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Cytological Diagnosis of Serous Effusions by Using Comparative Approach of Routine Staining and Cytospin Technique.

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ABSTRACT

Background: Cytological assessment of serous fluids is important not only for diagnosis of malignancies but may also give information of various inflammatory conditions like parasitic infestations, infection with bacteria, fungi or viruses and some immunological conditions. It helps in staging, prognosis and management of patients of malignancies. This study undertakes the assessment of the utility of cytospin method in increasing the sensitivity of cytodiagnosis of serous effusions in comparison to conventional staining method & bear its impact mainly in early and correct diagnosis of malignancies and hence patients management and prognosis. Aim: To study different body fluids for the presence or absence of local or systemic pathology of neoplastic, inflammatory, infective and immune mediated lesions, to evaluate the diagnostic efficacy and sensitivity of comparative approach of routine conventional smears and cytospin techniques. **Methods:** One hundred and fifty serous fluids were received for diagnostic evaluation. Along with conventional smear, fluids were cytocentrifuged. Smears obtained by each of techniques were scored for same four parameters. Statistical analysis with Wilcoxon rank sum test was performed and also Index of Qualitative Variation (IQV) was calculated to compare the results obtained by each of the above two methods. **Results:** Cellularity and additional yield for malignancy was obtained more accurately by Cytospin method than conventional method. **Conclusion:** The cytospin method provide high cellularity, better architectural patterns, morphological features and an additional yield of malignant cells, and thereby, increases the sensitivity of the cytodiagnosis when compared to conventional smear method.

Keywords: Cytospin, Conventional smear fluid cytology.

INTRODUCTION

Cytological assessment of serous fluids is important not only for diagnosis of malignancies but may also gives information of various inflammatory conditions like parasitic infestations, infection with bacteria, fungi or viruses and some immunological conditions. It helps in staging, prognosis and management of patients of malignancies.[1]

Cytologic evaluation is the best way to detect the presence of malignancy in body cavity fluids1.The general cytologic examination can be performed easily, quickly, and inexpensively by conventional smears. The accurate identification of cells in serous fluid as either malignant or reactive mesothelial cells are a common diagnostic problem on conventional cytological smears in day to day practice. Distinction between benign and malignant cellular changes requires meticulous screening, careful visualization of cellular features and an understanding of the range of changes in reactive process.[2]

Cytological examination gives the first indication of malignancy in one third of malignant effusions. However, to diagnose a cell accurately as either malignant, benign or 'reactive mesothelial cell in serous fluids is a real diagnostic challenge.[3]

Cytological assessment of effusion fluid is far better than the biopsy of the serous cavity lining for the diagnosis of malignancy affecting any of the cavities, as focal lesions on a serous surface may be missed by biopsy giving false negative results. But in an effusion, exfoliated malignant cells accumulate from all surfaces lining representating the entire serous cavity and are simple to collect.[4] This study undertakes the assessment of the utility of cytospin method in increasing the sensitivity of cytodiagnosis of serous effusions. This study will bear impact mainly in early and correct diagnosis of malignancies and hence patients management and prognosis

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Aim and Objectives

1. To study different body fluids i.e. pleural, peritoneal and pericardial, for the presence or absence of local or systemic pathology of neoplastic, inflammatory, infective and immune mediated lesions.
2. To evaluate the diagnostic efficacy of comparative approach of routine conventional smears and cytospin techniques.
3. To assess the increased sensitivity of cytospin method in cytodioagnosis of serous effusions.
4. To determine the primary malignancies in effusions.

MATERIALS AND METHODS

The present study is conducted in the department of Pathology, TMMC& Research centre, Moradabad, U.P. over a period of one and half years.

Inclusion criteria: All effusion samples of Pleural, Peritoneal, Cerebrospinal fluid, Pericardial fluid, Fluid aspirated from cystic lesions, Intraoperative and lavage fluid, Synovial fluid were included.

Exclusion Criteria: Clotted fluid specimen, Time between collection and processing more than one hour and Suboptimal preserved fluid specimens.

Sample size: All one hundred and fifty cellular samples comprising of inclusion criteria received over a period one and half years in the department of Pathology were included in the study.

Fluid sample was aspirated by wide-bore needle, i.e. 18-gauge needle, inserted under local anesthesia in sterile conditions. The samples of serous effusions were collected in the clean, dry, rubber stoppered, labelled large sterile glass containers, as well as in properly closed large jars in case of large volumes with properly filled requisition forms. Delayed samples were stored in refrigerator at temperature of 2-6^{o} C in volume ranged from 5ml to 2000 ml. All samples were processed by using routine CS method and cytospin method. In this study, freshly aspirated fluid specimens were used for preparing smears without addition of anticoagulant. In hemorrhagic fluid 0.1 ml of glacial acetic acid was added to haemolyze RBCs. Smears were prepared and fixed in ether alcohol. Air dried smears were also analyzed. The samples were examined by naked eye for physical characteristics and divided into two equal parts. One part of the specimens were processed by routine centrifuge practiced in our laboratory i.e. Thick and thin smears were prepared after centrifugation at 2500 rpm for 10 minutes.

In CS preparations, 5 ml fluid specimens were centrifuged at 2500 rpm for 10 minutes and then from sediment minimum 3 smears were prepared. One air dried smear was prepared and stained with the MGG stain. Other two smears were fixed in 95% alcohol, and stained with the H&E stain and Papanicolaou stain. Second part was subjected to cytocentrifuge i.e.300 microlitre of fluid was placed in cytospin funnel with the filter paper placed between the slide and the funnel, then subjected to centrifuge at 700 rpm for 6 minutes. The slide was then fixed in 95% ethanol for 15 minutes and stained with H&E.

The smears obtained by each of the above technique were evaluated for features such as background, cellularity, cell morphology and cell distribution and were scored from 0 to 2+ scale (Table-1) according to the Mair et. al.[5] scoring system -

Table 1: Scoring System.

Parameter	Quantative Assesment	Score
Background or proteinaceous material	Large amount, great compromise in diagnosis. Moderate amount, diagnosis possible. Minimal, diagnosis easy	0 1 2
Amount of cellular material	Minimal to absent, diagnosis not possible. Sufficient for cytodiagnosis. Abundant, diagnosis simple.	0 1 2
Cell morphology, cellular degeneration and trauma	Marked cellular degeneration, diagnosis not possible. Moderate cellular degeneration, diagnosis possible Minimal cellular degeneration, diagnosis easy.	0 1 2
Distribution of cells	Totally in the periphery or sparsly distributed. Combination. Evenly distributed	0 1 2

Morphological criteria including arrangement of cells, nuclear and cytoplasmic details and cellularity of smears, were put together for the categorization of the fluid specimens. Final diagnosis was made considering detailed clinical history, radiological examination; other laboratory tests, cytospin examination finding and then samples were categorized in benign, suspicious for malignancy and malignant effusion category. Final data was recorded in Microsoft Excel sheet 2007 for further analysis.

Statistical analysis: Wilcoxon rank sum test was used to determine the statistical significance of difference of each parameter between two methods.

Index of Qualitative Variation(IQV) was calculated to compare the results obtained by each of the above two methods.

RESULTS

In this prospective study total one hundred and fifty effusion samples from serous cavities were received and analyzed by routine centrifuge and cytospin. The result was recorded and evaluated. Maximum number of samples received was in age group of 41-60 years. Of the 150 samples 81(54%) samples were of age above 40 years and 69 (46%) samples below 40 years. Maximum samples were from age of 51-60 (in females) and 41-50 years (in males). Samples

were more from female as compared to male by 17.4%. Of the peritoneal fluid samples maximum numbers (n=17) were from the patients of age group of 41-50 years. Maximum no of pleural fluid (n=12) were in the age group of 61-70 years. Maximum sample of CSF (n=12) were from the age group of 0-10 years. [Table 2]

The most common effusion sample was peritoneal fluid 42.67 % (n=64) followed by pleural 28% (n=42) and CSF 26% (39). 2 Pericardial and Synovial and one BAL fluid were also received.

Out of 150 samples, 139 (92.66%) samples were benign and 11(7.3%) were malignant when examined by conventional method. Maximum number of cases of malignancy was found in pleural fluid (n=8, 72.72%), whereas maximum no of benign cases were found in ascitic fluid (n=64, 42.7%).Out of 150 samples, 135(90%) were benign and 15(10%) were of malignancy by cytospin method. 4 more cases of malignancy were diagnosed on cytospin method with total 15 cases diagnosed as malignancy. Maximum no of cases of malignancy were found in pleural fluid (n=11, 73.33%) with 3 more cases diagnosed on cytospin [Table 3]

In this study, out of 15 cases of malignant effusion, 11 (73.33%) were diagnosed as positive for malignancy on routine centrifuge compared to cytocentrifuge method which showed 15 cases of malignancy. [Table 4]

The smears obtained were evaluated for features such as background, cellularity, cell morphology and cell distribution and were scored from 0 to 2+ scale [Table 1] according to the Mair et. el.[5] (19) scoring system. By conventional method, maximum samples were in category 1 with respect to all parameters and DLC could be performed in only 112(74.7%) cases in contrast to cytospin method, where maximum samples were in category 2 with respect to all parameter except cell morphology, in which maximum sample was in category 1 and DLC could be performed in 149(99.3%) cases. [Table 5]

IQV (Index of Qualitative Variation) ranges from 0 (No variation) to 1 (Maximum variation). IQV was found minimum for the parameters in cytospin method. [Table 6]

Significance of difference of each parameter for two groups was performed using Wilcoxon rank sum test. The difference between CONVENTIONAL & CYTOSPIN for various parameters was found statistically significant i.e. ($p< 0.05$). [Table 7]

Table 2: Distribution of samples among different age and sex & fluid type (N=150).

Age (year)	Male (%)	Female(%)	Fluid type					
			Peritoneal	Pleural	CSF	Pericardial	synovial	BAL
0-10	9(10.22%)	6(9.68%)	2	0	12	0	1	0
11-20	9(10.22%)	6(9.68%)	1	4	9	1	0	0
21-30	9(10.22%)	11(17.7%)	7	4	8	1	0	0
31-40	11(12.5%)	8(12.9%)	14	1	4	0	0	0
41-50	18(20.45%)	10(16.12%)	17	10	1	0	0	0
51-60	13(14.77%)	12(19.35%)	10	11	4	0	0	0
61-70	15(17.04%)	7(11.29%)	8	12	1	0	0	1
71-80	3(3.4%)	2(3.22%)	5	0	0	0	0	0
81-90	1(1.13%)	0(0%)	0	0	0	0	1	0
Total	88	62	64	42	39	2	2	1

Table 3: Distribution of fluid specimen among Benign and Malignant by conventional method (N=150)

Fluid type	conventional method			cytospin method		
	Sample	Benign	Malignant	Sample	Benign	Malignant
Ascitic	64(42.7%)	62(44.60%)	2(18.18%)	64(42.7%)	61(45.18%)	3(20%)
Pleural	42(28%)	34(24.46%)	8(72.72%)	42(28%)	31(22.96%)	11(73.33%)
CSF	39(26%)	39(28.05%)	0	39(26%)	39(28.88%)	0
Pericardial	2(1.3%)	2(1.4%)	0	2(1.3%)	2(1.48%)	0
Synovial	2(1.3%)	2(1.4%)	0	2(1.3%)	0	0
BAL	1(0.7%)	0	1(9.09%)	1(0.7%)	0	1(6.66%)
Total	150	139	11	150	135	15

Table 4: Distribution of malignant cases diagnosed by various techniques

Malignant cases	On conventional method	On cytospin method
15	11	15

Table 5: Descriptive statistics for each of the four parameters by method of conventional and cytospin smear preparation

Method	Parameter	Frequency	Percentage
Conventional	Background		
	0	21	14
	1	93	62
	2	36	24
	Cellularity		21.3
	0	32	56.7

	1 2	85 33	22
	Cell Morphology 0 1 2	 69 79 2	 46 52.7 1.3
	Cell Distribution 0 1 2	 62 74 14	 41.3 49.3 9.4
	DLC Yes No	 112 38	 74.7 25.3
Cytospin	Background 0 1 2	 8 10 132	 5.4 6.6 88
	Cellularity 0 1 2	 4 39 107	 2.7 26 71.3
	Cell Morphology 0 1 2	 7 73 70	 4.7 48.6 46.7
	cell Distribution 0 1 2	 7 30 113	 4.6 20 75.3
	DLC Yes No	 149 1	 99.3 0.7

Table 6: Descriptive statistics for each of the four parameters by method of conventional and cytospin smear preparation

Method	Parameter	Mean ± S.D(Median)	IQV
Conventional	Background	0.70 ± 0.540	0.808
	Cellularity	1.01± 0.660	0.877
	Cell Morphology	0.55 ± 0.525	0.766
	Cell Distribution	0.61 ± 0.541	0.866
Cytospin	Background	1.42 ± 0.594	0.324
	Cellularity	1.69 ± 0.520	0.634
	Cell Morphology	1.42 ± 0.582	0.271
	Cell Distribution	1.48 ± 0.588	0.585

Table 7: Statistical significance of difference of each four parameter in two methods of comparison (conventional and cytospin)

Parameter	Comparison of method Conventional and Cytospin	
	z-value	p-value
Background	9.229	<0.05
Cellularity	7.992	<0.05
Cell morphology	9.773	<0.05
Cell distribution	10.777	<0.05

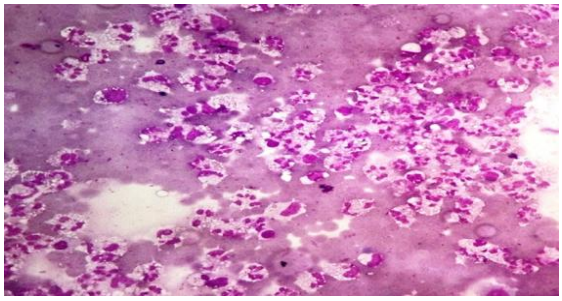

Figure 8: Photomicrograph shows plenty of polymorphs: negative for malignancy (giemsa stain, cytospin preparation, 400 X)

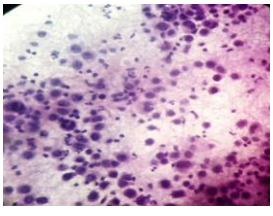

Figure 9A: shows mesothelial cell mixed with mononuclear inflammatory cells. (Giemsa stain, cytospin preparation, 400 X)

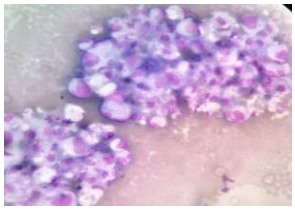

Figure 9B: shows rective mesothelial cell mixed with mononuclear inflammatory cells.

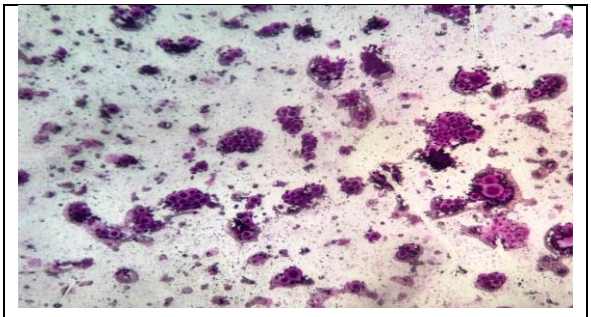

Figure 10 A: photomicrograph shows clusters of atypical epithelial cells admixed with mononuclear inflammatory cells. (Giemsa stain: 100 X, conventional preparation)

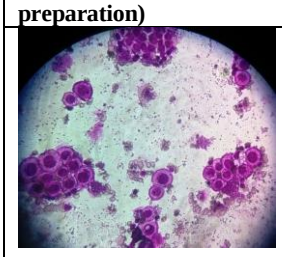

Figure 10 B: photomicrograph shows sheets of atypical epithelial cells having atypical nuclei (Giemsa stain: 400 X, conventional preparation)

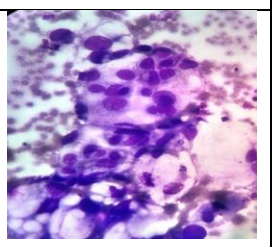

Figure 10 C: photomicrograph shows clusters of highly atypical epithelial cells. (Giemsa stain: 1000 X, cytospin preparation)

DISCUSSION

Cytological examination of serous effusions has increasingly gained acceptance in clinical medicine, to such an extent that a positive diagnosis is often considered as the definitive diagnosis and obviates explorative surgery. It is important not only in the diagnosis of malignant lesions, but also help in its staging and prognosis.[6]

In most of the cytology laboratories, cytologist prefers direct smear prepared from centrifuged deposits of effusion. Lack of good morphological details of the representative cells contributes to considerable difficulties in making conclusive diagnosis on conventional smears. In order to overcome these difficulties, in this study smears were prepared by both routine centrifuge and cytocentrifuge methods from the same sample and analyzed and then the results were compared. In this study due consideration was given to age, sex, site of effusion, clinical findings and other investigations to arrive at final diagnosis and also to identify primary site of malignant lesions. The use of cytocentrifuge not only increases the cellularity as compared to routine centrifuge, but also the cells were evenly distributed. The cellular morphology, nuclear and cytoplasmic details, was better represented on cytocentrifuge technique.[7]

In smears prepared by cytocentrifuge, cells are subjected to a centrifugal force and subsequently, flattening of cells occurs on the glass slide resulting in increased cellular area measurement.[8] The influence of centrifugal forces on cells tends to accentuate subtle variations in shape of the nucleus and as a result nuclear foldings are easily discerned. The presence of artifactual distortion of cells during cytocentrifuge smear can't be discounted this may however be minimized by lowering centrifugation speed. The degree of nuclear contour irregularity was much easily appreciated on cytospin preparation in comparison to CS.[9]

Thus routine centrifuge exhibits poor sensitivity and are less often positive than cytospin smears.[0,11] The presence of malignant cells in the pleural or the peritoneal fluids was indicative of metastatic lesions mostly, as in these fluids primary malignancies arising from the mesothelial cell lining were uncommon. In patients with known primary, positive effusion for malignant cells is a very important prognostic factor.[12] The presence of malignant cells in pleural effusion is a common complication and is an indication of advanced stages of lung, stomach and breast cancer, while malignant ascitic effusion is an indicative of liver, pancreatic colon and ovarian carcinoma. So, the serous fluids examination for the malignant cells presence has been accepted as a routine lab procedure for diagnosis of metastasis of unknown primary origin.[13,14]

Out of the total 150 fluids studied 64 (42.67%) were peritoneal fluid; 42(28%) pleural fluid and 2 (1.33%) were pericardial fluid. Maximum number of fluids were peritoneal i.e. 34(22.6%) in male and 30 (20%) in female. Out of a total of 150 specimen examined; 11(7.33%) were found to be positive for malignant cells by conventional method and 15(10%) by cytospin method. Our study is comparable to that of study conducted by Archana et. al.[15] which showed peritoneal fluid to be maximum amongst the samples studied and number of male patients were higher as compared to female.

In our study a significant difference between the results obtained by conventional smears as compared to cytocentrifuge which is in accordance with the study of Archana et. al.[15]

The most common effusion in the present study is peritoneal (42.67%) followed by pleural, csf and pericardial effusion. The same findings were found in the study by Archana et. al.[15] {Ascitic fluid (53.33%)}.

Present study studied 42 cases of pleural effusions; maximum numbers of patients lies in the age group above 4th decade. Pleural effusion is found common in age group between 40-60 years and the results of present study are comparable with Gerbes Al et.al. & Hymen S et. al. studies.[16,17]

Out of all 15 positive cases of malignancy maximum number (n= 12) of cases are found to be adenocarcinoma of lung which is slightly less as compared to the study by Khan N et. al.[18] and Gaur DS et. al.[19] Of all the positive ascitic fluids in this study maximum were due to adenocarcinoma of

ovarian origin (n=3), which is in concordance with the Monte SA et. al.[20] study, while maximum malignant cases were diagnosed in pleural effusions and is these cases are mainly due to adenocarcinoma of lung origin (n=12).

CONCLUSION

Routine centrifuge is not satisfactory in reporting fluids with scant cellularity. Hence for fluids with scant cellularity cytocentrifuge are useful methods. Also the morphology of the cells were well appreciated by cytocentrifuge as compared to routine centrifuge, thus aids in accurate diagnosis. In our study the diagnoses which were missed or incompletely diagnosed on routine centrifuge were diagnosed accurately by the other techniques. Also there was statistical difference between the results obtained by the two techniques. Thus cytocentrifuge proved to be superior method for the study of effusion as compared to routine centrifuge.

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