional smear and cell block along with clinical correlation

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Abstract
Background: The cytological analysis of serous effusions helps in diagnostic, therapeutic and prognostic implications especially in malignant effusions.
Aims: This study determines the comparison of precision in cytological analysis between conventional smear and cell block in body fluids with clinical correlation.
Study Design: The study was conducted in GMERS medical college Junagadh over a period of 2 years (2016-2018) in the department of pathologyon total of 100 fluid cytology samples.
Methods and Material: Conventional smears and cell block for the same fluid was performed and cytological analysis done. The cytological data was analyzed for significance in correlation between smear and cell block. Statistical analysis used. The results were tabulated and analyzed using SPSS version 15.0.
Results: Cell block was diagnostically superior in 53.11% of cases as compared to conventional smear which was 18.22%. However conventional smear was diagnostically inadequate in 33.98% of cases.
Conclusions: Cell block preparations can be combined with conventional smears wherever possible to improve diagnostic accuracy.

Keywords: Conventional smear, cell block, malignant effusions, cytology

Introduction
Diagnostic cytology is the interpretation of cells exfoliated from epithelial surface or removed from tissue. The cytological analysis of serous effusions helps in diagnostic, therapeutic and prognostic implications [1]. It is difficult to accurately identify the cellularity in conventional smears due to bland morphology of the cells, cellular overlapping, variation in laboratory techniques and useful material left back in the centrifuge tube in conventional smear method [2, 3].
This study determines the comparison of precision in cytological analysis between conventional smear and cell block in body fluids. Cell block was found to be a more specific technique for confirming the diagnosis given in conventional smear. However, both the techniques combined together gave highest sensitivity and specificity in detecting malignant cases. At times, lack of morphological details of the representative cells contributes to considerable difficulties in making diagnosis on conventional smears [2, 3]. An attempt was made to utilize cell block technique in addition to the routine centrifuge method to overcome this difficulty.

Materials and Methods
This study was conducted over a period of 2 years (2016-2018) in the department of pathology at GMERS Medical College and Hospital Junagadh. A total of 100 fluid cytology samples obtained from patients of various departments were analyzed. The clinical information including age, sex, history, provisional diagnosis was noted.
10 ml of fresh serous pleural, peritoneal, pericardial and cerebrospinal fluid samples received were first submitted for naked eye examination for physical characteristics and then subjected to conventional smear and cell block techniques. Around 5 ml of sample was taken in test tube and centrifuged at 2500 rpm for 15 minutes. A minimum of 2 thin smears were prepared from the sediment. The smears were stained with Haematoxylin and Eosin (H&E) stains.

The rest 5 ml of the sample was fixed with 5 ml of 10% alcoholic formalin (90% Ethyl alcohol and 10% formalin) for 24 hours. This cell button was processed by dehydration, clearing and embedding. 4 micron thickness cell block sections were prepared from the cell button and the smears were stained with Haematoxylin and eosin stain. All the samples were subjected to systematic examination and scored as per the scoring system by Thapar M *et al.* [4] morphologic criteria such as cellularity, architecture and smear background were described and categorized.

An impression of results were as per below:

1. Acute or chronic inflammation,
2. Reactive effusion,
3. Suspicious for malignancy
4. Positive for malignancy

The cytological diagnosis was correlated with clinical diagnosis and other specific laboratory investigations.

The results were tabulated and analyzed using SPSS version 15.0. Chi square test was used to analyse the benign and malignant lesions by CS & CB method.

Observation & Results

Total 100 samples were screened using conventional smear & cell block techniques simultaneously. The age of the patients range from 4-70 years, with maximum number of patients were in the age group of 40 -60 years. The samples of male patients (67) outnumbered the female patient's samples. Clinically, most common symptoms were abdominal distension, loss of weight, fever.

As shown in figure 1, we have included 52 cases of peritoneal fluids followed by 36 cases of pleural fluids. Majority of the cases with effusion showed reactive changes with the rest showing malignant effusion. Table 1 showing out of 52 cases of peritoneal fluids, 30 cases were associated with cirrhosis. 46 cases were diagnosed as reactive effusion while 6 cases were turned to be malignant. Table 2 showing 36 cases of pleural fluid, among which 16 cases were of tuberculous effusion and 12 cases were from patients of pneumonia. Majority of the effusions were peritoneal and pleura fluids of which 31% of the cases showed malignant cytology (Table 1 & 2). Lung carcinoma was the common cause for malignant pleural effusion and ovarian carcinoma was the most common cause in peritoneal fluids which were taken in the study respectively (Table 3 & 4).

Conventional smear showed high sensitivity in detecting malignant cell but was low in specificity in confirming the malignant nature of tumor cells. Cell Block was high on both sensitivity and specificity in detecting and confirming the malignant nature of the effusions. Cell block was diagnostically superior and adequate in detecting the nature of the malignant cells based on cell morphology, and adequate content of the cells from the effusion. (Figure 2)

The yield of cells with details of architectural pattern information about cellular with nuclear features, were more accurate in cell block as against the conventional smear method (Table 5, 6 & 7). It has been observed that CS show individually dispersed cells, clusters, papillary fragments and acinar formations & signet ring cells or keratinised cells in malignant fluid effusions, but the appreciation of architectural pattern of the malignant cytology, such as, three dimensional clusters, cell balls, sheets, cellular as well as nuclear pleomorphism, nuclear hyperchromatism, irregularity of the nuclear contours, type of chromatin, prominence of nucleoli, atypical mitotic figures and features of differentiation such as intracellular secretions, signet ring cells & evidence of keratinisation is much better in cell blocks (Figure 3 & 4). Chi square test was used to analyse the benign and malignant lesions by CS & CB method In which the p value was found to be highly significant.

Table 1: Peritoneal fluid analysis, CS - conventional smear CB - Cell Block

Clinical diagnosis	No of cases	Cytological Diagnosis	No. of Cases	
			CS	CB
Peritonotis	08	Acute Inflammation	08	08
Cirrhosis with ascites	30	Chronic Inflammation	30	30
Fatty liver	07	Chronic Inflammation	07	07
Malignancy	07	Reactive Effusion	01	01
		Suspicious for malignancy	04	00
		Positive for malignancy	02	06

Table 2: Pleural fluid analysis

Clinical Diagnosis	No. of cases	Cytological diagnosis	No. of cases	
			CS	CB
Pneumonia	12	Acute Inflammation	12	12
Tuberculosis	16	Chronic Inflammtion	16	18
Malignancy	08	Reactive	01	02
		Suspicious	05	-
		Positive	02	06
Total	36		36	36

Table 3: Malignant effusions diagnosed by cell block method in pleural fluids.

Malignant Effusion in pleural fluid	No. of cases		Total cases	Percentage
	Male	Female		
Lung	03	01	04	66.66%
Breast	-	02	02	33.33%
Total Cases	03	03	06	1000%

Table 4: Malignant effusions detected by cell block in peritoneal fluids

	Males Females	Total cases	Percentage
Stomach	01 -	01	10%
Colon	01 -	01	10%
Rectum	01 -	01	10%
Ovary	- 02	02	20%
Retro peritoneum	01 -	01	10%
Total	06	06	100

Table 5: Diagnostic material present.

Score	CS	N	CB	N
2	52%	52	65%	65
1	28%	28	20%	20
0	20%	20	15%	15

Table 6: Degree of cellular degeneration.

Score	CS	N	CB	N
2	35%	35	62%	62
1	50%	50	30%	30
0	15%	15	08%	08

Table 7: Retention of appropriate cellular architecture.

Score	CS	N	CB	N
3	10%	10	28%	28
2	58%	58	70%	70
1	32%	32	02%	02

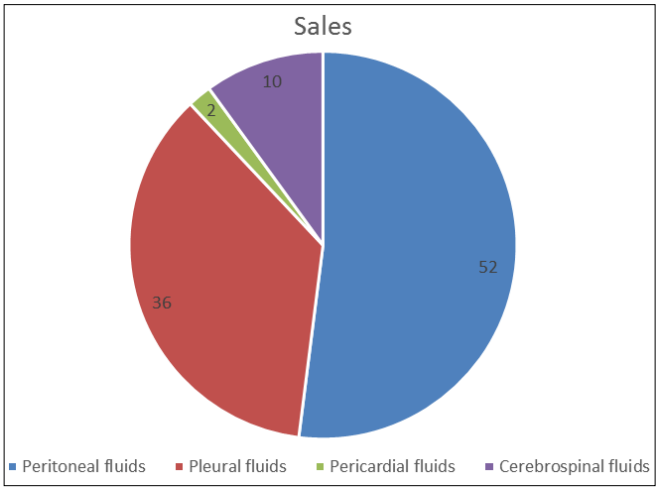

Fig 1: Distribution of cases among individual samples.

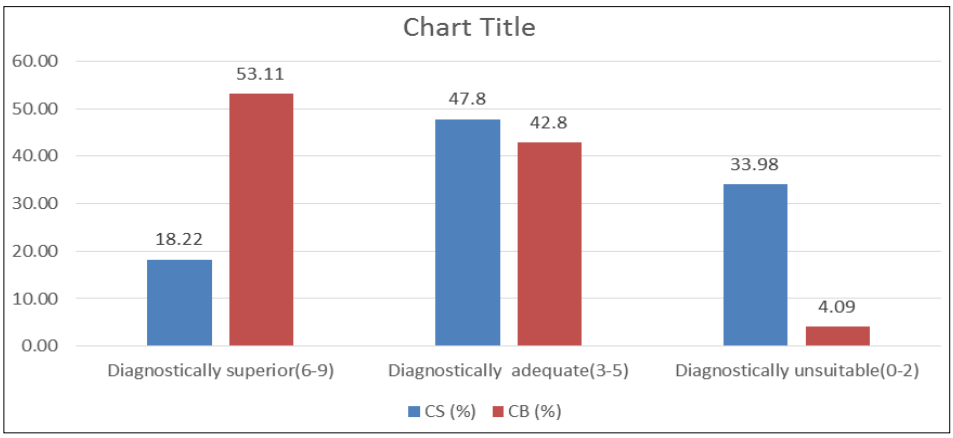

Fig 2: Showing diagnostic scores of CS and CB.

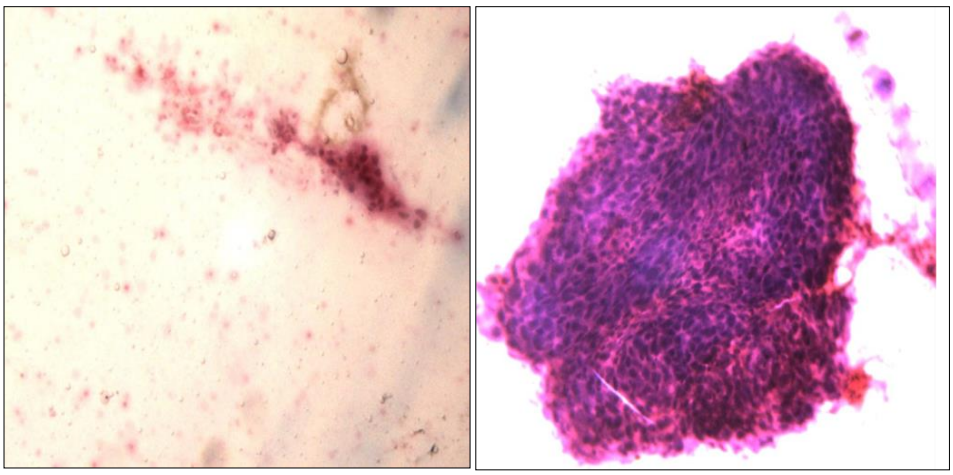

Fig 3: Pleural fluid malignant effusion.
(A) A smear showing few atypical cells arranged in clusters and singly. (H&E X 40)
(B) Cell block sections showing better cellularity and clear morphology of the atypical cells and clear background. (H &E X 100)

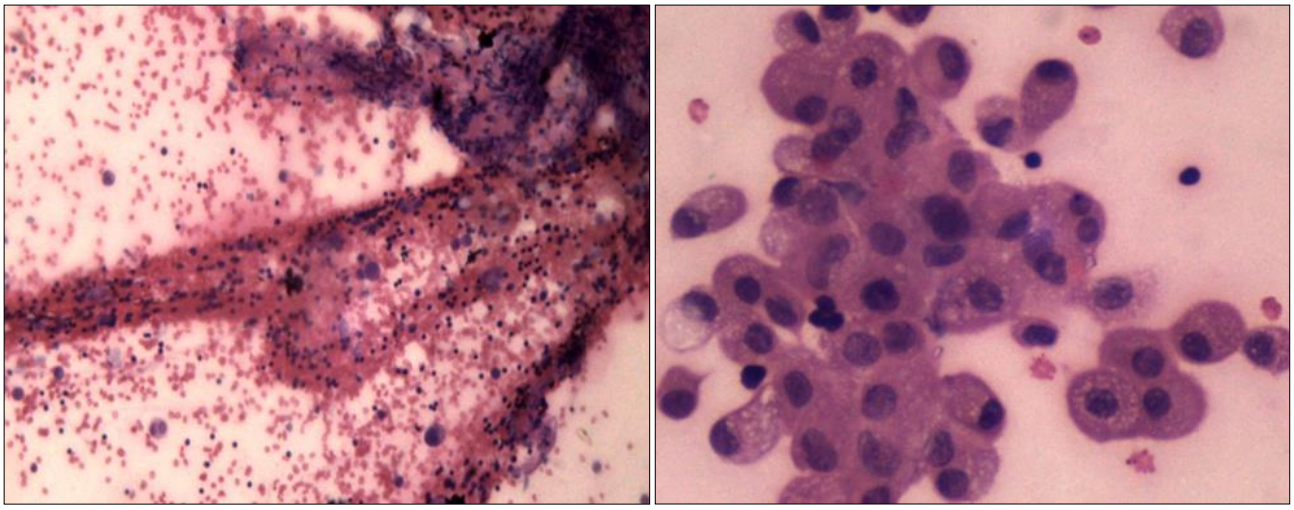

Fig 4: Peritoneal fluid in malignant effusion.
(A) Smear showing clumps of atypical cells arranged in loose clusters with poor cell morphology. (H&E X 40)
(B) Cell block section showing clear morphology of the atypical cells with clear background and good cell quantity. (H&E X 400)

Discussion

The cytological diagnosis of serous effusions is of paramount importance in diagnostic, therapeutic and prognostic aspects. Morphological details of cells are sometimes lacking in conventional smear which leads to difficult diagnosis [2, 3]. Cellblock technique was done to avoid difficult diagnosis in centrifuged samples. 10% alcohol-formalin as a fixative for cellblock preparation. Similar fixative was used in a study done by Bodele *et al.* and in similar studies [5, 6]. Out of 100 samples, 52 samples were peritoneal fluid, 36 were pleural, 10 were CSF and 2 were pericardial.

In analysis of 52 peritoneal fluid samples 30 cases were cirrhosis liver and 6 were alcoholic liver disease. Sujathan *et al.* [7] and Khan *et al.* [9] also observed the majority of cases in peritoneal fluid analysis the commonest being cirrhosis of liver with ascites. In pleural fluid analysis out of 36 samples, 16 cases were diagnosed as tuberculosis followed by pneumonia where the clinical cytological correlation was good in conventional and cellblock method which is similar to a study by Green LK *et al.* [8] and Humera Q.F *et al.* [10]. Cellularity was more in cellblock sections compared to conventional smears.

Cellblock sections identified better architectural patterns such as sheets, acini, cell balls and papillary formation whereas conventional smears showed only singly scattered cells and small clusters. Cell block sections were diagnostically superior as compared to conventional smears. These findings were consistent with the observations in the study done by Udasimath S *et al.* [11] The additional yield of malignancy was found to be 11% more by cell block technique compared to that obtained by conventional smear which is similar to a study by Richardson *et al.* [12] which reduced the false negative results.

Statistical analysis of malignant body fluids revealed significant difference of p value <0.05 in cellularity and diagnostic utility between conventional smear and cell block techniques.

Conclusion

We conclude that the cellblock technique is simple, inexpensive and does not require any special training or instrument. Cell block study has increased the diagnostic yield because of better preservation. It shows good architectural pattern, particularly in cases where there is a diagnostic dilemma between the malignancy and reactive changes. Conventional smear study is routinely practised since it is easier to perform and helps in diagnosis in short period of time. The overall diagnostic accuracy of conventional smear and cell block sections in body fluid analysis - 94%. Cell block preparations can be combined with conventional smears wherever possible to improve diagnostic accuracy.

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