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Pathology

ANALYSIS OF CELL BLOCK VERSUS SMEAR EXAMINATION IN EFFUSIONS

Dr.Jilla.Rajitha M.D.Pathology, Assistant Professor, Department of Pathology, Chalmeda Anand Rao Institute of Medical Sciences, Karimnagar,Telangana.

Dr. Madhavi Reddy Assistant Professor, Department of Pathology, M.N.R.Medical college, Sangareddy.

ABSTRACT

AIMS: To assess the utility of the cell block preparation method in increasing the sensitivity of cytodiagnosis of effusions & to evaluate the primary site malignant effusions wherever needed with the help of immunohistochemistry.

MATERIALS&METHODS: A total of 120 cases were studied in a period of Nov.2010-June 2012. They were subjected to routine smear examination as well as cell block preparation.

RESULTS: Out of 120 cases, 12 cases were found be malignant effusions. Among 120 cases 95 pleural,18 peritoneal,7 pericardial effusions. Using a combination of the cell block & smear technique yielded 3 more malignant cases than what were detected using smears by themselves.

CONCLUSION: Cell block technique provides high cellularity, better architectural pattern, morphological features & an additional yield of malignant cells &thereby increasing the sensitivity of the cytodiagnosis when compared with cytosmear technique.

KEYWORDS : Cell block, cytodiagnosis and immunohistochemistry.

INTRODUCTION

Cytological examination of serous fluids is of paramount importance not only in detecting cancer cells, but it also reveals information regarding various inflammatory conditions of serous membranes, various bacterial, viral, fungal infections and parasitic infestations.[1]

Accurately diagnosing cells as either benign or malignant or reactive mesothelial cells in serous effusions is a common diagnostic problem. The lower sensitivity of cytodiagnosis of effusions is mainly attributable to bland morphological details of cells, overcrowding or overlapping of cells, cell loss and changes due to different laboratory processing methods.[2]

Most of the fluids received in the cytology laboratory contain blood clots or small bits of tissue from the lesion. While preparing the slide they remain in bottle and are not available for microscopy. Cell blocks are also particularly useful when samples are heavily admixed with blood. Smears may show only blood and a few distorted cells. Surprisingly, good tissue fragments may be found in sections of the cell block.[3]

Apart from increased cellularity, better morphological details are obtained by cell block method which include preservation of the architectural pattern like cell balls and papillae and three dimensional clusters, better nuclear and cytoplasmic preservation, intact cell membrane and chromatin details.[4] Cell block method has many advantages like multiple sections of the same material can be obtained for special stains and immunohistochemistry.

Fluids which are received in the laboratory are evaluated in the form of physical, chemical and microscopic examinations. Physical examin ation includes volume, color and appearance. Chemical examination includes sugars, Proteins, Enzymes (LDH, ADA) Specific Gravity, PH, C-reactive protein, lipid analysis. For microscopy fluids are examined under the microscopy are in the form of cytosmears & cell block[5].

MATERIALS AND METHODS

The present study was done for a period of two years from October 2010 to September 2012. During this period, pleural, peritoneal and pericardial fluids obtained by aspiration were analysed. An analysis of 150 cases of various lesions of pleural, peritoneal and pericardial fluids during this period was done.

After clinical, biochemical and radiological investigation, fluids thus obtained were first examined by naked eye for physical characteristics and divided in to two halves. Fresh fluids were used for analysis.

Thoroughly mixed half of the specimen centrifuged at 1500 rpm for 15 min. Supernatant is discarded from that sediment. Smears are prepared and stained with Haematoxylin &Eosin and Romanowsky.

- For the cell block preparation, the other half of the fluid specimen is centrifuged at 2500 rpm for 10-15 min. The supernatant fluid is discarded following which a cell button is formed, to it, 2 to 3drops of outdated plasma, 2 to 3 drops of thromboplastin and 2 to 3 drops of calcium chloride are added and allowed to clot. Cell button along with the clot is formed which is then fixed in 10% Buffered formalin for 24 hours 6.
- Cell button with the clot is wrapped in a filter paper and processed in tissue processor. Cell block is prepared after embedding it in paraffin medium. Sections are cut and stained with Hematoxylin and Eosin. Special stains including, Periodic acid stain were done when needed.
- The slides were carefully evaluated for the following features: Background of the smear/cell block, predominant cell type, presence of aggregated/isolatedcells, predominant pattern of aggregate – spherules, loose clusters etc. presence of patterns such as Indian file arrangement, rosettes and acini, uniformity or pleomorphism, presence of vacuolated cells, presence of any irregularity nuclear membranes and chromatic pattern, presence/absence of nucleoli, abnormal mitosis, presence of any Multi nucleated cells/giant cells, presence of any other reactive/stromal elements. A comparative evaluation of smear versus cell block technique was done.

RESULTS

From October 2010 to October 2012, 190 samples of various fluids were received. We have studied 150 fluids for present study which constitutes 78% of total fluids. Among total no. of 150 fluids,122(81.3%) pleural, 23(15.35%) peritoneal, 5(3.33%) pericardial fluids.

In a total of 150 fluids, most of the patients between 41-50 years constituting 38 cases(25.3%).In this study most of the patients were males when compared to females, male to female ratio is 2.57:1.In a total of 150 fluids received, males were 108(72%) and 42 (28%) were females.

Out of 122 pleural fluids, 75cases showed lymphocytes by cell block technique in which 75cases showed lymphocytes on smear examination. 3 cases showed scant cellularity on cytosmears which shows mixed inflammatory cells & lymohocytes in cell block.2 cases of suspicious for malignancy by smear showed malignancy by cell block technique (Figure1).One of the pleura fluid cell block showed microfilaria (Wucheraria Brancrofti), which was missed on cytosmears (Figure 2). Table 1 shows comparision of smears with cell block in various fluids.

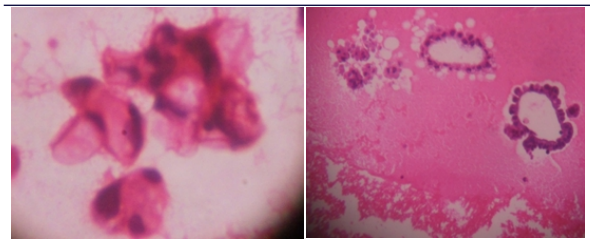

Figure 1: (a) Smears showing pleomprphic cells with eccentrically placed nuclei; Pleural fluid

(H&E stain,40X)
(b) Cell block showing cells are arranged in acinar pattern Pleural fluid (H&E stain,10X)

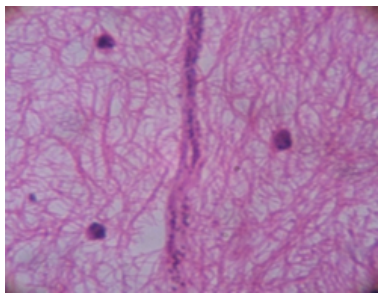

Figure 2: Cell block showing microfilaria Pleural fluid (H&E stain,10X)

Table 1: Efficacy of Centrifuged smears with cell block in detecting malignant cells

	Cell block		Total
Cytosmears	Positive for malignant cells	Negative for malignant cells	
Positive for malignant cells	13	0	13
Negative for malignant cells	03	134	136
Total	16	134	150

Sensitivity =81.25%
Specificity =100%
Positive predictive value = 100%
Negative predictive value = 97.81%
Accuracy = 98%

Out of 23 peritoneal fluids, 12 cases showed lymphocytes by cell block technique as well as in cytology. On cytosmears scant cellularity noted in a case, whereas cell block showed mixed inflammatory cells.1 case of suspicious for malignancy by smear showed malignancy by cell block technique (Figure 2&3).

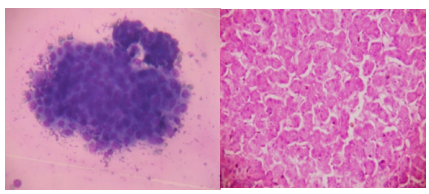

Figure 2: (a) Smears showing malignant cells in Peritoneal fluid (Leishman stain, 40X)

(b) Cell block showing malignant cells arranged in acinar formations; Peritoneal fluid
(H&E stain,10X)

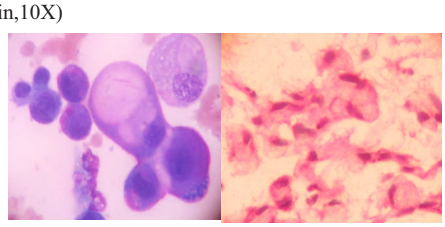

Fig 3(a): Smears showing signet ring cells; Peritoneal fluid (Leishman stain, 100 X)

(b) Cell block showing signet ring cells; Peritoneal fluid (H&E stain,40X)

In pericardial fluids, out of the 5 cases, 3 cases had predominantly lymphocytes and the other had mixed inflammatory cells. Table2 shows efficacy of centrifuged smears with cell block in detecting malignant cells.

Table 2: Comparision of smear versus cell block in various fluids

Cellularity	Pleural fluids		Peritoneal fluids		Pericardial fluids	
	Cytos mears	Cell block	Cytos mears	Cell block	Cytos mears	Cell block
Lymphocytes	75	76	12	12	03	03
Neutrophils	11	11	-	-	-	-
Mixed inflammatory cells	14	16	04	05	02	02
Blood elements	3	03	-	-	-	-
Mesothelial cells	3	03	03	03	-	-
Malignant cells	11	13	02	03	-	-
Suspicious of malignancy	2	-	01	-	-	-
Scant cellularity	3	-	01	-	-	-
Total	122	122	23	23	05	05

In this study most of the patients are clinically diagnosed as pleural effusion with 44.26% followed by tuberculosis with 24.59%, empyema with 9.83%, malignancy with 10.65%, cirrhosis of liver with 3.27%, alcoholic liver disease with 3.27%, congestive heart failure with 3.27%, pneumonia 0.81%.

Most of the patients of peritoneal fluids are clinically diagnosed as peritoneal effusions with 56.52%, alcoholic liver disease with 13.04%, tuberculosis with 8.69%, portal hypertension with 8.69%, malignancy with 8.69%

DISCUSSION

In the present study body cavity effusions are studied by using a comparative approach of routine cytosmears and cell block technique of pleural, peritoneal and pericardial fluids.Out of 190 cases of various fluids received, 150 cases were studied and analysed. Remaining cases were excluded as the material obtained was inadequate for cell block preparation. In the present study, the predominant lesion detected in the various fluids was inflammatory 134(89.33%) while malignancy was detected in 16(10.66%) of the cases. One of the pleural fluid cell block case showed Microfilaria (Wucheraria Bancrofti), which was missed on cytosmears.

The most common site of effusion was pleural, followed by peritoneal and pericardial effusion. Our results correlated with the studies done by Foot et al[7,8], van de Molengraft et al[9], Khan K et al[10] and Sears & Hajdu11,wherein the number of pleural effusion cases outnumbered the ascites cases. Majority of the patients in these studies were males and the primary site being the lungs and gastrointestinal tract.

In the study done by Stonifer et al[12], Sherwani R et al[13], James R. Hallman et al[14] and S. N. Booth et al[15] the most common site of effusion was peritoneal, followed by pleural and pericardial effusions. These results differed with our study which may be explained by the preponderance of females presenting with ascites in their studies.

In the present study the predominance of pleural fluids can be explained by the high prevalence of tuberculosis in the region of our study and lymphocyte rich effusion was noticed in 90 cases, among these 32 were tuberculosis.

In the studies done by Meenu et al2 and Melamed et al16,scanty cellularity was seen in 40(33.3%) and 21(34%) cases respectively. In the present study scanty cellularity was seen in 4 (2.98%) cases. Spieler et al observed the cytological features of tuberculous pleural effusion with moderate to high cellularity and predominance of lymphocytes.[17]

In a study done by Sujathan K et al.18 85 samples of pleural and ascitic fluid were examined over a period of 10 months and they concluded that out of 85 samples, 63 (74.12%) were inflammatory and 21(25.88%) were malignant.60In the present study, out of 134 inflammatory cases 11 cases (8.20%) were of acute inflammation,90 (67.16%) cases were found cytologically to be consistent with diagnosis of chronic inflammation,21(14%) cases were with mixed inflammatory cells,3(2.23%) cases were found with blood elements,6 cases (4.47%) were showing reactive changes.

In a study done by Nair et al.19 out of 171 samples, majority were pleural fluid 78% (133samples). Ascitic fluid comprised only 22 % (38 samples). Of the total samples, 44% were malignant effusions and 47% were reactive effusions. Out of the 75 malignant effusions, 15(20%) were ascitic fluids and 60(80%) were pleural fluids. Out of the total 81 samples of reactive effusions 74% were pleural effusion.

Out of 150 cases studied by Archana et al,4 39 (26%) were positive for malignancy by cell block method, while by routine method only 29 samples were reported as positive for malignant cells. Thus it was found that there was significant difference between the results obtained by direct smears method and cell block method.

Table 3: Comparison of the diagnostic yield of smear versus cell block in various studies

	Archana et al	Sujathan K et al	Present study
Total cases	150	85	150
Inflammatory	77	63	134
Positive for malignancy on smear	29	19	13
Unsatisfactory/negative on smear	10	2	3
Positive for malignancy on cell block	39	21	16

In the present study, out of 150 cases, 16 cases of malignancy were detected by using cell block method, while by using routine cytosmears, only 13 cases were diagnosed as malignant. Thus the use of cell block increased the diagnostic yield of malignancy from 13 to 16 samples showed 10% more diagnostic yield in cell block technique.

CONCLUSION

Pleural fluids accounted for the majority of the effusion fluids. Majority were in the age group of 41-60years.Inflammatory effusions outnumbered the malignant cases. Among the inflammatory effusions lymphocytic predominance is noted in majority of cases. Malignant pleural effusion was more common in males, the primary tumor was in the lung. Malignant ascites was more common in females, with the primary lesion in the ovary. Cell block technique increased the diagnostic efficacy by 6.5% when compared to cytosmears. We conclude that the cell block technique when used as an adjuvant to routine smear examination increases the diagnostic yield because of availability of more material for evaluation and better preservation of the cytoarchitectural pattern.

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