Mediscope The Journal of GMC

ORIGINAL ARTICLE DOI: https://doi.org/10.3329/mediscope.v10i1.65397

Diagnostic Efficacy of Conventional Cytological Smears and Cell Block of Ascitic Fluid for Detection of Malignant Cell

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Abstract

Introduction: For identification of malignant cells in effusion, cell block is a simple, inexpensive method and no additional expatriation is needed. In the identification of malignant cells in effusion, its differentiation from cells showing reactive and degenerative changes were diagnostic difficulties in some of the cases. The cell block method yielded more cellularity and provided better morphological details. Multiple sections could be obtained for special stain and immunohistochemistry when required. **Methods:** This cross-sectional observational study was carried out at the Department of Pathology, Bangabandhu Sheikh Mujib Medical University (BSMMU), Dhaka, Bangladesh; from March, 2017 to December, 2018. The sample size was 101 and the study material was ascitic fluids of clinically suspected malignancy cases. Conventional smears and cell block were prepared in every cases. Both conventional and cell block slides were scored according to Miar's point scoring system and analyzed according to diagnostic categories. **Results:** By conventional smear, 86 cases were diagnosed as negative for malignancy, four cases were diagnosed as suspicious for malignancy and remaining 11 cases were diagnosed as positive for malignancy. After analyzing cell blocks, three more malignant cases were diagnosed, which had been diagnosed as suspicious for malignancy by conventional smear. Cell block technique showed significant differences in the diagnosis of suspicious cases of effusion in comparison to conventional smear ($p<0.05$) **Conclusion:** Cell Block technique could be considered as a useful adjunct in evaluating malignant cells in malignant ascitic effusion for a final cytodiagnosis, along with the routine conventional method.

Keywords: Diagnostic efficacy, Cytological smears, Cell block, Ascitic fluid, Malignant cell

Introduction

The word ascites came from the Greek word 'Askos', meaning a bag or sack.[1] Ascites is one of the most common clinical presentations of various underlying pathologies. Ascitic fluid analysis is the most effective way to diagnose the cause of ascites.[2] Normally the peritoneal cavity is collapsed with a small amount of fluid content and lined by single layer of mesothelial cells known as serosa. This fluid helps to lubricate the adjacent surfaces. A greater amount of fluid accumulation in disease states is known as an effusion.[3] When the balance between plasma flowing into and out of the blood and lymphatic vessels is disrupted, ascites forms. This imbalance may be due to increased capillary permeability, increased venous pressure, decreased protein or lymphatic obstruction. Malignancies account for approximately 10% of cases. The other types of ascites are categorized as cardiogenic, nephrogenic, infectious,and miscellaneous.[1] Cytological examination of body

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fluid is gaining acceptance in clinical medicine. Once positive diagnosis is made it is often considered as definitive diagnosis. To identify primary site and type of malignancy is of paramount importance. This helps to obviate the proper surgical management, chemotherapy and radiotherapy. So, it results in increase patients' survival rate.[4]

In cytological study, the diagnostic performance may be attributable to several facts. The cell population of the sediment is representative of a much larger surface area in comparison to needle biopsy. So, collection of cells from any fluid and keep them on the slide during staining is very important. Otherwise this may cause unsatisfactory smear, reported as inconclusive without a definitive diagnosis, or even a false negative diagnosis.[5]

In conventional cytological smears, the accurate identification of cells as either malignant or reactive mesothelial cells is a diagnostic problem. To differentiate benign from malignant cellular changes, it requires meticulous screening, careful scrutiny of cellular features and an understanding of the range of reactive changes. Lower diagnostic yield may be due to cellular overlapping, delaying artifact, suboptimal processing and leaving behind useful material.[6]

Adjunct to conventional smears, cell block is embedded in paraffin and examined for types of cells in fluid. Cell block technique increases cell yield. It increases the sensitivity of the test and decreases the number of false positive and false negative results. A modified cell block technique using alcohol formalin fixative, followed by routine tissue processing offers a better preservation of architectural details of cells. This can be used for special stains and immunocytochemistry also. This method is a simple and inexpensive technique and only needs routine laboratory chemicals.[7]

Presently, in our country only conventional smears are made in almost all the laboratories. The study has been undertaken to assess the utility of cell block in cytological diagnosis of suspected malignant ascitic effusions and compare the diagnostic efficacy of conventional cytological method versus cell block techniques in effusions.

Materials and methods

This was a cross-sectional observational study carried out at the Department of Pathology, Bangabandhu Sheikh Mujib Medical University (BSMMU), Dhaka, Bangladesh; from March, 2017 to December, 2018. The sample size was 101. The study material was ascitic fluids of clinically suspected malignancy cases. The fluid was taken from about 100 clinically suspected malignant cases from department of pathology, BSMMU.

About 20 ml fluid was taken in two separate containers from the sample. A detailed history regarding age, sex, relevant investigations, clinical diagnosis and general and systemic examination findings was taken. Conventional smear slides were prepared by cell centrifuge and stained with Papanicolaou and hematoxylin and eosin stain.

Cell blocks were prepared by cell centrifuge and stained with hematoxylin and eosin stain. Both conventional and cell block slides were scored according to Miar's point scoring system.4 Miar's point scoring system includes background, cellularity, cytoplasmic and nuclear details (cellular morphology) and architecture (acini, papillae, cell balls, and proliferation spheres). Each parameter was scored separately. Conventional smears and cell block were analyzed according to diagnostic categories.

Some of the cases were stained with PAS and PAS-D when required. A few cases were randomly selected for immunohistochemistry by epithelial membrane antigen (EMA) and calretinin (CAL) to observe their positivity to find out whether these were metastatic adenocarcinoma or malignant mesothelioma.

Study Procedure

Processing of fluid:

Conventional Smear Preparation:

- Fluid from one container was transferred to centrifuge tube labeled with the specimen identifier and then was centrifuged for 5 minutes at 2000 rpm.
- Supernatant fluid was discarded and the sediment was taken on the slide with the help of glass rod and spread by thick and thin method.
- Two smears were prepared.
- Two slides were fixed in 95% ethanol and stained with Papanicolaou and H & E stain.

Cell block preparation:

- The fluid specimen reserved for cell block was fixed in ethanol formalin fixative (9 parts absolute alcohol & 1 part 10% formalin) in the ratio of 1:1 for one hour.
- Centrifugation was done at 2000 rpm for 10 minutes.
- Supernatant was poured off and sediment was drained by inverting the tube on Whatman filter paper.
- The sediment was then wrapped in the same filter paper and embedded in paraffin.
- Multiple thin sections of 4-5-micron thickness from paraffin blocks were obtained, stained with H &E stain and examined microscopically.
- Special staining like PAS, PAS-D were done whenever required.[8]

Observation and Interpretation

Each individual slide was objectively analyzed for background, cellularity, cytoplasmic and nuclear details (cellular morphology), architecture (acini, papillae, cell balls, and proliferation spheres), using the Miar's point scoring system. According to the criteria mentioned in the table below, comments will be rendered on the quality of the slides by qualitatively grouping them into three categories.

Quality of slide:

1. Diagnostically unsuitable (0)
2. Diagnostically adequate (1-5)
3. Diagnostically superior (6-8)

These smears were observed and diagnosed on conventional smears and cell block separately.

Diagnostic categories:

1. Negative for malignancy
2. Suspicious for malignancy
3. Positive for malignancy

Cellularity	Point score		
	0	1	2
1. Volume of Blood/ clot obscuring background	Large: diagnosis greatly compromised	Moderate: diagnosis possible	Minimal: diagnosis easy, textbook quality
2. Amount of diagnostic cellular material present	Minimal: diagnosis not possible	Moderate: sufficient for diagnosis	Abundant: diagnosis simple
3. Degree of cellular degeneration and cellular trauma	Marked: diagnosis not possible	Moderate: diagnosis possible	Minimal: good preservation
4. Retained architecture / Cellular Arrangement	Minimal: diagnosis not possible	Moderate: some preservation	Excellent architectural display

Results

In this study, 101 cases of clinically and radiologically suspected malignant ascitic effusion fluid were included. Diagnostic efficacy of cell block method in contrast to conventional smear was assessed. For this, Miar's scoring system was used to evaluate the diagnostic yield of conventional smear and cell block. In this study most of the patients were over the age of 40 years (70%) (Table 01). By conventional smear, 86 cases were diagnosed as negative for malignancy, 04 cases were diagnosed as suspicious for malignancy and remaining 11 cases were diagnosed as positive for malignancy. After analyzing cell blocks, three more malignant cases were diagnosed, which had been diagnosed as suspicious for malignancy by conventional smear.

The remaining suspicious case by conventional smear was diagnosed as negative for malignancy. Cell block technique showed significant differences in the diagnosis of suspicious cases of effusion in comparison to conventional smear (Table 02). Of the malignant cases, all were diagnosed as metastatic adenocarcinoma. Five malignant cases were selected randomly for immunohistochemistry by epithelial membrane antigen (EMA) and calretinin (CAL) to confirm the positivity. Among them all the cases (n=5) were strongly EMA positive and CAL negative in tumour cells (Table 03). A critical analysis along with p-value detection based on Miar's scoring system background, cellularity, cellular morphological details and pattern of cellular arrangements was done, to compare the diagnostic yield between two methods. Paired t-test was applied to compare the diagnostic yields between conventional smear and cell block in cases of ascitic fluid. There is significant difference in the distribution of scores between two methods for all criteria ($p<0.05$) (Table 04). Again, paired t-test was applied to compare the diagnostic yields between conventional smear and cell block in cases of malignant ascitic effusion. There was significant difference in the distribution of scores between two methods for all criteria ($p<0.05$) (Table 05).

Table 01: Age and sex of the patients

Age (Years)	Male	Female	Total
≤ 20	2	7	9
21-40	10	11	21
41-60	17	18	35
>60	23	13	36
Total	52	49	101

Table 02: Differences in the diagnosis of the ascitic fluid by conventional smear and cell block

Category	Conventional smear	Cell block
Negative for malignant Cell	86 (85%)	87 (86%)
Suspicious for malignant cell	04 (4%)	00 (0%)
Positive for malignant cell	11 (10.8%)	14 (13.8%)

Table 03: Immunohistochemistry in 5 cases of ascitic fluid positive for malignancy

Immunomarkers (n=5)	Positive	Negative
EMA	5	0
Calretinin	0	5

Table 04: Statistical comparison between conventional smear and cell block preparation in ascitic fluid in terms of Miar's scoring system (n=101)

Traits	Conventional Smear (Mean score ± SD)	Cell block (Mean score ± SD)	P-value
Background	1.53 ± 0.59	1.76 ± 0.51	0.008
Cellular Yield	1.40 ± 0.53	1.59 ± 0.57	< 0.001
Cellular Morphology	1.01 ± 0.38	1.59 ± 0.55	< 0.001
Architecture	1.72 ±0.56	1.86 ± 0.47	< 0.001

Table 05: Statistical comparison between conventional smear and cell block preparation in malignant ascitic effusion in terms of Miar's scoring system (n=14)

Traits	Conventional Smear (Mean score ± SD)	Cell block (Mean score ± SD)	P-value
Background	1.5	1.92	< 0.001
Cellular Yield	1.42	2	< 0.001
Cellular Morphology	1.07	1.78	< 0.001
Architecture	1.07	2	< 0.001

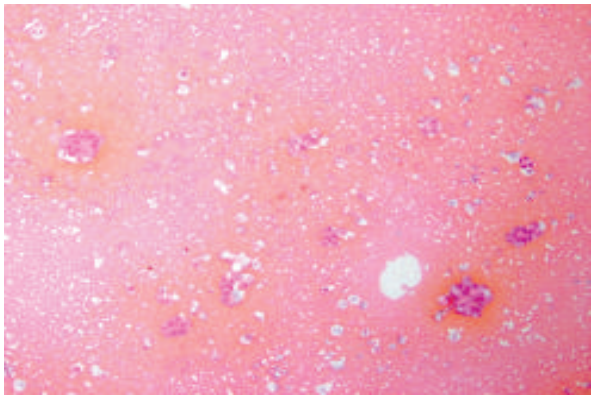

Figure 01: Photo micrograph of conventional smear of metastatic adenocarcinoma, showing background blood and moderate amount of diagnostic material (Case no 3, Papanicolaou stain x100)

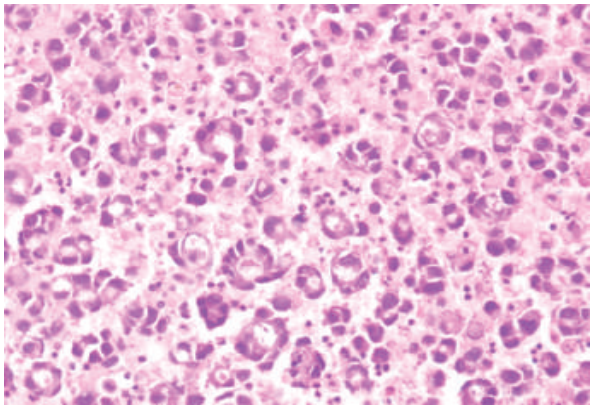

Figure 02: Photo micrograph of cell block of metastatic adenocarcinoma, showing abundant diagnostic material and excellent glandular architecture (Case no 3, H&E x400).

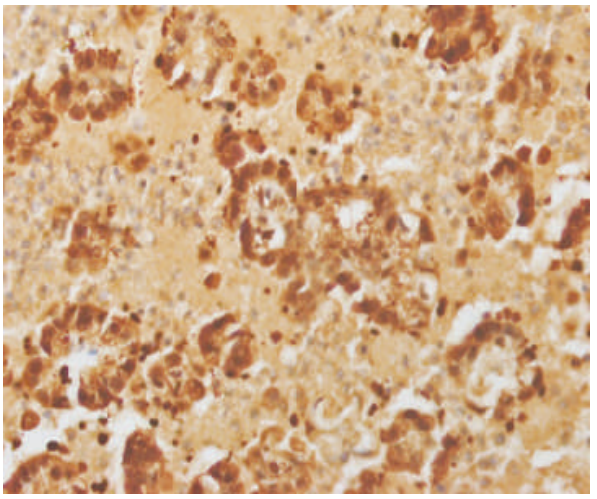

Figure 03: Photo micrograph of Epithelial membrane antigen (EMA) positivity in tumour cells in metastatic adenocarcinoma (Case no 39, EMA immunostain x400 H&E x400)

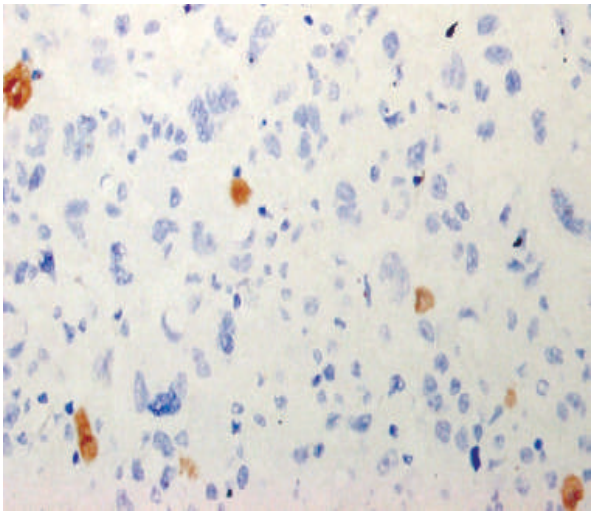

Figure 04: Photo micrograph of Calretinin (CAL) negativity in tumour cells in metastatic adenocarcinoma (Case no 46, CAL immunostain x400 H&E x400)

Discussion

The cause of ascites is a common diagnostic challenge. The initial management of these patients should be through history with examination and this should then direct to further laboratory assessment, with cell count and differential, albumin and culture being mandatory. Runyon suggested the following three criteria as

indications to proceed with cytology: (i) a history of cancer, (ii) no physical findings suggestive of liver disease, and (iii) an initial ascitic fluid sample with a high lymphocyte count (500 cells/mm3) and few neutrophils.[9]

The difficulty is secondary to the marked atypia of the mesothelial cells which is caused by the microbiological, chemical, physical, immunological, or the metabolic insults to the serous membranes or due to the subtle cytomorphological features of some malignant neoplasms. The problem may become more compounded due to the artifacts which are caused by poor fixation, preparation, or staining techniques.[10]

In the present study, 101 cases of suspected malignant ascites were included. The age ranged from 18 to 101 years. The maximum numbers of cases were over 40 years (70%) with a slight male predominance (51.5%). Among the positive cases, most of the patients were over 40 years (85%) and female patients outnumbered male patients (71%). These findings correlated with some other studies.[11,12]

In present study interpretation of slide was done based on Miar's point scoring system which was followed by Shubhada, et al. (2015) and Thapar, et al. (2009).[4,13] Each slide was observed and scored based on background, cellularity, cellular morphology and architecture. Preparation and analysis of smears and cell block from the same specimen was done. Due consideration was given to age, sex, clinical and radiological findings to arrive at final diagnosis.

The conventional method shows minimal background obscurance in 57% cases and moderate background obscurance in 37% cases. In comparison cell block shows minimal background obscurance in 80% cases and moderate background obscurance in 16% cases. The conventional method shows maximum cellular yields in 41% cases. In comparison cell block shows maximum cellular yields in 63% cases. The cell block concentrated the cellular material into a small area which was useful in screening the material in lesser time. Similar findings were noted in some other studies.[13,14]

When conventional smears were compared with cell block preparation for morphological preservation, the cell block sections showed clearly recognizable cells with minimal shrinkage and aberrations. The conventional smear shows only 8% cases with excellent cellular morphological features compared to 62% cases in cell block method. The cytomorphologic features were well maintained with minimal shrinkage and aberration, better nuclear and cytoplasmic preservation, intact cell membrane and crisp chromatin details in cell block method. Similar findings were noted in some other studies.[13-15]

Cytological examination of the serous effusions is a routinely done procedure in cytology laboratories of the department of pathology everywhere. It is a very important tool to differentiate various benign conditions like hepatic cirrhosis, pleurisy and pulmonary infarcts from suspicious and known malignant conditions.[16]

Conventional cytological examination of effusions has a sensitivity of only 40-70% for detecting the presence of malignant diseases. In the CS method, overcrowding of the cells, reactive mesothelial cells, cell loss due to different laboratory processing methods, abundance of inflammatory cells and a paucity of representative cells contribute to the considerable difficulties which are faced in making conclusive diagnosis.[17] Cell block method in addition with conventional methods increases both sensitivity and specificity of cytological examination.[18]

Apart from increased cellularity and better morphological details, cell block method also showed preservation of the architectural pattern such as, cell ball, well-formed glands, papillae and three-dimensional clusters. These features have increased the sensitivity of the diagnosis of malignancy by the cell block method. All of these were helpful to diagnose the positive cases as

well as their types. Reactive mesothelial cells are responsible for simulating malignancy in conventional smear, largely due to the formation of rosettes, pseudo acini or acini, with or without the presence of prominent nucleoli. The cell block effectively puts both the features in their proper prospective, that is, the nucleoli do not appear as prominent as in conventional smear and the pseudoacinar or acinar structures can be better appreciated when present in the cell block. Similar findings were noticed in another study.[14] More important is, this cell block is a valuable tool in the evaluation of well differentiated adenocarcinoma in contrast to conventional smear. Sears & Hajdu (1987) have suggested a clear preference for cell block sections in cytological examination of effusions.[19]

To measure the diagnostic yields total score of Miar's scoring system were evaluated in conventional smear and cell block. In conventional smear 66% cases were diagnostically superior and scored more than 5. About 28% cases were diagnostically adequate. In cell block method 88% cases were diagnostically superior and scored more than 5. This correlates with the study of Shubhada, et al. (2015) and Sumitha, et al. (2017).[4,8]

The criteria were statistically evaluated by paired t-test on each criterion of Miar's scoring system. Each criterion such as background, morphology and architecture showed significant differences in the distribution of scores between conventional smears and cell block.

The criteria were further evaluated in only positive cases (n=14). These also showed the same type of findings. P-value for each criterion was found to be statistically significant (<0.05) favoring cell block over conventional smear.

Conventional smear and cell block techniques were used in all the 100 samples included. In conventional smears 86 of the fluids were diagnosed as negative for malignancy (85%). In conventional method, the cellularity of most of the negative cases revealed mostly lymphocytes. In cell block, the inflammatory cells presented as flat sheets with a thick amorphous background. The diagnosis of negative cases was reconfirmed in cell block.

In conventional methods, 11 cases were diagnosed as malignant which was reconfirmed by cell block. Four other cases were diagnosed as suspicious for malignancy in conventional smear, three of which were diagnosed as positive for malignancy by cell block method. The remaining suspicious for malignancy case was diagnosed as negative for malignancy by cell block method. Cell block method helped in these cases by adding additional information of architectural pattern, cellularity and nuclear features of anaplasia. These findings correlate with the study of Shivkumarswami, et al. (2015) (15%), Khan, et al. (2006) (20%), Bodele, et al. (2003) (7%), Richardson, et al. (1995) (5%).[11,20-22]

Five positive cases were randomly selected for immunohistochemistry with epithelial membrane antigen (EMA) and calretinin (CAL) to find out whether these were metastatic adenocarcinoma or malignant mesothelioma. All of these five cases showed positivity with EMA supporting a diagnosis of metastatic adenocarcinoma. Calretinin was negative in all five cases excluding the diagnosis of malignant mesothelioma.

Conclusion

Cytological examination of serous fluid is easily performed, less expensive, most specific test to diagnose malignancy. It also helps in staging and as well as prognosis of the disease. The present study assessed the role of cell block in suspected malignant ascitic effusion. The cell block showed statistically significant diagnostic yield with minimal obscurance by background material, more yield of cellularity with preserved architecture and cellular details. Cell block technique in selective cases could contribute to make a definitive diagnosis. Cell block technique is not routinely practiced in Bangladesh. But considering the additional benefits that can be

obtained from cell block methods, a routine practice of keeping the pellets of cells after centrifugation of effusion fluids can be incorporated in laboratory practice.

Limitations of this study

There were some limitations of the study

1. Small sample size
2. Study period was short for proper follow up of the patients.
3. Inadequate clinical information in some cases.

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