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Original Article

Comparative Study of Serous Effusions Using Conventional Smear, Cytospin and Cell Block Technique.

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ABSTRACT

Background: Cytological examination of serous effusions is a widely recognized method and has gained increased acceptance. Diagnostic problems occur while trying to differentiate reactive mesothelial cells and malignant cells by conventional smear method, as a result of which new diagnostic modality such as cytocentrifuge (cytospin) has been incorporated along with cellblock method thereby increasing the efficacy of cytodiagnosis. In this context the present study is undertaken to study the morphological and cytological features of effusions by using conventional centrifuge, cytospin and cell block technique.
Methods: A prospective study was conducted on 200 samples of serous effusion received in the Department of Pathology, ESIC Medical College, Bangalore for a period of one and a half years. Each sample were subjected to conventional, cytospin and cell block technique. Further IHC and special stains were performed on cases of malignancy.
Result: Of the 200 samples of serous fluids, 61% were pleural fluids, 36% were peritoneal fluids and 3% were pericardial fluids. In the benign effusions, majority were pulmonary tuberculosis (39%), followed by pneumonia (23%) and nonspecific inflammation (23%). Transudative effusions were more (54 %) compared to exudative effusions (46%). Most of the exudative effusions were due to tuberculosis followed by malignancy. 24 cases of malignant effusion were detected and confirmed by cellblock technique.
Conclusion: A combined approach by conventional smears, cytospin and cell block technique provides high cellularity, better architectural patterns, morphological features as compared to conventional method.

Keywords: *Conventional smear, Cytospin, Cellblock, serous effusion.*

INTRODUCTION

Aspiration of excess amount of fluids within the serous cavities is a simple and relatively non invasive technique to achieve a diagnosis. Cytological examination of serous fluids is one of the commonly performed investigations.[1] The information provided by body fluid analysis assists the clinician in formulating, in order of priority a list of differential diagnoses.[2] Cytological examination is a complete diagnostic modality which aims at pointing out the etiology of effusion as well as prognosis of disease. [3]

Serous effusions are most often the first clinical symptoms of malignancy or their metastatic manifestation. Cytological techniques have been universally recognized as the most important diagnostic tool in the recognition of malignant tumors in effusions. Conventional method of preparing and interpretation of serous effusions is the one of the most commonly applied methods. However the accurate identification of cells as either malignant or reactive mesothelial cells is a diagnostic problem in conventional cytological smears. Thus, the cytocentrifuge preparations and cell block preparations are commonly used presently as they can preserve the cellular details and reduce the overlapping of cells, enabling precise interpretation than conventional smears.[1] In this context the present study is undertaken to study the morphological and

cytological features of effusions by using conventional centrifuge, cytospin and cellblock technique. It also aims to compare the sediments obtained in relation to cell yield, distribution and preservation of cell morphology.

MATERIALS AND METHODS:

A prospective study was conducted on 200 samples of serous effusion received in the Department of pathology, from Nov 2015 to June 2017 for a period of one and a half years. Complete clinicopathological details as per the proforma was collected. Fresh samples of pleural, peritoneal and pericardial fluids were evaluated for the study. These were taken and divided into three equal parts. Each sample was subjected to conventional, cytospin and cell block technique. One part was for conventional cytology and the other two parts were equally distributed for cytospin and cell block technique respectively.

For conventional smear 2 ml of fluid was centrifuged at 2500 rpm for 10 minutes and a minimum of two thin smears were prepared from the sediment. One smear was air dried and stained with Leishman stain and the other smear was immediately fixed in 95% alcohol and stained with hematoxylin and eosin stain.

For cytocentrifuge 300 µl of fluid was placed in 2 cytospin funnel with the filter paper placed between the slide and the funnel, then subjected to centrifugation at 1500 rpm for 10 minutes. One slide was air dried and stained with Leishman stain and the other slide was fixed in 95% ethanol for 15 minutes and stained with haematoxylin and eosin.

For Cell block technique, remaining sample of fluid was immediately fixed in 10% alcohol-formalin in 1:1 proportion for one hour. After fixation, it was centrifuged at 2500 rpm for 15 min. After centrifugations the supernatant was discarded and 3ml fresh 10% alcohol-formalin was added to the sediment and was kept for minimum of 24 hours. Next day, sediment was scooped out on the filter paper. The filter paper containing the sediment was processed like routine histopathological specimens. Paraffin embedded 4-5µ thick sections were routinely stained with Haematoxylin and Eosin stain. Special stains such as PAS and Alcian Blue were done wherever required. Further IHC and special stains were performed on cases of malignancy.

RESULTS:

Of the 200 samples of serous fluids, 61% were pleural fluids, 36% were peritoneal fluids and 3% were pericardial fluids. Age group of patients ranged from 10 to 80 years with the youngest patient aged10 and the oldest was 80 years with a mean age of 41.52 years. A mild male preponderance with male to female ratio 1.7: 1 was seen. Out of the 200 cases subjected to conventional centrifuge, cytospin and cell block procedures, majority of the samples were non neoplastic for each method [87.5%, 88% and 88%] respectively. Whereas malignancy identified on conventional centrifuge was 4.5%, cytospin was 8.5% and cell block was 12%.

FIGURE [P1.1] WITH LEGENDS [

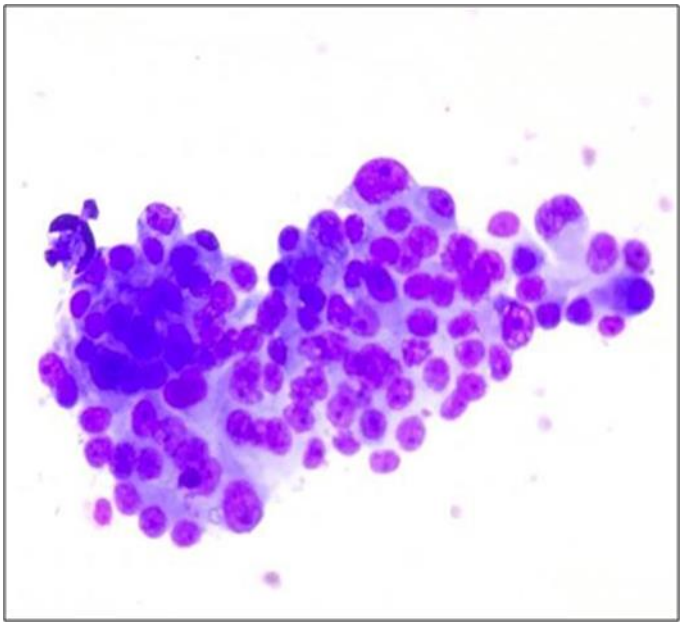

Fig 01 Photomicrograph of malignant cells in clusters in cytospin smear [Leishman 40X]

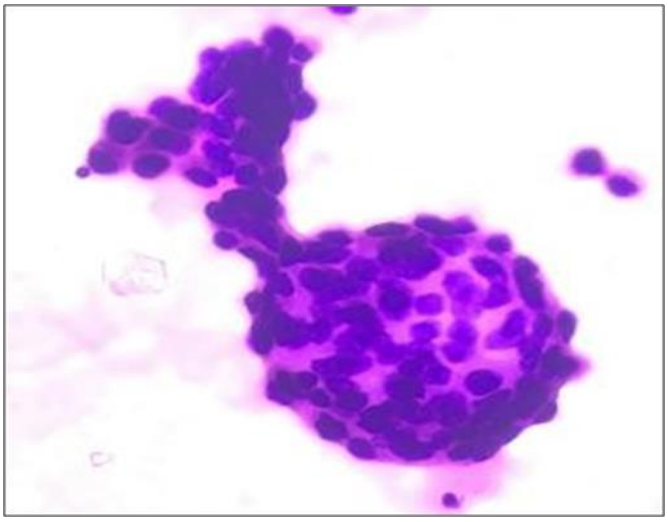

Fig 02 Photomicrograph of metastatic deposit of adenocarcinoma ovary arranged in papillae in cytospin smear [Leishman 40X]

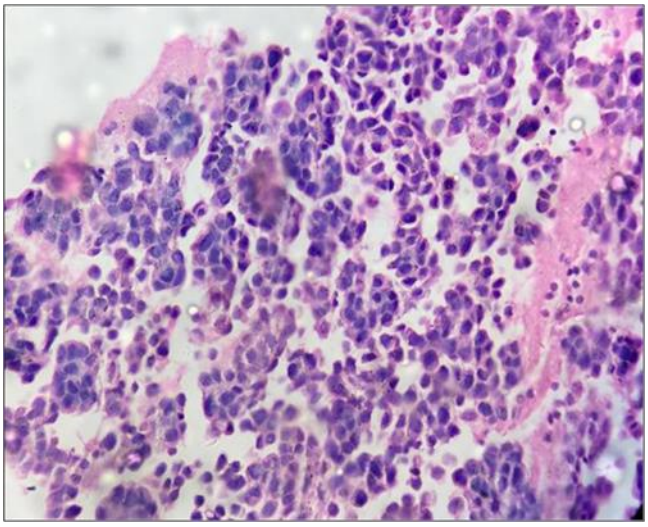

Fig 03 Photomicrograph of metastatic deposit of adenocarcinoma ovary in small clusters and acini in cell block [H&E 10X]

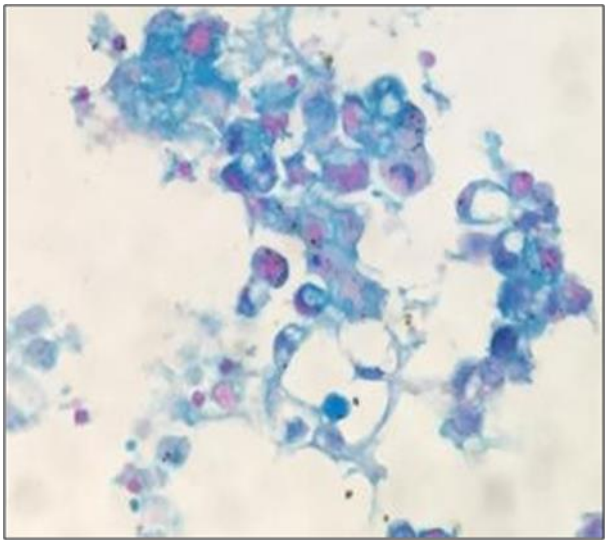

Fig 04 Photomicrograph of metastatic ovarian adenocarcinoma deposit exhibiting blue colored intracytoplasmic mucin [Alcian Blue 40X

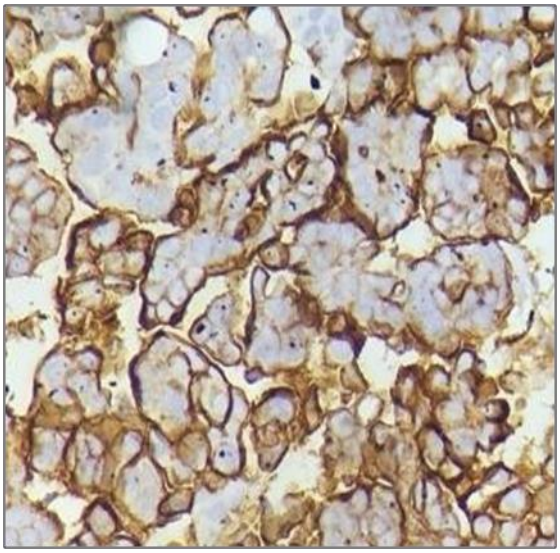

Fig 05 Photomicrograph of metastatic adenocarcinoma deposits of ovary showing strong membranous positivity for CA125 [40X]

TABLES:

Table 01 Distribution of tumor types based on Diagnostic Procedure performed

	Distribution of tumor types based on diagnostic procedure performed					
	Centrifugal		Cytospin		Cell Block	
Tumors	n	%	n	%	n	%
Non neoplastic	174	87.5	176	88.0	176	88.0
Suspicious malignancy	17	8.5	7	3.5	0	0.0
Malignancy	09	4.5	17	8.5	24	12.0

Table 02: Distribution of malignant tumor based on primary.

Distribution of malignancy based on types and their primary site of occurrence		
Malignant tumors	n	%
Adenocarcinoma	21	87.5%
Squamous cell carcinoma	2	8.30%
Angiosarcoma	1	4.20%
Primary Site	n	%
Lung	16	66.70%
Ovary	5	20.80%
Heart	1	4.20%
Breast	1	4.20%
GIT	1	4.20%

Table 03 Comparison of study characteristics using Kruskal Wallis test

Comparison of study characteristics between different procedures using Kruskal Wallis Test followed by Mann Whitney post hoc Analysis								
Characteristics	Procedure	Mean	SD	Median	IQV	Min	Max	P-Value
Background	Centrifugal	1.04	0.49	1	0.848	0	2	<0.001*
	Cytospin	1.67	0.47	2	0.765	1	2	
	Cell Block	1.70	0.46	2	0.579	1	2	
Cellularity	Centrifugal	1.31	0.46	1	0.856	1	2	<0.001*
	Cytospin	1.76	0.43	2	0.730	1	2	
	Cell Block	1.85	0.36	2	0.510	1	2	
Morphology	Centrifugal	1.38	0.49	1	0.942	1	2	<0.001*
	Cytospin	1.78	0.42	2	0.698	1	2	
	Cell Block	1.84	0.37	2	0.538	1	2	
Distribution	Centrifugal	1.30	0.47	1	0.640	0	2	<0.001*
	Cytospin	1.86	0.35	2	0.482	1	2	
	Cell Block	1.91	0.29	2	0.328	1	2	

In the benign effusions, majority were pulmonary tuberculosis (39%), followed by pneumonia (23%) and nonspecific inflammation (23%). Transudative effusions were more (54%) compared to exudative effusions (46%). Most of the exudative effusions were due to tuberculosis followed by malignancy. 24 cases of malignant effusion were detected and confirmed by cell block technique and all were metastatic deposits. Out of which majority was adenocarcinoma (87.5%) followed by squamous cell carcinoma (8.3%) and one case each of ductal carcinoma (4.2%) and angiosarcoma (4.2%). majority of the cases had primary in the lung [66.7%] followed by ovary [20.8%] and one case each in heart [4.2%], breast [4.2%] and GIT [4.2%]. Additional diagnostic yield of 7.5% was obtained for malignancy on cell block method. In the present study on comparing the cytological details brought out by each technique, cytospin and cell block was superior in demonstrating cellularity, cell retrieval , less cellular crowding, better cytoplasmic and nuclear preservation than routine method. Mann Whitney post hoc test was performed for comparing between methods and revealed a statistically significant p-value <0.005 when conventional centrifuge was compared with cytospin and cellblock respectively. However no statistical significance was obtained on comparison between cytospin and cell block.

DISCUSSION:

Majority of the cases in our study were pleural [61%] followed by peritoneal fluid [36%] and pericardial fluid[3%] which was similar to the studies by authors like Singh M et al [4] and Aggrawal T et al. [5] . In our study transudates [54%] were more common than exudates [46%]. Exudates were commonly caused by infection, TB and malignancy and Transudates were due to chronic liver disease, chronic obstructive pulmonary disease, renal failure, congestive cardiac failure and hypoalbuminemia which was similar to the study conducted by Mulwalkar M et al [6]and Sherwani M et al [7]

In our study, the age range from 10 to 80 years with common age group being 41-50 years a mean age of 41.5 years which is almost similar to the study undertaken by Aggrawal T et al [5]with a mean age of 45.5.Our study showed a higher male preponderance, with Male to Female ratio 1.7:1, similar to study by Udasimathetal et al[8] and Poorana Petal et al[9]

In our study on conventional smear examination, 175[87.5%] of the cases were benign, 16[8%] were suspicious for malignancy and 9 [4.5%] were positive for malignancy. On cytocentrifuge method, 176 [88%] cases were benign, 7[3.5%] suspicious and 17 [8.5%] were malignant. Cell block confirmed the diagnosis in 176[88%] of benign effusion and 24[12%] of malignant effusion. This finding is comparable with the studies done by Joshi et al [10] and Singh M et al [4] where the positive cases of malignancies were higher on cell block than conventional smear method.

In our study, comparative evaluation was done for samples processed by conventional and cell block technique, where cell block was able to confirm the diagnosis of malignancy in 17 suspicious cases detected on conventional smear and 1 suspicious case of malignancy was confirmed as non-neoplastic on cell block and additional 9 [4.5%] cases of malignancy diagnosed on conventional centrifuge were confirmed on cell block. No suspicious cases were detected on cell block. Our study was similar to the study done by Shivkumarswamy et al [8] all suspicious cases were confirmed as malignant on cell block.

In our study we found that cellular morphology, nuclear and cytoplasmic details were better appreciated on cytocentrifuge and cell block technique. And comparing the cytological details brought out by each technique, cytospin and cell block was superior in demonstrating cellularity, cell retrieval, less cellular crowding, better cytoplasmic and nuclear preservation than routine method. Various statistical tests were applied on the data obtained and the results were compared which was similar to the statistical evaluation done by Joshi et al[10]. A significant p- value was also obtained [p<0.001]

CONCLUSION:

Thus utility of cell block method in cytodiagnosis of malignant effusion is highly significant as compared to conventional smear method and the accuracy of diagnosis and diagnostic yield can be further increased when these three techniques are used together. Morphological features like architectural pattern - cell balls, papillae and three dimensional clusters with intact cell membranes and crisp chromatin details are well appreciated on cell block and cytospin when compared to conventional smear method. So a combined approach of conventional smears, cytospin and cell block technique helps to get an additional diagnostic yield for malignant effusions

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Competing Interests: None

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