

COMPARATIVE STUDY OF CELL - BLOCKS & ROUTINE CYTOLOGICAL SMEARS OF PLEURAL & PERITONEAL FLUIDS IN SUSPECTED CASES OF MALIGNANCY

Geethu G Nair^{1,*}, Anupama Achyuthan Manjula²

¹Assistant Professor, Department of Pathology, Malabar Medical College,
Modakkallur P.O. Atholi, Kozhikode, Kerala- 673315

²Associate Professor, Dept. of Pathology, Govt. Medical College,
Kozhikode, Kerala- 673008

***Corresponding Author:**

E-mail: drggnair573@gmail.com

ABSTRACT

Cell-block preparations made from sedimented cells can be useful adjunct to the routine cytological methods used for pleural and peritoneal fluids. There are only few studies evaluating its diagnostic efficacy and additional benefits when used with routine cytology. This study was conducted to compare the diagnostic efficacy parameters of cell-blocks and routine cytological smears of pleural and peritoneal fluids in suspected cases of malignancy. A one year study was done in a Gov. Medical College on pleural and peritoneal fluids in cases of suspected malignancy. Total number of cases studied was 148. Cytology smears were stained with Papanicolaou stain and cell-block preparations of centrifuged deposits were processed, cut at 5 micrometers and the sections stained by Hematoxylin and Eosin in every case. Additional stains and immunohistochemistry were done in the cell-block slides as required. Diagnosis of malignancy by any tissue based method within a period of three months of follow up was taken as the gold standard for analysis. Sensitivity of cell-blocks (0.6714%; 95% CI 0.5476-0.7762) was nearly double that of routine cytology (0.3230; 95% CI 0.2154-0.4517). Both methods had very high specificity. Cell- blocks proved to be superior to smears in pattern recognition and are advantageous when there's need for immunohistochemistry. Use of cell-blocks as an adjunct to routine cytology smears of body fluids can increase the sensitivity to a considerable extent. It is of further use in pinpointing a diagnosis by pattern recognition or immunohistochemistry.

Keywords: Cell blocks; Cytological smears; Pleural & Peritoneal fluids

INTRODUCTION

Diagnosis of malignant cells in effusions is important for staging procedures and resulting therapeutic decisions. According to Marel M et al cytologic study is considered to be the best for establishing a diagnosis of malignancy of pleural fluid. [1] Various methods are available like routine smears, cellblocks, thin preps etc. for cytological diagnosis. Of these, the cell block technique itself is not new and takes an intermediate position between histological and cytological techniques.

Cell blocks prepared from residual tissue fluids and fine-needle aspirations can be useful adjuncts to smears for establishing a more definitive cyto-pathologic diagnosis. They can be particularly useful for categorization of tumors that otherwise may not be possible from smears themselves. It also plays an important role when there is a need for special stains or immunohistochemistry. There are many studies done to compare the usefulness of cell blocks with that of smears in fine needle aspiration materials, but only a few in the case of serous fluids. In this context the present study has been undertaken to assess the utility of the cell block preparation method in increasing the sensitivity of cyto-diagnosis of serous fluids.

AIMS & OBJECTIVES

1. To compare the sensitivity, specificity and positive predictive value of cell-blocks and routine cytological smears of fluid specimens in diagnosing malignancy
2. To know the efficacy of cell-blocks in typing malignancy

MATERIALS & METHODS

171 fluid samples (both pleural and peritoneal) from 148 patients sent to cytopathology laboratory of a Govt. Medical College constituted the material for the present study. Only those patients who had clinical or radiological evidence of malignancy were included. Duration of study was 1 year. No separate consent was needed from patients other than that obtained before fluid tap. The study was approved by Institutional Ethics Committee.

All fluid samples were initially examined for color and appearance. The samples were centrifuged at 2000 rpm for 5mt. The supernatant discarded and routine smears were prepared from the cell button. Smears were stained with Papanicolaou stain. Remaining cell button was centrifuged with 2-3 drops of supernatant [2000 rpm, 10mt]. Fixative AAF (absolute alcohol, glacial acetic acid and 40% formaldehyde) added thrice the volume of material. Again centrifuged for 10mt at 2000 rpm. The test

tube was kept in slanting position for 4-6 hrs. Cell button was scraped; wrapped in filter paper & gauze piece and paraffin embedded in same way as that of routine biopsy specimens. Sections were taken from these blocks and slides stained with Hematoxylin and Eosin stain. Both smears and blocks were examined separately.

Anyone of the following was considered to be the gold standard for diagnostic confirmation.

1. Direct FNAC or biopsy of lesion
2. FNAC or biopsy of lymph nodes
3. Sputum cytology
4. Bronchial Washing
5. Peritoneal washings

If there were sufficient clinical and radiological feature of malignancy and the fluid samples revealed malignant cells it was considered as true positives. In suspicious cases, immune-histochemistry done for confirmation. In a negative result, the cases were followed up for a period of 3 months.

ANALYSIS

The results of both smears and cell-blocks were analyzed to calculate their sensitivity, specificity, positive predictive value and negative predictive value in correlation with the gold standard.

RESULTS

The total number of cases studied was 148 (90 males, 58 females). From these 148 cases, a total of 171 samples were collected. Out of the 171 samples, majority were pleural fluid (133samples, 78 %). 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. The age structure of sample is depicted in figure 1.

The group of 'malignancy' includes all those patients, who had a positive tissue diagnosis. There were 75(43.8%) cases of malignant effusion. All cases of reactive effusions (47.4%) are included in the category of 'no malignancy'. In 8.8% of the samples there was either no follow-up or the patients had expired. Expired means all those cases who died before a definite tissue diagnosis could be established. These cases were omitted from further analysis.

The actual diagnosis in smears and cell-blocks in relation to the eventual diagnosis (gold standard) is given in tables 1 and 2. The sensitivity, specificity, positive and negative predictive values for diagnosis of malignancy, calculated from the above tables for smears and cell-blocks are shown in table 3 and 4. To calculate these values, only two of the above parameters were considered (malignant cells and no malignant cells). Diagnosis of atypical cells, suspicious cells and no material were omitted. The diagnostic efficacy parameters for diagnosing malignancy in smears and cell-blocks were compared in specific group's also viz ascitic fluid, pleural fluid, and epithelial malignancy (Table 3 & 4).

Comparing the results of whole samples, cell-blocks had a higher sensitivity of 67.14% while the smear showed nearly half of it, i.e. 32.3%. Specificity of both was nearly equal.

Table 1: Actual diagnosis in smears in relation to final diagnosis

Smear Diagnosis	Final diagnosis		Total
	Malignant	Non Malignant	
Malignant cells	21	0	21
Atypical cells	6	1	7
Suspicious for malignancy	4	0	4
No malignant cells	44	80	124
Total	75	81	156

Table 2: Actual diagnosis in cell-blocks in relation to final diagnosis

Cell-block diagnosis	Final diagnosis		Total
	Malignant	Non Malignant	
Malignant cells	47	1	48
Atypical cells	2	2	4
Suspicious for malignancy	0	0	0
No malignant cells	23	72	95
No material	3	6	9
Total	75	81	156

Table 3: Diagnostic efficacy parameters in smears (95% CI)

Parameter	Whole cases	Ascitic fluid	Pleural fluid	Epithelial malignancy
Sensitivity	0.3230 (0.2154-0.4517)	0.3333 (0.1298-0.6131)	0.32 (0.1992-0.4683)	0.2950 (0.1886-0.4274)
Specificity	1 (0.9429-1)	1 (0.7907-1)	1 (0.9261-1)	NaN (NaN-NaN)
Positive predictive value	0.1448 (0.0939-.2151)	0.1470 (0.0554-0.3183)	0.1414 (0.0871-0.2265)	0.2950 (0.1886-0.4274)
Negative predictive value	0.8551 (0.7848-.9060)	0.8529 (0.6816-0.9445)	0.8558 (0.7734-0.9128)	0.7049 (0.5725-0.8113)

(The entry 'NaN' in above cells means that the calculation cannot be performed because the values entered during calculation include one or more instances of zero and 'CI' means confidence interval)

Table 4: Diagnostic efficacy parameters in cell blocks(95% CI)

Parameter	Whole cases	Ascitic fluid	Pleural fluid	Epithelial malignancy
Sensitivity	0.6714 (0.5476-0.7762)	0.8866 (0.5838-0.9765)	0.6181 (0.4037-0.5962)	0.6769 (0.5482-0.7845)
Specificity	0.9863 (0.9157-0.9992)	1 (0.7812-1)	0.9818 (0.8900-0.9990)	NaN (NaN -NaN)
Positive predictive value	0.3356 (0.2602-0.4200)	0.3939 (0.2342-0.5776)	0.3181 (0.2344-0.4147)	0.6769 (0.5482 -0.7845)
Negative predictive value	0.6643 (0.5799-0.7397)	0.6060 (0.4223-0.7657)	0.6818 (0.5852-0.7655)	0.3230 (0.2154-0.4517)

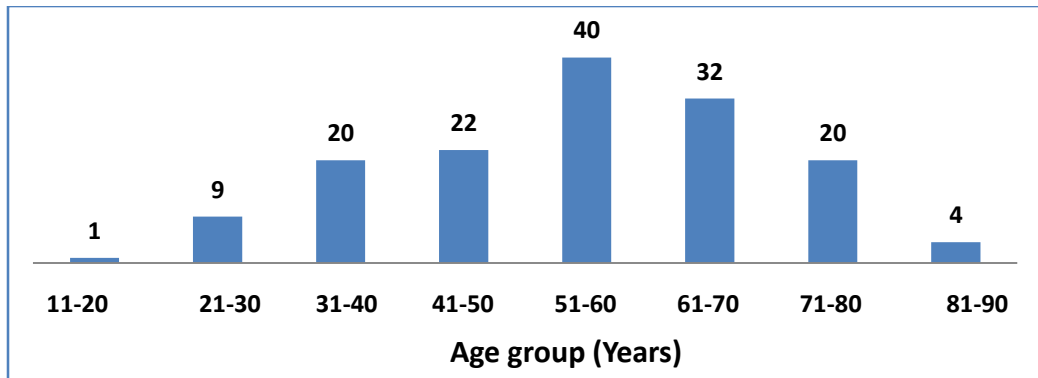


Fig.1: Age structure of sample

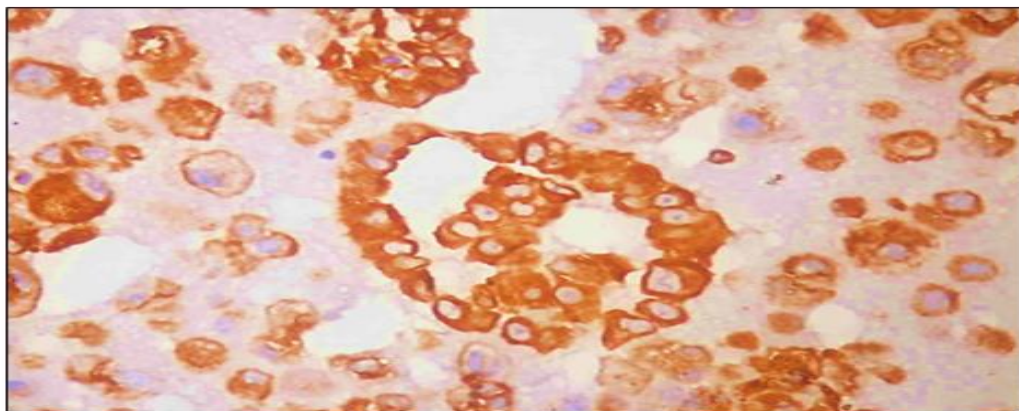


Fig 2: Case of mesothelial hyperplasia; reactive mesothelial cells are positive for Vimentin, x400

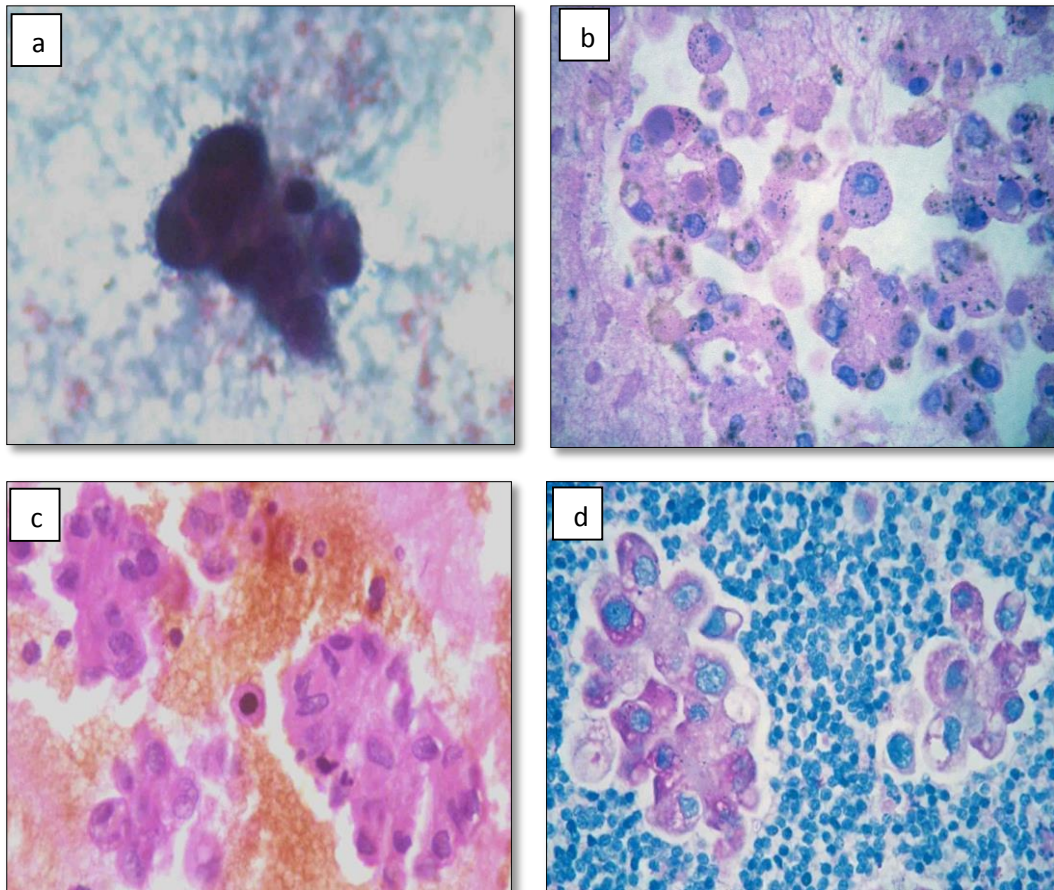


Fig 3: Adenocarcinoma cells. a) Routine cytological smear- Papanicolaou stain, x400 b) Cell-block-H & E stain x400 c) H &E stain x400 d) PAS stain x400

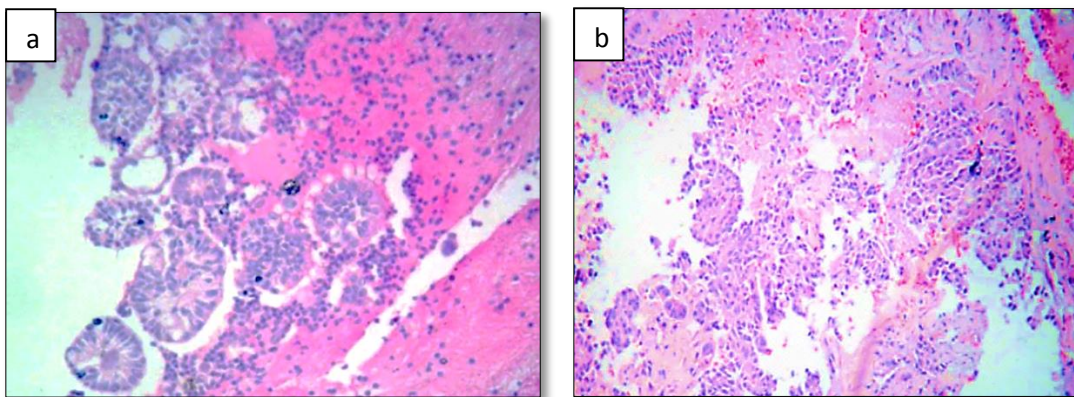


Fig 4: a) Carcinoma breast with lung metastasis. Adenocarcinoma cells in cell-block of pleural fluid. H&E x100 b) Pleural biopsy of same case showing metastasis from Infiltrating duct carcinoma. H&E x100

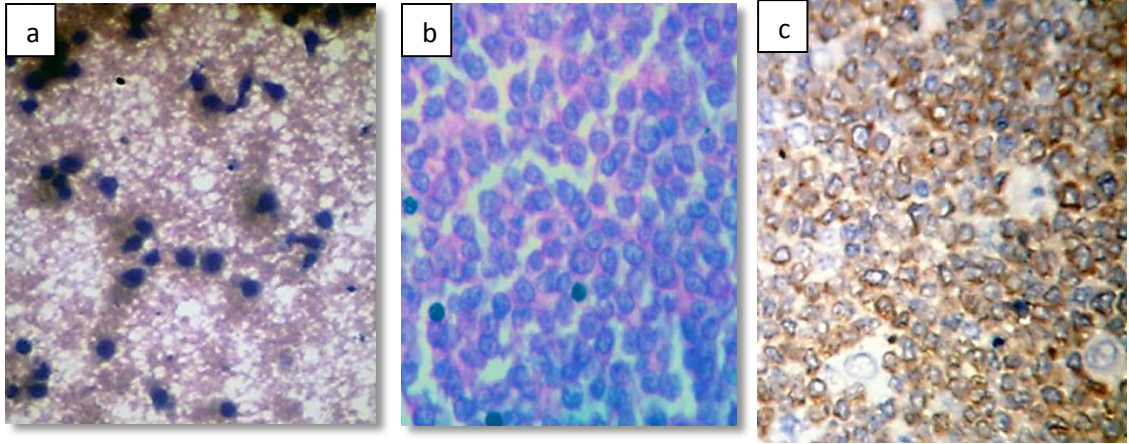


Fig 5 : a) Lymphoma cells in smear. Papanicolaou x100 b) Cell-block showing monotonous population of lymphoid cells. H&E x400 c) Lymphoid cells are positive for CD3 establishing diagnosis of T-cell lymphoma, Cell-block x400

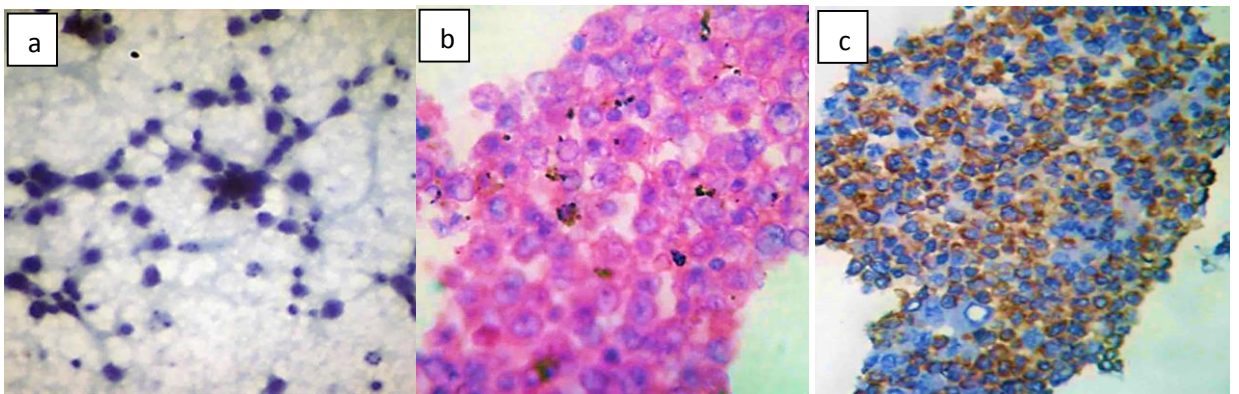
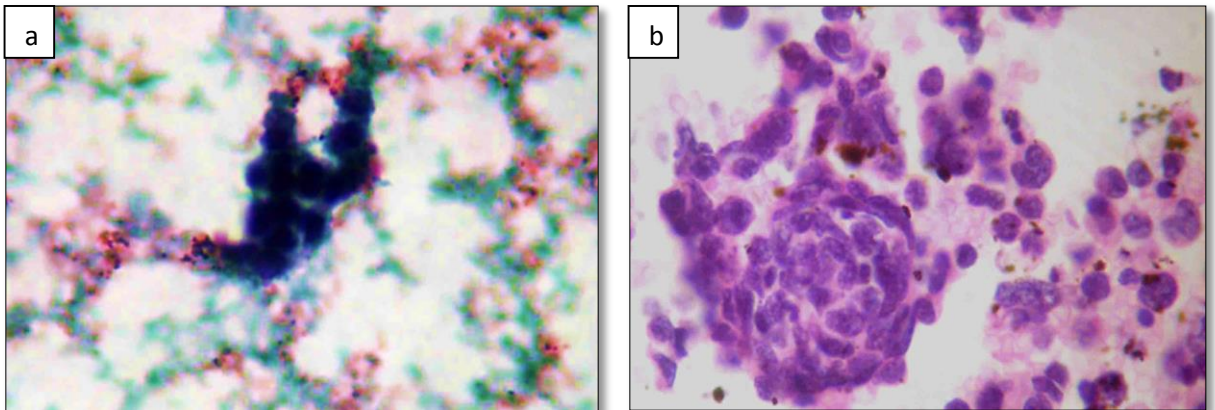


Fig 6: a) Smear reported as Lymphoma /poorly differentiated carcinoma. Papanicolaou x100 b) Same case as in cell-block. H&E x100 c) Cells are positive of CD20. Diagnosed as Non Hodgkin Lymphoma. CD-20 x 100



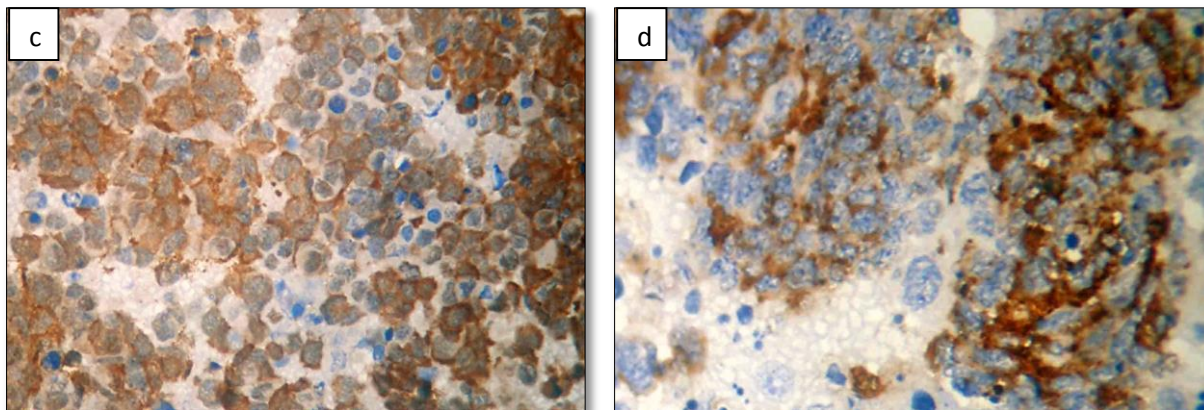


Fig 7: a) Case of small cell carcinoma in smear. Papanicolaou x100. b) Corresponding cell-block. H&E x 400. c) IHC for Chromogranin in the cell-block x400. d) IHC for NSE in cell-block, x100.

DISCUSSION

Cytological evaluation is the best way to detect the presence of malignancy in body cavity fluids. Results of many studies support the superiority of fluid examination in the diagnosis of malignancy.^[2] The diagnostic yield is dependent on such factors as the extent of disease and the nature of primary malignancy. Materials from patients submitted in the form of fluid for cytological examination can be evaluated in many ways. Most of the laboratories prefer routine cytological smears for this purpose. A study by Oyafuso et al on 4297 fluid samples showed the sensitivity, specificity, efficiency as well as positive and negative predictive values of smears as 44.55%, 95.7%, and 50.1%, 98.7% and 20% respectively.^[3] Similar results were obtained with Mother by et al also.^[4] These data from the literature show that the diagnostic accuracy of effusion cytology by means of routine smears is unsatisfactory and should be improved. Therefore the use of different adjuvant methods is recommended. The cell-block technique has been in use for many years and has gained considerable acceptance. In this context the present investigation was initiated to assess whether the diagnostic accuracy can be increased by concomitant use of smears and cell-blocks.

In a study of effusions, conducted by Meenu Thaper et al^[5] out of 190 cases studied, 120 (63.15%) cases were of different reactive effusions and 70 (36.85%) cases were of malignant effusions. Out of the 120 cases of reactive effusions, 48.3% cases were of pleural effusion followed by peritoneal (45%) and pericardial fluids (6.7%). The majority (22, 18.33%) of cases were due to tuberculosis. In our study, proportions of reactive and malignant effusions were nearly equal. Tuberculosis remains the most common cause of reactive effusion. This might be because of the high prevalence of tuberculosis and fact that majority of the pleural fluid samples are sent from

Regional Institute of Chest Diseases where a large number of TB patients are admitted.

In the case of pleural fluids, 60 samples (45.1%) were malignant and 62 samples (46.61%) were reactive effusions. Adenocarcinoma was the most common cyto-pathologic diagnosis rendered in malignant pleural effusions in this study. Primary adenocarcinoma of lung comprised almost 50% of the diagnoses made. In 3 cases the diagnosis was given as non-small cell carcinoma because the differentiation was not clear. In many cases though adenocarcinoma was confirmed, the site of primary could not be identified. Primary site of tumor was identified as breast in 7 cases. Lymphomatous involvement was seen in 5 cases. As would be expected, squamous cell carcinoma was an uncommon cause of malignant effusion. Same was the case of small cell carcinoma. Both of these were identified in 3 cases each (Fig.7). Of the reactive effusions nearly half of the cases were due to tuberculosis.

Regarding ascitic fluids, 39% were malignant and 58% were reactive. Adenocarcinoma of gastrointestinal tract accounts for most of the cases of malignant ascites followed by carcinoma ovary. In the case of reactive effusions, cirrhosis accounts for 36.8% followed by tuberculosis (4 samples).

Out of the 75 samples of malignant effusions, 21(28%) samples were reported to be positive for malignant cells by routine cytological smears. But cell-block method picked up malignant cells in 47 cases (63%). Compared to Thaper's study our figures are similar for cell-blocks, but much lower for smears. Sensitivity of smears in detecting malignancy is 32.30% in our study. But for both smears and cell-blocks the specificity is very high. The sensitivity of smears in our study is lower than those reported by Shafiqh et al^[6] and Nathan et al also.^[7] In these studies the sensitivity of smears and cell-blocks tended to be similar whereas the cell-blocks proved to be superior in our material.

According to literature, the low sensitivity of smears in our study may be due to limitations in the methodology, invasive features of neoplasia and sampling errors.^[3, 4] In our study this may be the result of following reasons.

- 1) Only one specimen was examined in majority of the cases.
- 2) Pick up rate may be increased if 4 slides examined in each case. In our case the number of slides examined was 1 or 2.
- 3) Preparation technique may have some faults requiring correction.
- 4) Cell morphology is difficult to interpret in the presence of a hemorrhagic background

The sensitivity of cell-blocks in ascitic fluid is high compared to pleural fluid which is in accordance with Mother by et al.^[4]

Specificity of both smears and cell-blocks were nearly equal. There was only one case which was falsely reported as malignant in cell-block. Actually it was a case of tuberculous effusion and there was monotonous population of reactive mesothelial cells showing anisonucleosis. Distinction of reactive mesothelial cells from malignant cells is always a diagnostic concern in cytodiagnosis of serous fluids. In such situations immunohistochemistry may be helpful.^[8-15] One example is illustrated in Fig: 2. this is a case of mesothelial hyperplasia showing glandular pattern in cell-block causing diagnostic confusion with adenocarcinoma. But these cells were strongly positive for vimentin. Positivity for vimentin is helpful in differentiating mesothelial cells.^[12, 16]

Examination of Papanicolaou stained smears has certain advantages. It is a quick procedure. It does not need any extra time for processing and cutting of paraffin embedded methods. All that it requires is the expertise to identify the malignant cells in smear.

Even though the preparation of cell-blocks takes time, it has many advantages. As evident from Fig: 3 & 4 they bring out architectural patterns of tumor beautifully. If the material submitted is very copious, it may be impossible to sample it completely by means of smears. By preparing cell-blocks, this problem can be solved. Cell-block is also useful when there is a need for special stains (Fig: 3d). The blocks can be stored and multiple sections can be taken. Role of cell-blocks in immunohistochemistry should also be mentioned. Destaining cytology slides for immune-staining is laborious and results in the loss of what may be the only conventionally stained cytological material. In addition the results from immune-staining smears tend to be poor unless the procedure is frequently practiced. In particular there tends to be a high level of background staining. Fig: 5 are from a case of lymphomatous pleural effusion. Even though smear examination identified lymphoma

cells, it was typed as T cell only after IHC. Another example is shown in Fig: 6. it was reported as poorly differentiated carcinoma/lymphoma in smear. After doing IHC it was confirmed as a case of B cell lymphoma.

In our study, most of the malignant effusions were adenocarcinoma either primary or metastasis. Sensitivity of cell-blocks was better than smears in detecting epithelial malignancies. There were 3 cases of squamous cell carcinoma. But neither smears nor could cell-blocks pick them up. Regarding lymphomas there were 3 cases of T cell lymphomas, one case of follicular lymphoma and one case of diffuse large B cell lymphoma (DLBCL). Smears showed a higher sensitivity in picking up lymphoma cells than cell-blocks.

Thus it is concluded from this study is that for cytological examination of all fluid samples, smears should be supplemented with cell-blocks to increase the pickup rate especially if there is suspicion of malignancy. This becomes all the more imperative if study of repeated cytology samples is not feasible.

CONCLUSION

Use of cell-blocks as an adjunct to routine cytology smears of body fluids can increase the sensitivity to a considerable extent. It is of further use in pin-pointing a diagnosis by pattern recognition or immunohistochemistry.

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