Utility of the Cell Block Preparation as an Additive Tool to the Conventional Cytological Smears

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Abstract

Background: For the detection of malignancy in the serous fluids of the body, cytological examination of the fluid is very important. It is also of prime importance and seems difficult to differentiate the malignant cells from reactive mesothelial cells during fluid cytology. The cell block preparation is a useful and additive diagnostic tool when cellular features in cytology are inconclusive or suspicious. The aim of the study was to assess the utilization of the cell technique as an additional tool along with the conventional smears that we are using in routine practice.

Methods: 200 body (effusion) fluids were examined by the conventional cytological smears as well as by the cell block technique.

Conclusion: The diagnostic yield increases when we use the cell block preparation method along with the conventional smears because the architectural patterns and cell morphology are very well preserved in cell block as compared to the smears.

Keywords: *Conventional smear, Cell block, body (effusion) fluids, Fluid Cytology.*

Introduction

Cytological examination of serous body fluids causes detection of cancer cells and it also gives us the details about inflammation and various infections[1]. The most important reason for cytological examination of the effusion fluid is to determine if it contains malignant cells or not [2]. It is difficult to differentiate a malignant cell from reactive mesothelial cell in day to day practice. The sensitivity of cytological diagnosis of effusion is low, which is attributed to benign morphology of the cells and changes that have occurred during processing.[1]

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Cell block technique is the unique and one of the oldest techniques for preparation of the material for microscopy.[1] We can diagnose the cytological abnormalities like reactive mesothelial cells in the fluid or adenocarcinoma by using the cell block technique.[1] We can obtain increased cellularity as well as better morphological details because there is good conservation of architectural features in the cell block. [1] Many sections can be prepared from the block for further study like immunohistochemistry and this is the major advantage of cell block method.[1] The cell block method was described in the literature as early as 1900 and it is one of the traditional methods used for processing cytological material. 10% alcohol-formalin is used for fixation. The principle of the fixative is cross linkage of the proteins present in the effusion material and formation of gel that can be used for cell block formation. We can evaluate the usefulness of the cell block method in the diagnosis of serous fluids by present study.

Aims and Objectives

The present study done with the view to:

· To establish utility and accuracy of cell block method

· To study the cytological findings of serous fluids by conventional smears and compare it with the cell block preparation.

Material and Method

The present study included 200 cases of effusion fluids, out of which 117 were pleural, 82 were peritoneal and 1 was pericardial from a tertiary care hospital, Vadodara. The patient presented with complains of ascites, pleural effusions or pericardial effusions were included in the study. The patients were subjected to fluid analysis, by both conventional smears as well as cell block technique. [5] The clinical features of the patient and the laboratory reports of the patient were obtained. The received sample was separated into two parts for different processes. Half of the fluid was put into the centrifuge, the supernatant fluid was discarded, and smears were prepared; stained with Hematoxylin & Eosin and MGG. PAP stain was used only if there is a need. The other half of the sample was centrifuged at 1500 rpm, we discarded the supernatant and the sediment obtained after centrifugation was fixed in 10% formal alcohol for 24 hrs [1]. The sample obtained by this method is further processed as a normal histology sample. The sections were cut, prepared and stained with H&E for microscopy. Some of the sections were stained with the special stains like PAS and Mucicarmine wherever necessary. The slides prepared from the block were examined for cellularity, arrangement, nuclear and cytoplasmic features. The comparison of conventional smear and cell block technique was done.

Results and Discussion

We examined 200 effusion fluid samples, in which 117 were pleural fluids, 82 were peritoneal fluids and 1 was pericardial fluid. Out of 200 cases, there were 110 (55%) males and 90 (45%) were females. We also classified the cases according to the age of the patient and 98 (49%) cases of the total were noted in age group of 41-60 years and only 1 (0.5%) case was noted in age group 0-10 years. The cases of pleural effusion were more common amongst the males i.e. 77 (38.5%) whereas only 40(20%) cases were noted among the female. Our study showed more cases of inflammation i.e.70 (35%) and only 07 (3.5%) cases of malignancy. Age group of 41-50 years was having the highest number of cases and the age group of 0-10 years was having the lowest number of cases (Table 1). In case of pleural fluid, 4 smears were diagnosed as suspicious on cytology and the same cases turned out to be malignant by cell block technique (Table 2).

In case of ascitic (peritoneal) fluid, the higher numbers of cases were inflammatory cases i.e. 69 (34.5%) and 13 (7.5%) were malignant (Table-3). Age group of 51-60 years was having the highest number of cases and the age group of 71-80 years was having the lowest number of cases (Table-1). In case of ascitic fluid, 6 (3%) smears were diagnosed as suspicious on cytology and the same cases turned out to be malignant by cell block technique (Table-3).

In the pericardial effusion case, there was an only inflammatory change and it was in the age group of 41-50 years. It was having mixed inflammatory cells.

Table 1 Sample distribution according to age and sex for the effusion fluids

Age Group→ (In years)		0-10 years	11-20 years	21-30 years	31-40 years	41-50 years	51-60 years	61-70 years	71-80 years
Pleural Fluid	Male	01	03	14	16	17	10	11	05
	Female		02	05	06	10	07	08	02
Peritoneal Fluid	Male		03	03	05	10	08	04	
	Female		01	02	06	12	23	02	03
Pericardial Fluid	Male								
	Female					01			
Total (200)		01	09	24	33	50	48	25	10

Table 2 Comparison of Pleural fluid cases diagnosed by smear and cell block technique with sex wise distribution

Diagnosis			Diagnosis by smear	Diagnosis by cell block technique
Inflammatory Cases	Male	71(35.5%)	106	106 (no cellularity cases are included)
	Female	35(17.5%)		
Malignancy Cases	Male	04(2%)	07	07
	Female	03(1.5%)		
Suspicious Cases	Male	03(1.5%)	04	04 (Diagnosed as malignant)
	Female	01(0.5%)		
Total cases	Male	78(39%)	117	117
	Female	39(19.5%)		

Table 3 Comparison of Ascitic fluid cases diagnosed by smear and cell block technique with sex wise distribution

Diagnosis			Diagnosis by smear	Diagnosis by cell block technique
Inflammatory Cases	Male	28 (14%)	69	69 (no cellularity cases are included)
	Female	41 (20.5%)		
Malignancy Cases	Male	02 (1%)	07	07
	Female	05 (2.5%)		
Suspicious Cases	Male	02 (1%)	06	06 (Diagnosed as malignant)
	Female	04 (2%)		
Total cases	Male	32 (16%)	82	82
	Female	50 (25%)		

The cell block technique was described by Mandlebaum in 1900 for studying the exudates [4]. The fixative used for fixation of the smears is 10% alcohol-formalin and the formalin present in the fixative solution causes cross-linkage of the protein material present in the fluid and forms a gel which can be used for further processing [5].

In our study of 200 cases, maximum numbers of cases were inflammatory 177 (88.5%) while malignancy was detected in 27 (11.5%) cases. Pleural effusion cases were the commonest among all the cases followed by peritoneal effusion cases and pericardial effusion cases. We have compared our study results with, Archana et al. [1], Meenu et al. [3] and Bhavana et al. [8]. Because of high prevalence of tuberculosis in the region of the study, there is predominance of pleural fluids in present study (Table 4).

Table 4: Various studies showing distribution of the cases

Name of the study	Pleural fluid	Ascitic fluid	Pericardial fluid	Total
Bhavana et. al. 8	139 (61.78%)	84 (37.34%)	2 (0.88%)	225 (100%)
Foot et al. 6	1301 (64.12%)	700 (34.5%)	28 (1.4%)	2029 (100%)
Van de Molengraft 7	171 (67.32%)	83 (32.68%)	-	254 (100%)
Present study	117 (58.5%)	82 (41%)	1 (0.5%)	200 (100%)

Lymphocytes were predominantly seen in 62 (31.3%) cases. 7 (3.5%) and 40 (33.3%) cases were noted showing low cellularity in various other studies like Bhavana et. al. [8] and Meenu et. al. [3] respectively (Table 5). Inflammatory smears prepared from pleural effusion were mainly showing leucocytes. Our study has more numbers of pleural effusion cases showing chronic inflammation with lymphocytes predominant and very less/ no mesothelial cells.

Mesothelial cells show a characteristic feature that is gaps or window formation due to microvilli that separates the cells [9]. Bedrossian described that in benign mesothelial cells, the microvilli are evenly distributed around the cells and they are slender; whereas in adenocarcinoma, the microvilli if present, are concentrated at the poles and are short [10]. In present study, predominantly mesothelial cells were present in 21% (42 cases) of inflammatory effusion cases. Generally the malignant cells may have molding of the nucleus, irregular nuclear membranes and prominent nucleoli with no gaps or windows.

The cell block technique can be used in the diagnosis of early stages of adenocarcinomas because there may be very less malignant features in the conventional smears but we can identify the features of malignancy by cell block and it is possible to stain and confirm the malignancy in the same sample with various special stains [11].

In the cell block, the carcinomatous cells have variable sizes, nucleus showing pleomorphism and overlapping, hyperchromatic nucleoli, intracytoplasmic vacuoles and occasional multinucleated cells. The malignant cells may form tubular structures with central lumina or show gland like arrangement. The nuclei of the individual malignant cells show abnormal mitoses, granularity of the chromatin and prominent nucleoli [1]. There are many advantages of Cell block technique as they can be utilized for immunohistochemistry. Eight to ten sections can be cut and used for evaluation of a large number of antigens. We can store the cell blocks very easily as compared to the conventional smears.

In the study done by Archana et al [1], out of 150 cases, only 29 cases were diagnosed as malignant by conventional cytological smears but 39 cases were found to be malignant by cell block technique and in our study, 27 malignancies were detected by cell block technique, but routine conventional smear could diagnose only 12 malignancies (Table- 6). Thus we can increase the diagnostic yield by using the cell block technique along with the conventional smears. Only 5 cell blocks in our study showed no cellularity. Technical errors such as degenerated samples or inadequate sampling can produce lack of cellularity in cell blocks.

Table 5: Various studies showing comparison of cellular component present in inflammatory smears

Cases	Bhavana8et al	Meenu3et al	Present study
Smears with Neutrophil Predominant	43 (21.7%)	26 (21.7%)	38 (21.5%)
Smears with Lymphocyte Predominant	62 (31.3%)	16 (13.3%)	71 (40.1%)
Smears with Low cellularity	7 (3.5%)	40 (33.3%)	8 (4.5%)
Smears with Mixed cellularity	40 (20.2%)	24 (20.0%)	33 (18.6%)
Smears with Mesothelial cells (Predominantly)	46 (23.2%)	6 (5.0%)	27 (15.3%)
Total	198 (100%)	120 (100%)	177 (100%)

Table 6: Various studies showing comparison of Diagnostic yield by smear and cell block technique

	Bhavana8et al	Archana1 et. al.	Present study
Cases of Effusion	225	150	200
Diagnosed as Inflammatory	183	77	177
Diagnosed as Suspicious on smear	15	10	10
Cell block showing no cellularity	7	34	5
Diagnosed as malignant on smear	12	29	14
Diagnosed as malignant by cell block technique	27	39	24

Conclusion

We can conclude that there is increased diagnostic yield with the use of cell block technique because the architecture can be preserved and the same technique can be used in some cases where there is confusion between the malignancy and reactive / inflammatory changes. For the accurate diagnosis, IHC can also be performed on the tissue from the cell block.

Conflict of Interest: Nil

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