Original Article

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"Diagnostic Utility of Cell Block Preparation with Conventional Cytological Smears. A Cross Sectional Study"

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ABSTRACT

Background: Cytological examination of serous fluids aspirated is a simple and relatively non-invasive technique to diagnose whether the effusion is malignant or benign. Cell block preparation along with conventional smear increases the sensitivity of detecting malignancies, and also has the ability to reduce false-positive interpretations.

Methods: A total 68 samples of body fluid (pleural and ascitic) specimens were examined for conventional cytological smear (CS) and cell block method (CB) over a period of one year. Out of 68 fluids, 40 were pleural fluid and 28 were ascitic fluid. Each fluid specimen was examined by conventional smear technique as well as cell block technique. The morphological details, cellularity, architecture, nuclear and cytoplasmic details were studied in both CS and CB techniques.

Result: A total 82.35% smears had adequate material; while of the total cell blocks, 75% cell blocks had adequate material. A total of 11.76% cases were malignant on smears,5.88% were suspicious of malignancy, 64.7% were benign/non-neoplastic lesions. A total 13.2% cases were malignant on cell block, 1.47% were suspicious of malignancy, 60.29% were benign/non-neoplastic lesions.

Sensitivity, positive and negative predictive value and accuracy of cell block technique were greater than that of FNAC smears

Conclusion: For the final cytodiagnosis of body fluid, there is statistically significant difference between the two techniques. Cell blocks prepared from the residual fluid specimen can be useful for more definitive diagnosis, with advantage of IHC and special stains where required.

Keywords: *Cell Block, Cytodiagnosis, Effusion*

Introduction

Clinical cytopathology is firmly established as a simple, reliable, rapid and cost effective diagnostic tool. Cytological examination of serous fluids is one of the commonly performed investigation to diagnose whether the effusion is malignant or benign. Microscopic examination of fluids collected from the serous cavities and other bodily secretions can offer very useful information facilitating the process of diagnosis and pointing out the etiology of effusion and list of differential diagnoses. Secondly, it allows one to follow the results of therapy and prognosis.[1]

Malignancies can be diagnosed using conventional cytological smears and also the cell block techniques. Accurately diagnosing cells as being either malignant or benign 'reactive mesothelial cells' in serous effusions is a common diagnostic problem.[2] The cell block (CB) technique is one of the oldest methods for the evaluation of body cavity fluids.[1] Cell blocks are particularly useful when the cytological abnormalities are misleading, such as in reactive mesothelial cells, or obscure as in occasional well differentiated adenocarcinoma. [2]

CB method has many advantages. Sensitivity and specificity of cell block is reported to be better than conventional cytology preparations. It is virtually a mini biopsy and has the same advantages as that of routine histopathological sections. [2]

A new method of cell block preparation by using an improvised ethanol formalin fixative "Nathan alcohol formalin substitute"-is recommended by Nathan and Narayan followed by a simple paraffin processing schedule which gives equally good morphological results.[3] The main advantages of the CB technique are preservation of tissue architecture and obtaining multiple sections for special stains and immunohistochemistry.[1,4]

The objective of this study was to assess the utility of the cell block preparation method in increasing the sensitivity

of cytodiagnosis of serous fluids and if possible, to know primary site of malignant effusions. The other objective was to compare the morphology in the cell block preparation with the conventional processing of fluids with regard to cell pattern, nuclear and cytoplasmic morphology.

In this study the merits and demerits of this method are explored in an effort to better the diagnostic performance by both the methods.

Materials and Methods

This study was conducted at Tertiary care hospital and Medical College in Central Gujarat from October 2015 till October 2016. A total 68 samples of body fluid (pleural and ascitic) specimens from patients admitted to Medicine, Surgery, Pediatric, ENT, TB & Chest, Obs and Gynecology and Outdoor patient were collected and sent for further investigations to pathology department. All cytological samples including pleural and ascitic fluids received within 4 hours of aspiration were included; and fluids received after four hours of collection, fluids showing degenerative changes or inadequate material in conventional smears were excluded.

The clinical data of patients viz…date of admission, age, sex, relevant investigations and diagnoses were abstracted from the case record forms and entered in MS Excel. The data was analysed using…MS office.

Process of preparing the smears and cell blocks:

Cytology Smear Preparation: Conventional smears were obtained by centrifuging the fluid. Two sets of slides were prepared from centrifuged samples at 4,000 rpm for 6 minutes. 3 smears were prepared,2 fixed in methanol for H & E stain and Pap stain and 1 air dried for Giemsa stain.

Cell Block Preparation: After making smears ; residual clot in the vacuette was removed carefully in the laboratory and mixing it with 5 ml of 10% alcohol-formalin(i.e,9 parts of 90% alcohol and one part of 7.5% formalin).This fluid was centrifuged at 2500 rpm for 15 minutes after one hour. A further 3 ml of fresh 10% alcohol-formalin was again added to the sediment after discarding the supernatant. The sediment which contained the cell button of fluid sample was scooped out on to a filter paper. This cell button was processed along with histological specimens after paraffin embedding. Subsequent steps in the preparation of cell block slides were identical to the one used in the routine Histopathological slides.

Staining Method Used: A routine Haematoxylin& Eosin (H&E), Papanicolaou, Giemsa stain was used for smears and Haematoxylin& Eosin (H&E) for cell blocks. The smears & cell blocks were evaluated independently of each other and the observations were recorded. The observations on smears & cell blocks were then correlated. The results obtained were compared with those of other studies.

Results

The present study is a cross sectional study conducted over a period of one year viz. from October 2015 to October 2016. There were 68 samples of fluids collected during the study period. Out of 68 fluids, 40 were pleural fluids (58.82%) and 28 were ascitic/peritoneal fluid (41.17%) specimens. Out of total 68 patients, 45 were males and 23 were females with a male to female ratio of 1.9:1. The youngest patient was 18 year old (male) and the oldest patient was 85 year old (male). Almost two thirds of the samples (64.7%) came from patients in the age group of 31 to 60 years.

A total 56 smears had adequate material (82.35%), while of the total cell blocks, 51 cell blocks had adequate material (75%). A total of eight cases were malignant on smears (11.76%), 4 were suspicious of malignancy(5.88%), 44 were benign/non neoplastic lesions(64.7%). A total nine cases were malignant on cell block (13.2%), 1 were suspicious of malignancy (1.47%), 41 were benign/non-neoplastic lesions (60.29%). Shown in Table 1

Among malignancies reported on smears from centrifuged deposits, the findings on corresponding cell blocks were nine malignant (13.2%), 1 suspicious of malignancy(1.47%), 41benign/non neoplastic (60.29%) and 17were inadequate(25%).Among four cases suspicious of malignancies reported on smears from centrifuged deposits, the findings on corresponding cell blocks were one malignant(1.47%) and two cases were diagnosed as benign on cell block. Among benign/non-neoplastic lesions reported on smears from centrifuged deposits, the findings on corresponding cell blocks were consistent. Among the inadequate smears on centrifuged deposits the corresponding cell blocks were also inadequate.

This information is shown below in the table 2

Among a total of 68 specimens studied by both techniques, 8 fluid specimens were clearly malignant on conventional smears (11.7%). The diagnoses on cell blocks were concurrent with the smears. Among a total of four conventional smears raising suspicion of malignancy (5.8%), one remained suspicious of malignancy (1.45%) even on cell block, while one cell block confirmed the malignancy (1.45%). The remaining two blocks turned out to be benign on cell block (2.9%).

Among 41benign/non-neoplastic lesions diagnosed by smears (60.2%),37 cell block concurred with smears (54.4%), no malignancy was detected or suspected in any, but four cell blocks were inadequate (5.8%). Fifteen

smears were inadequate or scantily cellular, but cell blocks were prepared from the sediment diagnosed as only 1 was clearly benign (1.45), while the others showed very few cells and were undiagnostic.

Positive cases were confirmed by histopathological examination and IHC; wherever possible.

The statistical analysis of the 68 fluid specimens showed a higher cellular yield by the CB method than by the CS method. Therefore, in this study, the utility of the CB method in the cytodiagnosis of malignant effusions was found to be highly significant as compared to the CS method.

Table 1: Results of conventional smear & cell block examination of 68 cytologic specimens:

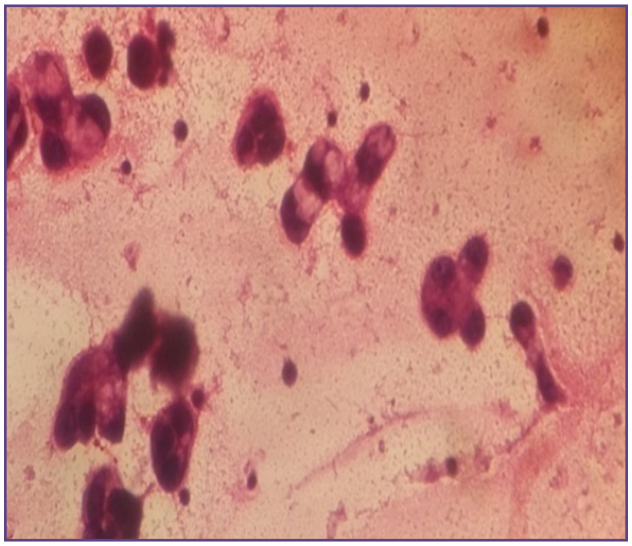
	Malignant	Suspicious of malignancy	Benign/non-neoplastic	Inadequate
SMEARS				
Fluids (n=68)	8	4	44	12
CELL BLOCKS				
Fluids (n=68)	9	1	41	17

n = number of fluids for cytology

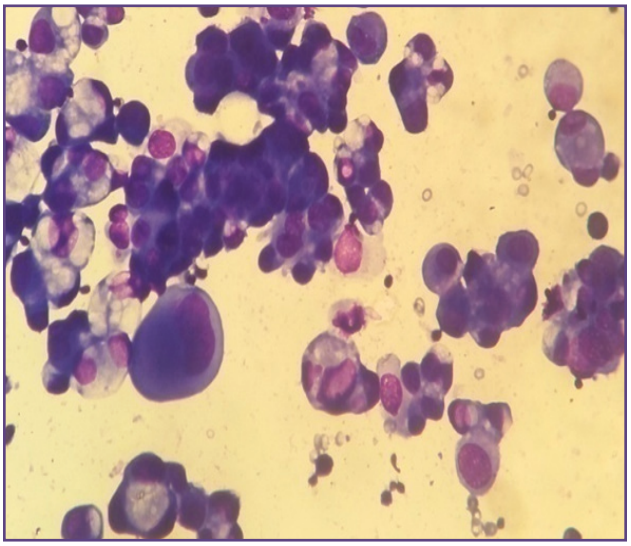
Table 2: Comparison between performance of direct smears v/s cell block.

		Cell block				
		M	**S/o M**	**B/NN**	**In**	**Total**
	Conventional smear					
1.	M	08	00	00	00	**08**
2.	S/o M	01	01	02	00	**04**
3.	B/NN	00	00	37	04	**41**
4.	In	00	00	01	14	**15**
	Total (n=68)	**09**	**01**	**40**	**18**	**Total:68**

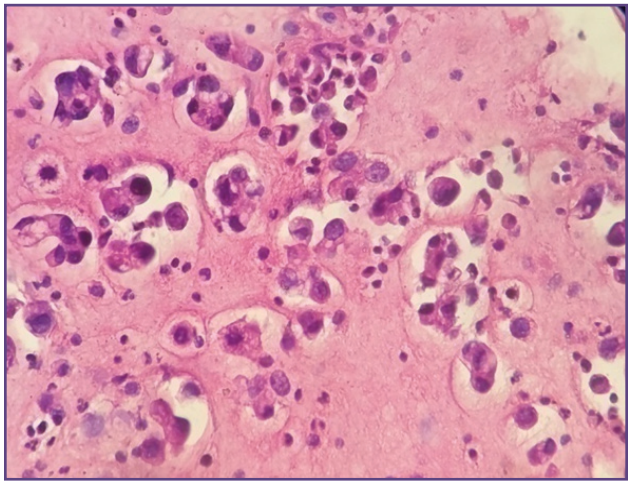
(M) = Malignancy
(S/o M) = Suspicious of malignancy
(B/NN)= Benign / non neoplastic
(In) =Inadequate material



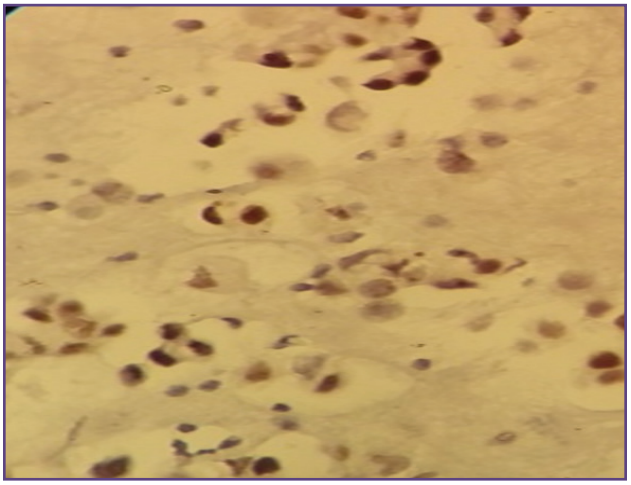
METASTATIC ADENOCARCINOMA ASCITIC FLUID (45 year female) CONFRIMED BY IHC



Photomicrograph showing metastatic adenocarcinoma conventional smear (GIEMSA ,X100)



Metastatic adenocarcinoma on cell block (H&E ,X100)



Metastatic adenocarcinoma of ovary on cell block (WT1 POSTIVE , X400)

Discussion

The cell population in sediment of body fluids represent a much larger surface area than obtained by needle biopsy. Since the introduction of the CB technique by Bahrenburg nearly a century ago, it has been used routinely for processing fluid.[5] Cell blocks prepared from residual tissue fluid can be used as adjuncts to smear for establishing a more definitive cytopathological diagnosis. The accurate identification of cells as either malignant or reactive mesothelial cells is a diagnostic problem in conventional cytological smears. The main advantages of the CB technique are preservation of tissue architecture and obtaining multiple sections for special stains and IHC. [6] To attain the best possible results; both smear and CB should be prepared from the same fluid specimen whenever possible.[7]

Various studies have included different serous body cavity fluids viz pleural fluids, peritoneal fluids, pericardial fluids. [3, 8 ,9 ,10] The criteria of selection of material also are varied. In some studies proved/suspected malignancies only are included.[11]In the others, the selection is random & in still others it included consecutive cytological material received.[3,12] In the present study, out of the total 68 specimens studied, 40(58.82%)) were of pleural fluid and 28(41.17%) were of ascitic fluids. 45 (66.18%) males and 23 (33.82%) female cases were recorded. Two third of cases were seen in the age group of 31-60 years.

There are many methods for cell block preparation like Plasma thrombin clot method, bacterial agar method, compact block technique, cell block from milipore filter method.[5] Different fixatives and different embedding media with many modifications have been used by different workers.[2,6,8,9,13,14] In the present study, alcohol formalin mixture(9 parts of 90% alcohol and 1 part of 7.5% formaldehyde) was used.[2] It was used as fixative for better preservation, when the material was adequate. Drawbacks were cellular shrinkage & deposition of formalin pigment. A few of the samples (5 in number) were scantily cellular on conventional smear and so plasma thromboplastin was used to form a pellet cell block in these situations.

On CS, 64.7% were benign, 5.8% were suspicious for malignancy and 11.7% were clearly malignant. Other studies reported 72% -90% as benign,2%-8% as suspicious for malignancy and 18%-22% as clearly malignant. [6,13,15,16]

On CB, 60% were benign, 1.4% were suspicious for malignancy and 13% were clearly malignant. Other studies reported 70% -83% as benign,1%-2% as suspicious for malignancy and 17%-28% as clearly malignant. [6,13,15,16]

No convincing explanation is possible for the varying performance of smears & cell blocks. It could be because of the difference in the case selection & loss of cellular material during processing for cell block.

Some workers have reported that by using both smears from centrifuged deposit & cell block method, the diagnostic efficacy improves, especially in the diagnosis of malignant effusions.[5,6]

Direct smears proved to be very useful in diagnosis of malignancy. Cell block helped in resolving the grey zone of suspicious direct smears into definite malignancy or into benign lesions due to better architectural and morphological features seen in cell block. After the study with CB method, out of 4 CS specimens reported as

Suspicious of malignancy; 1 specimen of peritoneal fluid turned to be adenocarcinoma; two specimens turned out to be benign and one specimen remained suspicious. Thus by cell block method, additional diagnostic yield can be obtained, which is in line with the study done by different authors.[2,5, 17, 18]

The Advantages of CB are better recognition of the histological patterns e.g. glandular structures, papillary structure can be more reliably seen in cell block method of diseases, possibility of studying multiple sections by routine staining, histochemical staining and by IHC studies, lesser cellular dispersal, and the possibility of storing the slides for retrospective/further studies.

A few limitations are delay in the diagnosis when compared to the conventional smears and, sometimes, the risk of losing material during the processing. Cell blocks are possible only in cases in which some material or blood is visible to the naked eye in the specimen container.

CS can be better than CB in certain conditions. This is because smear examination of centrifuged deposits of fluids is a reliable, quick and easy method for diagnosis and can be repeated if need.

Cell block is essentially a mini biopsy and the effort and time taken is about the same as that of biopsy processing. There is also a risk of loss of material during processing. Preservation of cellular morphology is better with smear in most cases. Thus, we may evaluate the use of cell blocks in future studies with larger samples. Also, the method of preservation of cell blocks for future confirmation of malignancies if need be; may be cost and time saving, which paves the way for future research.

Conclusion

Cell block may offer some advantage e.g. for special stain and IHC if suspicious for malignancy and no advantage when body fluids are clear with scanty cellularity in conventional smears.

For cellular smears, cell blocks are definitely superior for special stains and assigning a primary site in case of metastasis by using IHC.

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