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COMPARISON OF CONVENTIONAL CYTOLOGY AND CELL BLOCK PREPARATION IN SEROUS EFFUSIONS

Pathology

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ABSTRACT

Background: Effusion is the accumulation of fluid in body cavity. Cytological examination has lower sensitivity due to overcrowding of cell, cell loss. So, cell block should be used for making the exact diagnosis.
Aim: To study the spectrum of cytological diagnosis of serous effusion and to compare the diagnostic accuracy of conventional smear versus cell block technique.
Setting and design: Hospital based prospective cross-sectional study.
Material and Methods: A 2 years prospective study was conducted with a sample size of 801 cases. For cytological examination of fluid 2 dry and 2 wet smears were prepared and stained. Cell blocks were prepared in 125cases. Cases which had low/no cellularity, or in which cell button was not formed were excluded.
Statistical analysis: Data were analyzed by SPSS (Statistical Package for the social service) software version 26 and applied statistical test of significance was Chi-square.
Results: On microscopic examination, cell block sections revealed better morphological details which could help to find out the primary site and to differentiate reactive proliferation from malignancy. The additional yield of malignancy by cell block method was 1.6% as it helped in diagnosing one additional case of malignancy. Moreover, most of the atypical and suspicious cases of conventional smears showed features of malignancy on cell block. One of the case diagnosed as atypical on conventional smear showed reactive mesothelial proliferation on cell block.
Conclusion: Cell blocks should be used along with conventional smears to pick up more cases of malignancy which could be missed on conventional smears.

KEYWORDS

Cell Block, Serous Effusion, reactive mesothelial proliferation.

INTRODUCTION

Effusion is the accumulation of fluid in the body cavity. The common causes of pleural and peritoneal effusion are non-neoplastic conditions such as congestive heart failure, hepatic cirrhosis, hypoproteinemia, infections and neoplastic condition like mesothelioma and metastatic carcinoma. [1]

Effusion can be divided into exudative and transudative types. Transudative has less protein content, low total cell count while exudative has high protein content and raised total cell count. Moreover, most of the reactive mesothelial cell proliferation and malignant cases are exudative in nature. [1]

The cytological examination of serous fluids is one of the commonly performed investigation.[2] The conventional smear provides faster results, is easy to perform, cost effective, shows good sensitivity and specificity in diagnosing primary as well as metastatic pleural malignancies. [2,3]

However, the sensitivity of conventional smear is lower compared to cell block technique as a large amount of fluid is left after smear preparation that might contain valuable diagnostic material which may lead to false negative result on smear preparation.

Moreover, in conventional smear there is overcrowding of cells, cell loss, lack of architecture, an abundance of inflammatory cells and paucity of representative cells. To overcome this problem cell block method is preferred along with conventional smear preparation as it ensures full utilization of sample and also concentrates the minimal amount of sample in a small area that can be evaluated at a glance, thus increasing the accuracy of results.[4,5]

The cell block technique enables cells to be retrieved from a fluid specimen to form a paraffin block, which concentrates the cells in the limited field without loss of cellular material. The cell block technique enables to distinguish between reactive mesothelial cells from metastatic neoplasm as nuclear and cytoplasmic details are well preserved and recognition of the histological pattern of diseases is better with cell block. It also allows to study multiple sections by histochemical staining and immunohistochemistry procedure.[6] Immunohistochemistry (IHC) is a tool to investigate those cases that cannot be accessed well by histological examination. [6,7]

D2-40 is found to be 100 % sensitive and specific for mesothelial cells as compared to MOC- 31, EMA, Calretinin and WT-1 marker. [6,7] The combination of MOC- 31 and D2-40 has been used and their specificity and sensitivity in the diagnosis of adenocarcinoma are 100% and 99 % respectively.[7] It is a recently developed, commercially available monoclonal antibody directed against M2A antigen, a Mr 40000 surface O-linked sialoglycoprotein.[8]

The present study was formulated to study the spectrum of cytological diagnosis of serous effusions and to compare the diagnostic accuracy of conventional smear and cell block technique.

MATERIAL AND METHODS

A 2-year hospital based prospective cross-sectional study was done in department of pathology from June 2018 – May 2020 with a sample size of 801 cases of serous effusion. The study was conducted with clearance from the ethics committee of the institute.

Samples from pleural, peritoneal, and pericardial effusion were included. Fluids with scant / no cellularity, whenever proper cell button was not formed were excluded. Also, D2 – 40 IHC was performed on cases with florid mesothelial proliferation and malignancy.

Smears were prepared from centrifuged deposits and stained with May - Grunwald Giemsa stain, PAP stain and H&E stain. Microscopic

examination was done according to Effusion Guidelines of Indian Academy of Cytologist. [9]

Cell blocks were prepared in 125/801 cases based on exclusion criteria .5ml of fluid was taken and mixed with 5 ml of 10% alcohol formalin (9 parts of 90% isopropyl alcohol and 1 part of 10% formalin).

Briefly, after centrifugation at 2500 rpm for 15 minutes the supernatant was discarded and cell button was fixed over night with 3ml 10% formalin. The cell button was then removed with the help of wire loop and was kept in a universal container along with 10% formalin for histo-processing. The slides obtained from cell block technique and conventional smears were compared to access the quality and reliability of the cell block technique based on cell morphology, nuclear and cytoplasmic detail.

D2-40 IHC was applied in reactive mesothelial proliferation and malignant cases and was considered positive when moderate to strong expression of only membranous or membrano-cytoplasmic pattern was observed in ≥5% mesothelial cells. [8,10]

For statistical analysis data was analysed by SPSS software version 26 and p-value (< 0.0001) was calculated using Chi-square ($X^2$247.828). Sensitivity, specificity, positive predictive value, negative predictive value and diagnostic accuracy of cell block were calculated.

OBSERVATIONS AND RESULTS

Total of 801 fluids from pleural, peritoneal, and pericardial cavities were studied over a period of 2 years of which 429 (53.6%) were pleural, 362 (45.2%) were peritoneal and 10 (1.2%) were pericardial. The age ranged from 5months to 93 years with maximum number of cases in 41 to 60 years with a mean age of 48.3 years. Male patients outnumbered female patients by a 1.6:1 ratio.

They were categorized as Unsatisfactory for evaluation (Category 1), no malignant cells/Benign Changes (category 2), Atypical Cells: Not Otherwise Specified (category 3), Atypical Cells: Suspicious for Malignancy (category 4) and Malignant Cells (category 5).[9]

Majority of the cases 787/801(98.2%) were in category 2, of which 68/801(8.5%) cases showed variable number of mesothelial and inflammatory cells (category 2A) and 719/801 (89.7%) showed specific cellular changes (category 2B). 593 cases revealed lymphocyte rich effusion, 119 cases revealed neutrophilic rich effusion and one case showed eosinophil rich effusion, 6 (0.7%) showed reactive mesothelial cell proliferation. 11/801 (1.4%) cases showed equivocal features not sufficient to categorise as reactive or malignancy (category 3) One case was suspicious for malignancy (category 4) and two cases showed features of malignancy (category 5). Cases of Category 1 were excluded (table 1).

Table 1: - Categorization of cases on conventional fluid cytology

Category	CYTOLOGICAL DIAGNOSIS	Cases No.	%
Category 1	**Unsatisfactory for evaluation**	-	-
Category 2A	**A - No Malignant Cells Detected**	68	8.5
Category 2B	**B - Benign Changes**		
	I - Reactive Mesothelial Cells	6	0.7
	II - Inflammatory Cells	120	14.9
	III - Lymphocyte rich Effusion	593	74.1
	IV - Specific Infections	0	0
Category 3	**Atypical Cells, Not Otherwise Specified**	11	1.4
Category 4	**Atypical Cells, Suspicious For Malignancy**	1	0.1
Category 5	**Malignant Cells**	2	0.3
	Total	801	100

Biochemical analysis for protein and sugar was available in 538 out of total 801 cases. Based on biochemical analysis fluids were divided into exudative and transudative types of effusion. 337 (62.6%) were transudative in nature and 201 (37.4%) cases were exudative in nature. Majority of the exudative cases (53.7%) had cell count > 1000 cells/mm^3 and maximum number of transudative cases (45.4%) showed cell count in the range of 3 -100 cells/$mm.^3$

3/4 (75%) reactive mesothelial proliferation and 9/10 (90%) atypical, suspicious and malignant cases (Category 3, 4 and 5) were exudative in nature. A pattern was observed of increasing trend of exudative nature of fluids from Category 2 to Category 5.

Table 2: - Histopathological Diagnosis On Cell Block

Histodiagnosis	Number of cases	%
Negative for malignant cells	104	83.2
Reactive mesothelial proliferation	7	5.6
Malignant	14	11.2
Total no of cell block preparation	125	100

Cell blocks were prepared in 125/801 cases based on cytological findings, amount of fluid received and formation of adequate cell button. Out of 14 malignant cases,13 showed morphological features favouring adenocarcinoma and one was metastatic squamous cell carcinoma (table 2).

Table 3: - Cyto – Histopathological Correlation

Cytodiagnosis			**Histodiagnosis**		
Category		Number of Cases	Negative for malignant cells	Reactive mesothelial proliferation	Malignant
Category 1	**Unsatisfactory for evaluation**	-	-	-	-
Category 2A	**A - No Malignant Cells Detected**	**4**	**3**	**0**	**1**
Category 2B	**B - Benign Changes**				
	I - Reactive Mesothelial Cells	6	0	6	0
	II - Inflammatory Cells	17	17	0	0
	III - Lymphocyte rich Effusion	84	84	0	0
	IV - Specific Infections	0	0	0	0
Category 3	**Atypical Cells, Not Otherwise Specified**	**11**	**0**	**1**	**10**
Category 4	**Atypical Cells, Suspicious for Malignancy**	1	0	0	1
Category 5	**Malignant Cells**	2	0	0	2
Total		125	104	7	14
$X^2$247.828, p values < 0.0001					

Cyto-histopathological concordance was seen in all cases except two. One was reported negative for malignant cells (Category 2A) on cytopathology and showed features of malignancy on cell block. Other was reported as Atypical: NOS on conventional smear (Category 3) revealed reactive mesothelial proliferation on cell block which was further confirmed by IHC D2-40.

10/11 cases of category 3 and 1 of category 4 were diagnosed as malignant on cell block. Both cases in category 5 were diagnosed as malignant on both conventional smear and cell block method (table3).

D2- 40 immunostaining was applied on 7 reactive mesothelial proliferation and 13/14 malignant cases. **3/7** (42.8%) cases of reactive mesothelial proliferation showed D2-40 positivity and none of malignant cell showed D2-40 positivity. In **4/13** malignant cases, background mesothelial cells stained with D2-40 IHC.

STATISTICALANALYSIS

Out of 801 cases of serous effusion, cell blocks were prepared in 125 cases. Cyto-histopathological correlation was seen in all cases except two. Considering the cell block as gold standard, the statistical parameters – sensitivity, specificity, positive predictive value, negative predictive value, and diagnostic accuracy of conventional smear were

92.9%, 99.1%, 92.9%, 99.1% and 98.4% respectively.

Diagnostic yield for malignancy is increased by **1.6 %** when cell block method was examined along with the conventional smear as it helped in diagnosing one additional case of malignancy which was diagnosed as negative (Category 2A) on conventional smear.

DISCUSSION

Diagnostic cytology is the scientific art of interpretation of cells that exfoliate or are removed from their physiological milieu. [11] Cytological examination of serous effusion is of importance in diagnostic, therapeutic and prognostic implication. [12] Cytodiagnosis by conventional smear has got lower sensitivity due to overcrowding of cells, cell loss, different laboratory processing methods, [13] presence of reactive mesothelial cells, abundance of inflammatory background, drying artefacts, poor fixation and leaving behind useful material. [14]

Accurate identification of malignant or reactive mesothelial cells is a diagnostic problem in conventional smear. To overcome this problem, cell blocks prepared from residual fluids can be useful. The cell block technique provides better morphological details and better preservation of architectural pattern like in 3- dimensional clusters, acinar pattern compared to conventional smear. [15]

In the present study, out of total 801 serous fluids, majority 429/801(53.6%) were pleural, followed by 362/801(45.2%) of peritoneal and 10/801(1.2%) of pericardial fluid (Table 4) which was in concordance with other studies where pleural effusion accounted for more than 50% of total cases [3,5]. *Thapar M et al,[4] Madakshira MG et al,[6] Khan N et al,[16] Ranade RS et al*[12] showed peritoneal effusion predominance. This difference could be because of nature of cases received in healthcare facilities or selection of cases based on inclusion and exclusion criteria.

According to literature, patients aged ranged from 1 – 80 years with highest number of cases in 6th decade and least number of cases in 1st decade. [15] Majority of our patients were in 5th and 6th decade and minimum number of cases were in 1st decade.

Male predominance was seen in 490/801 cases (61.2%) with M: F ratio of 1.6:1 which was similar to other studies. [5,17] While studies conducted by *Madakshira MG et al,[6] Tayel HY et al,[18] Khan N et al,[16] SharmaM et al* [14] showed female predominance.

Biochemical analysis for protein and sugar was available in 538 cases. 337/538 (62.6%) were transudative and 201/538 (37.3%) were exudative. 3/4 (75%) reactive mesothelial proliferation and 9/10 cases of 3-5 categories revealed exudative effusion. Similarly, Tarn AC et al [19] concluded that majority of reactive mesothelial proliferation and malignant cases were exudative in nature.

While evaluating the cytology smear, our primary goal was to study the spectrum of cytological diagnosis in serous effusion and to compare the diagnostic accuracy of conventional smear with cell block technique. Out of 801 cases, majority 593 cases under category 2B showed lymphocytic rich effusion which could be because of chronic inflammatory and infective conditions like tuberculosis, being more prevalent in India. 119 cases showed neutrophilic rich effusion which could be due to primary and secondary infections. One case of pneumothorax showed eosinophilic effusion and had >10% eosinophils. [12]

6 cases showed reactive mesothelial proliferation. Reactive mesothelial cells tend to round up giving plumped appearance with enlarged nuclei that can be mistaken for cancer cells, and therein lies the essence of the diagnostic dilemma in fluid cytology.[20]

We encountered 11 cases under category 3 which may be considered as a borderline category because cells show cytological atypia that quantitatively or qualitatively do not favour malignancy. Similarly, one case under category 4 showed cytological atypia that quantitatively or qualitatively fell short to make the diagnosis of frank malignancy and 2 cases under category 5.[9]

Cyto-histopathological concordance was seen in all except two case. One was reported negative for malignant cells on cytopathology and showed features of malignancy on cell block. This was because of presence of large size coagulum in this case which could have entrapped the cytological material into it. [21] Other was reported as Atypical: NOS on conventional smear which revealed reactive mesothelial proliferation on cell block and was further confirmed by D2-40 IHC. To overcome this problem of under diagnosing the atypical and suspicious cases, preparation of cell blocks along with conventional smear in useful. Ancillary studies in the form of IHC on cell block is recommended.

The cell blocks were prepared using 10% alcohol-formalin embedded in paraffin wax which provided better cellularity by forming protein cross links that minimized the cell loss. Also, it is an easy and cost-effective method. [15] The only disadvantage with this technique was it required overnight formalin fixation which may cause delay in diagnosis. In other studies, also alcohol-based paraffin embedded cell blocks were prepared.[4,21]

Due to many diagnostic dilemmas in effusion cytology, a definitive diagnosis is often difficult based on morphology alone. Therefore, combined use of both conventional smear and cell block technique along with use of ancillary techniques is recommended. [18]

Immunohistochemistry has proven to be valuable in the differentiation of reactive mesothelial, mesothelioma and metastatic adenocarcinoma cases, no single antibody has demonstrated absolute sensitivity or specificity in making the diagnosis. [8]

We performed D2-40 immunostaining on 7 reactive mesothelial cases and 13/14 malignant cases.

None of the adenocarcinoma cases showed D2-40 positivity. However, in **4/13** malignant cases, background mesothelial cells stained with D2-40 IHC. This was in concordance with other studies. *[8, 22,10]*

Thus, to conclude cell block examination is a simple, inexpensive method which gives better architectural patterns, morphological details and should be used along with conventional smear to find out primary site and to differentiate reactive proliferation from malignancy. In present study, increased diagnostic yield of 1.6% was noted by cell block method. It also has an added advantage that multiple sections can be obtained for immunohistochemistry. However, biopsy was not available in majority of malignant cases. Future studies with a large sample size are needed for statistically significant results.

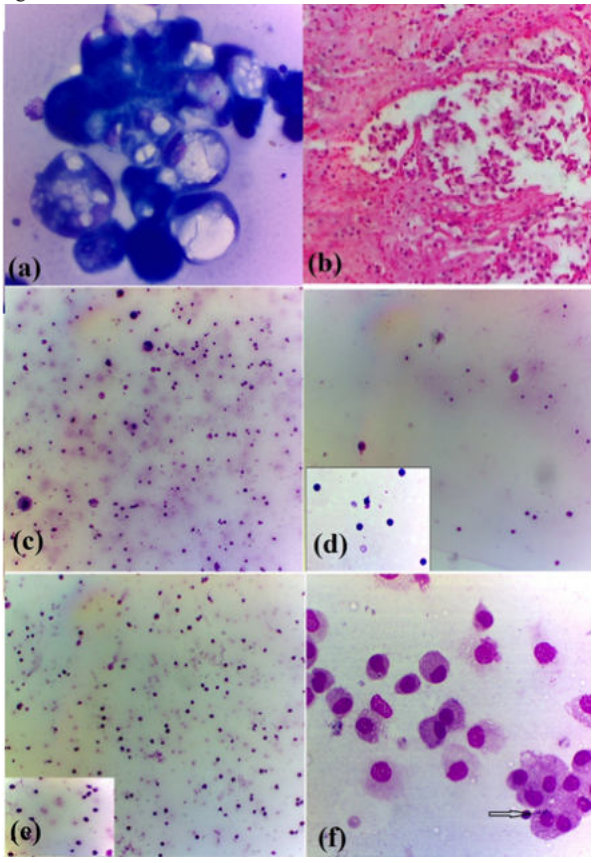

Figure 1: (a) - Reactive mesothelial cell cluster on conventional smear with enlarged nuclei and vacuolation (MGG, x400). **(b)** Reactive mesothelial cells on cell block admixed with inflammatory cells (H&E, x100). **(c)** Neutrophil rich effusion with few mesothelial cells on conventional smear (MGG, x100). **(d)** Lymphocyte rich effusion with mesothelial cell on conventional smear (MGG, x100). **(e)** Eosinophil rich effusion with other inflammatory cells on conventional smear (MGG, x100). **(f)** Clusters of macrophages (arrow) with abundant vacuolated cytoplasm, vesicular chromatin and hemosiderin pigment (MGG, x400).

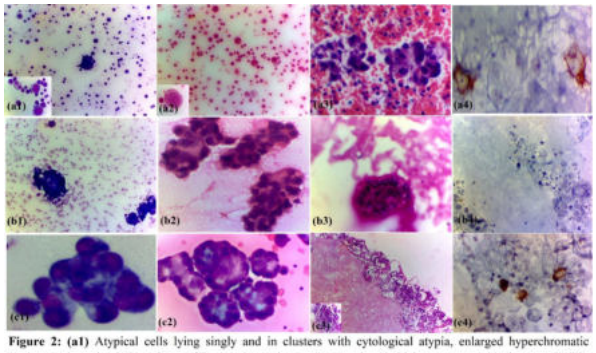

Figure 2: (a1) Atypical cells lying singly and in clusters with cytological atypia, enlarged hyperchromatic nucleus and amphophilic to basophilic cytoplasm along with cytoplasmic blebs on conventional smear (MGG, x100, 400). **(a2)** Atypical cells on conventional smear (H&E, x100, 400). **(a3)** Cell block showing tumour cell in vague glandular pattern with enlarged nucleus (H&E, x400). **(a4)** IHC D2-40 Negative in tumour cells. Mesothelial cells having membranous positivity (D2 – 40, x400). **(b1)** Clusters of atypical cells and mesothelial cells in conventional smears (MGG, x100). **(b2)** Atypical cells cluster have enlarged hyperchromatic nucleus (H&E, x400). **(b3)** Cell block exhibiting tumour cell cluster with large hyperchromatic nucleus and increased N:C ratio (H&E, x100 **(b4)** IHC D2-40 on cell block - Negative in tumour cells (x400). **(c1)** Malignant cell clusters admixed with enlarged hyperchromatic nucleus, basophilic to vacuolated cytoplasm along with mesothelial cells (MGG, x400). **(c2)** Malignant cell clusters on conventional smear (H&E, x400). **(c3)** Malignant cells on cell block having enlarged hyperchromatic nucleus and high N:C ratio (H&E, x100, 400). **(c4)** Corresponding area on IHC shows D2 – 40 positivity in mesothelial cells and tumour cells stained negative for D2 – 40 (x400).

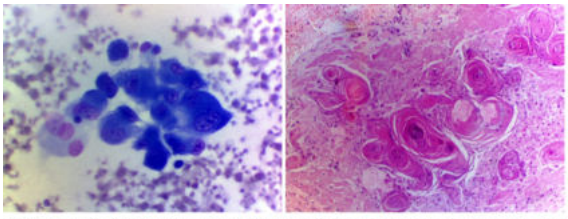

Figure 3: (a) Malignant cells cluster with hyperchromatic nucleus and abundant basophilic cytoplasm. (MGG, x400). **(b)**Well-formed keratin pearls on cell block (H&E, x400).

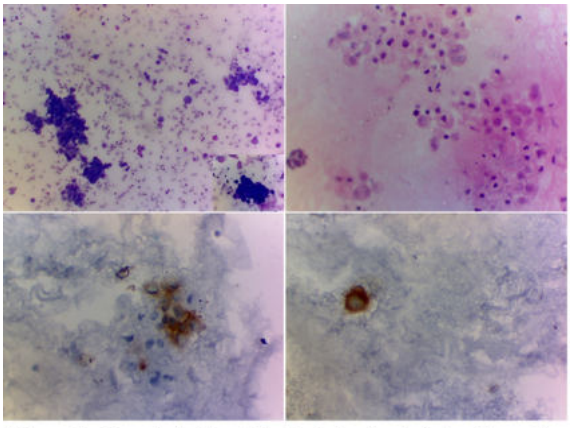

Figure 4: Discordant Case (a) Atypical cell admixed with reactive mesothelial cells and macrophages on conventional smears (MGG, x100) Inset: atypical cells with high N:C ratio (MGG, x400). **(b)** Cell block revealed clusters of reactive mesothelial cells admixed with macrophages (H&E, x400). **(c)** IHC-D2-40: Mesothelial cell cluster exhibiting cytoplasmic and membranous positivity (x100). **(d)** D2-40 showing positivity in mesothelial cell (x400).

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