



Diagnostic Efficiency of Cell Block Method over Conventional Smear Method in the Cytology of Malignant Effusions– A Prospective Study

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Abstract

Background: Cytological examination of body fluids has increasingly gained acceptance in clinical medicine to such an extent that a positive diagnosis made is often considered definitive diagnosis. It is of utmost importance to identify primary site and type of malignancy. The aim of this study is to compare the diagnostic efficiency & morphological features of cell block & conventional smear method in malignant fluid samples. This study also evaluates the usefulness of immunohistochemistry on cell blocks in identifying the primary site of malignancy

Methods: Cell blocks & conventional Smears from 30 malignant fluid samples were prepared. Morphological features (like cellularity, nuclear features, cytoplasmic features & pattern) and final diagnosis were compared in both. Immunohistochemistry was performed on cell blocks to identify the primary site of malignancy. The results obtained by immunohistochemistry were compared to the final histopathological diagnosis of the primary malignancy.

Results & Discussion: The morphological features were found to be superior in cell block method compared to conventional smear method. The diagnostic efficiency of cell block was 86.7% whereas that of conventional smear was only 80%. Combined diagnostic efficiency of the two methods was 96.7%. Immunohistochemistry done on cell blocks could identify the primary malignancy or atleast suggest two or three possible sites of primary in all 30 cases.

Conclusions: Cell block in conjunction with smears helps increase the accuracy of fluid diagnosis. The better morphological features and the use of immunohistochemistry to help identify primary site of malignancy gives a more definitive diagnosis by the cell block method.

Keywords: Cell block, Conventional smear, Immunohistochemistry.

Introduction

The cytological examination of serous fluids is of utmost importance in the diagnosis, staging and prognosis of malignant lesions⁽¹⁾. The development of malignant pleural effusion is a common presentation of cancers like pulmonary, breast and

gastric carcinoma⁽²⁾. If malignant cells are seen in the pleural, pericardial or ascitic fluid, it almost always points out to metastatic tumors. While screening the effusion cytology smears for malignancy, reactive mesothelial cells is a commonly encountered problem. The lower

sensitivity is mainly attributable to bland morphological detail of cells, overcrowding or overlapping of cells, cell loss and changes due to different laboratory processing in the conventional smear method⁽³⁾.

The cell block method is one of the oldest methods for the evaluation of body fluids. It can be used as an adjunct to smears for establishing a more definitive cytopathological diagnosis. Its main advantage is increased cellularity, the preservation of tissue architecture, and obtaining multiple sections for special stains & immunohistochemistry. Patterns of arrangement of cells are better appreciated in cell block⁽⁴⁾. There are several methods of the cell block preparation like fixed sediment method, plasma thrombin clot method, bacterial agar method, cell block from millipore filter method, etc. The most widely accepted method for cell block preparation uses 10% alcohol formalin as fixative, which is a very simple and inexpensive method. It does not require any special training or sophisticated instrument. Aspiration biopsy material (FNA), sputum, effusions, urine sediment, and material from the gastrointestinal tract, can also be subjected for cell block processing, as are all tissue fragments incidentally obtained during any other diagnostic cytologic procedure⁽⁵⁾.

Methods

All pleural fluid, pericardial and ascitic fluid samples received in the Department of Pathology, Jubilee Mission Medical College for the next one and a half years were subjected to cell block and conventional smear examination.

The samples were collected in clean test tubes or containers, half of which were used for conventional smear method and the other half for cell block method.

In the conventional smear technique, the fluid sample were centrifuged at 2500 rpm for 15 mins. A minimum of 3 smears were prepared from the sediment. Two of the smears were immediately fixed in 80% isopropyl alcohol and stained with Papanicolaou and Haematoxylin-Eosin stain. The

third slide was air dried and stained with Leishman stain.

For the cell block technique, the fluid was centrifuged for 15 minutes at 2500 rpm after adding 10% alcohol formalin fixative. The supernatant was poured off. 10% alcohol formalin was again added to the sediment and left overnight. On the following day, the sediment was scooped out on to filter paper and this sediment was processed along with other routine histopathological specimens. Paraffin embedded sections of 4-6 micro meter thickness were prepared and stained with hematoxylin and eosin stain. The slides were examined under the microscope⁽¹⁾.

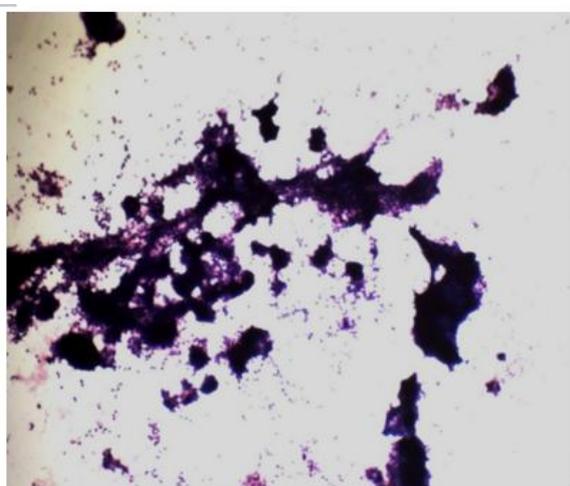
The slides were studied in detail taking into account the available clinical data, various investigation reports & morphological details. The samples were categorised as benign, suspicious for malignancy or malignant. All the samples that are suspicious for malignancy or were malignant by either the cell block method or the conventional smear method and where there was a biopsy correlation were included in the study population. Cytomorphological features like cellularity, arrangement of cells, cytoplasmic and nuclear details were compared for the above two methods. Immunohistochemistry was performed on the cell blocks to identify the primary site of malignancy using markers that included CK 7, CK 20 if the cytomorphology is that of a carcinoma. Additional markers will be used wherever relevant. The results of the fluid cytological examination & cell block immunohistochemistry were compared with the final histopathological diagnosis of the primary tumor.

Results

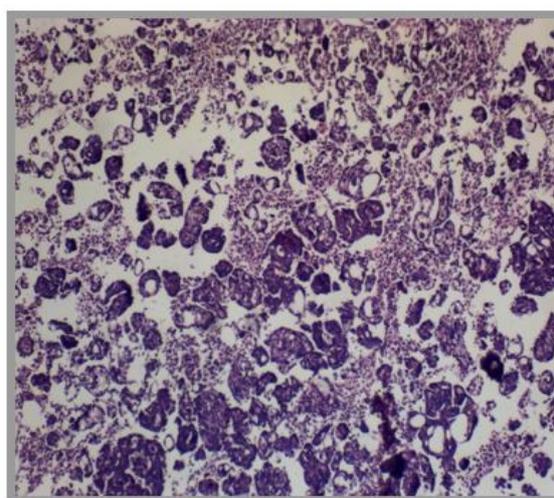
A total of 30 fluid samples were observed. The age of the patients range from 33-89 years. 7 cases (23.3%) were males and 23 cases (76.7%) were females. Among the 30 cases, there were 16 ascitic fluid samples, 8 pleural fluid, 2 pericardial fluid and 4 peritoneal washings.

Cellularity was found to be better in the cell block method in 14 (46.7%) of the cases and by conventional smear analysis in 6 (20%) of the cases. In 10 (33.3%) of the cases, there was not much difference in the cellularity. Nuclear features were found to be better by the Cell Block method in 16/30 cases (53.3%) and Conventional Smear method in 5/30 cases (16.7%). There was no considerable difference between Cell Block and Conventional Smear method in 9/30 cases (30%). In the present study with 30 malignant fluid samples, cytoplasmic features were found to be better in 15 (50%) cases by the cell block

method and in 6 (20%) cases by the conventional smear analysis. There was not much difference in the cytoplasmic features in 9 (30%) of the cases. These findings are illustrated in Graph 1. The pattern of arrangement of cells (eg. glandular pattern) was better demonstrated by cell block method in 16/30 (53.3%) and by Conventional Smear analysis in 7/30 (23.3%) fluid samples. There was no considerable difference in the pattern of arrangement of cells in 4/30 (13.3%) samples. 3/30 (10%) samples showed no specific pattern of arrangement of cells.



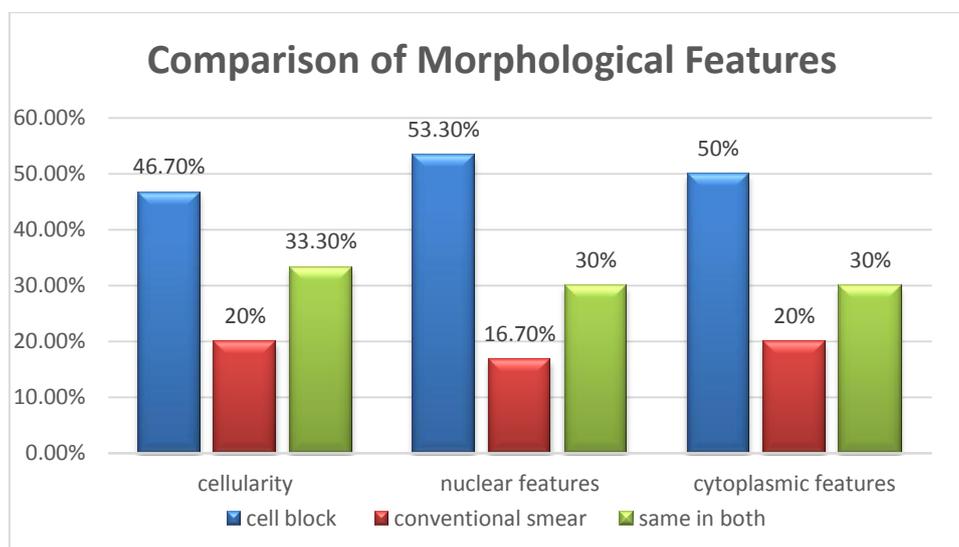
Picture 1a



Picture 1b

Cellularity in conventional smear (1a) compared to cell block (1b), 10x, H&E Ascitic fluid from a case of Serous Carcinoma Ovary

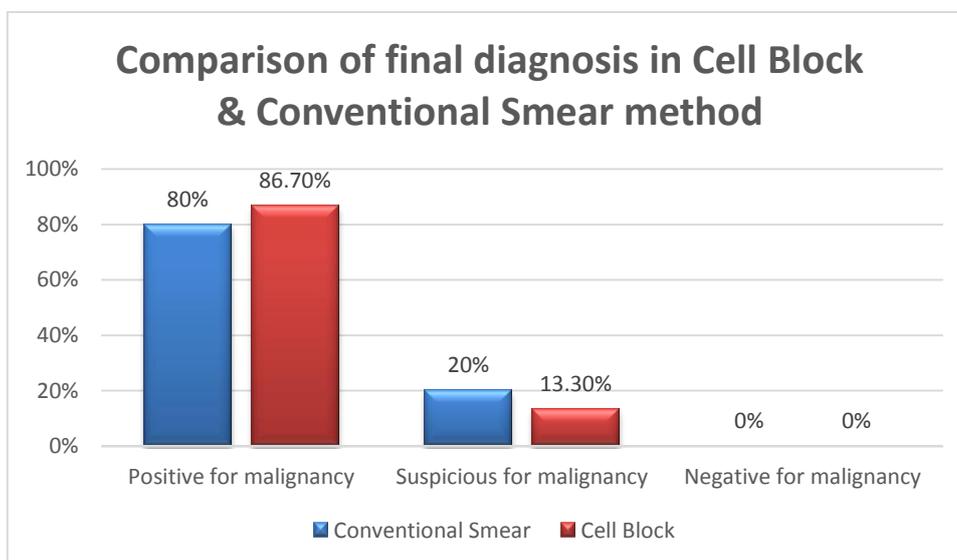
Graph 1



The final cytological diagnosis was positive for malignancy in 26/30 (86.7%) cases by the cell block method and 24/30 (80%) cases by conventional smear analysis. 4/30 (13.3%) cases

were suspicious for malignancy by cell block method whereas. 6/30 (20%) cases were suspicious for malignancy by conventional smear analysis. (Graph 2)

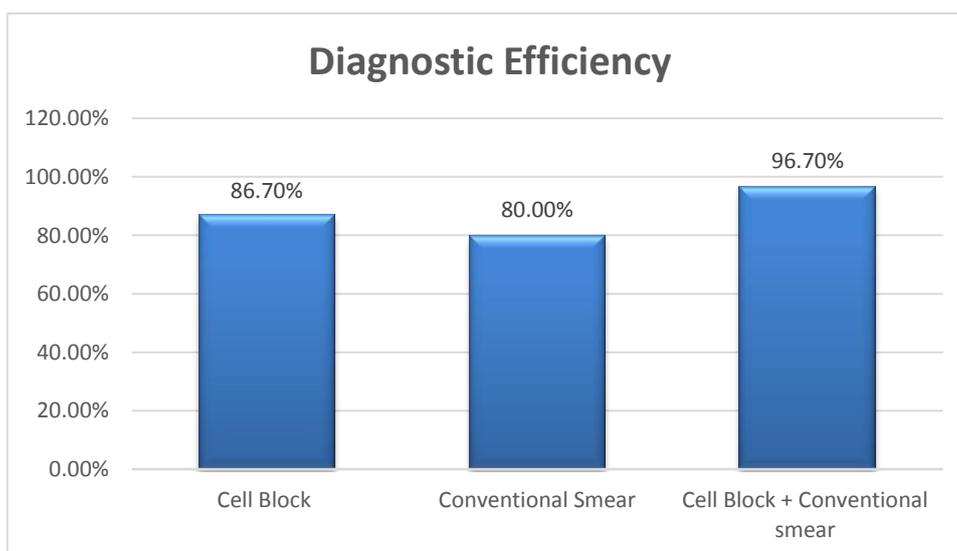
Graph 2



Only 1/30 sample was suspicious for malignancy by both cell block and conventional smear method. The diagnostic efficiency of cell block method was 26/30 (86.7%) and of conventional smear method was 24/30 (80%). (Graph 4) There was no statistically significant increase in diagnostic efficiency by cell block method over conventional smear analysis (p value by Fisher's Exact Test: 1)

But on combining these two tests, the efficiency became 29/30 (96.7%) which also showed no statistically significant increase in diagnostic efficiency when compared to cell block method (p value: 0.1) or conventional smear analysis (p value by Fischer's Exact Test: 0.2) alone. (Graph 3)

Graph 3



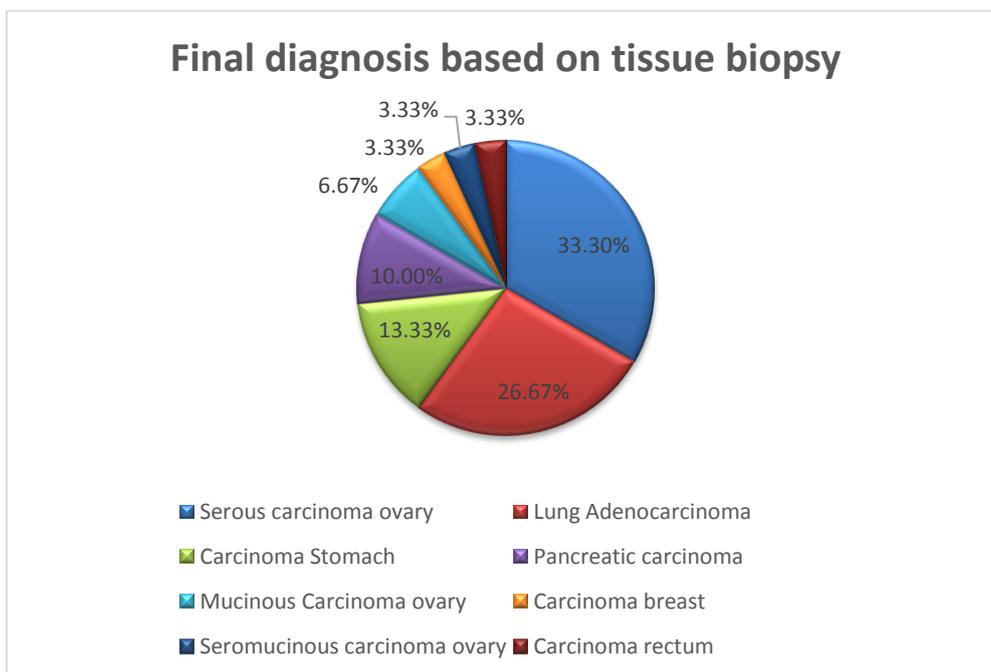
The sensitivity of cell block method was found to be 86.6% which was more than that of conventional smear method which was 80%.

Of the 30 fluid samples studied, most common primary was Serous Carcinoma Ovary (10/30 cases). Other primary malignancies included Lung

Adenocarcinoma (8/30 cases), Carcinoma stomach (4/30 cases), Adenocarcinoma pancreas (3/30 cases), Mucinous carcinoma ovary (2/30

cases), Carcinoma breast (1/30 cases), Seromucinous carcinoma ovary (1/30 cases) and Carcinoma breast (1/30 cases) (Graph 4)

Graph 4

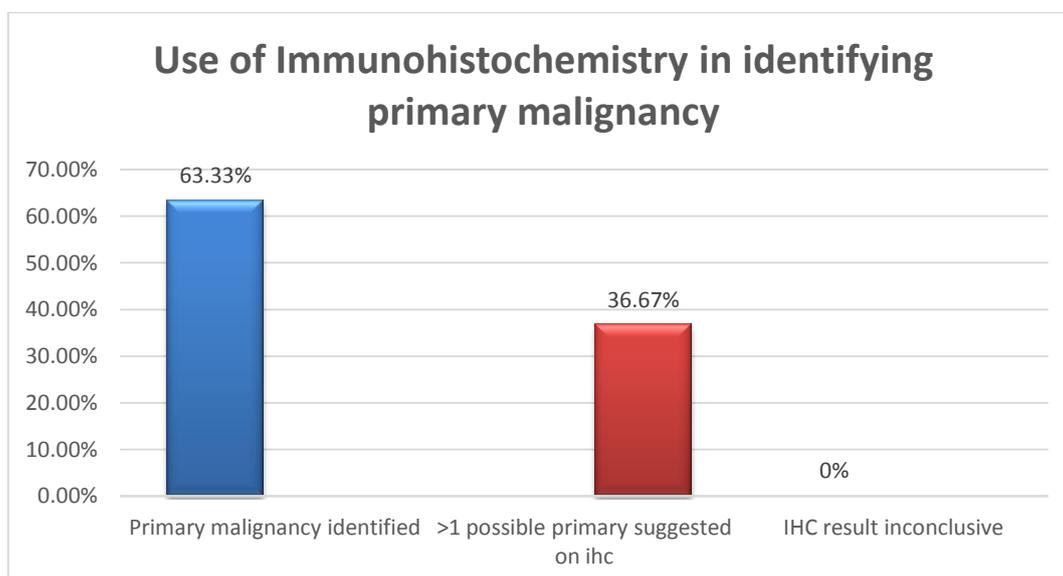


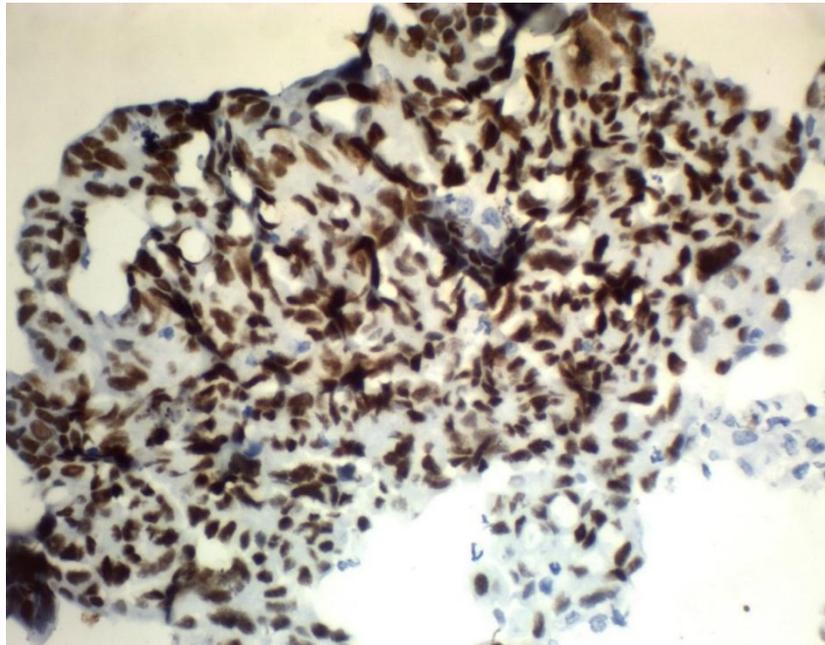
The most common primary malignancy in the female population was Serous Ovarian Carcinoma (10/23 cases) and in the male population, Adenocarcinoma lung (4/7 cases). The most common malignancy in the ascitic fluid sample was from Serous carcinoma ovary (8/16 cases). Among pleural fluid samples, 8/8 cases were from Adenocarcinoma Lung.

19/30 (63.33%) cases, it was possible to narrow down to a single primary malignancy. In 11/30 (36.67%) cases, immunohistochemistry could help in suggesting >1 possible primary malignancies. There were no cases (0%) where primary malignancy could not be identified. (Graph 5). In all 30 cases, the IHC diagnosis of primary malignancy correlated with the final histopathological diagnosis of the same.

Immunohistochemistry was done on the cell block of all the cases to find the primary malignancy. In

Graph 5





Picture 2: WT1 positive tumor cells from serous carcinoma ovary, 10X

Discussion

Cytologic examination has been used since a long time in the evaluation of the cellular components of body cavity fluids, including pleural, peritoneal, and pericardial effusions. Reactive mesothelial cells with atypical morphologic characteristics, however, sometimes are not easily distinguished from malignant cells by morphologic characteristics alone. The distinction between mesothelial cells and malignant cells in body cavity fluids remain a common challenging task. The purpose of this study was to analyse the effectiveness of cell block method over the conventional smear method. A total of 30 cases for which histopathological correlation or FNAC correlation from the primary tumor was available for comparison were included in this study.

In the present study, a total of 30 fluid samples were subjected to the conventional smear preparation and cell block techniques. Out of this 16 were ascitic fluids, 8 were pleural fluids, 2 were pericardial fluids and 4 cases were peritoneal washings. Peritoneal effusions (66.6%), including ascitic fluid (53.3%) and peritoneal washings (13.3%) outnumbered pleural effusions (26.7%) and pericardial effusions (6.7%) in this study. Age of the patients ranged from 33-89 years. Mean age was 59.57 years and standard deviation 16.164

years. The mean age was found to be similar to most of the previous related studies. As regards gender, there was a very strong female preponderance (76.7%) similar to the studies by Mohamed Fagere⁽⁶⁾ and Shivakumarswamy⁽⁷⁾. Malignant effusions were observed to be mostly macroscopically hemorrhagic (63.3%).

Cellularity was found to be better in most cases (46.7%) by the cell block method as it concentrates cellular material, increases cell yield and forms a cell button. Architecture of cells was also found to be better in the cell block method. Features like cell balls, glandular and papillary pattern which are indicative of adenocarcinoma were better appreciated in the cell blocks. There was an ascitic fluid sample with cells in glandular pattern & with numerous psammoma bodies which were more easily picked up on cell block. This was a strong clue to the primary tumor which was Serous Carcinoma Ovary. Nuclear & cytoplasmic features were also better appreciated in the cell blocks. This could be due to the resemblance to tissue H&E staining.

In the present study diagnostic yield for malignancy was increased by the cell block method. The present study identified additional 6.67% (2 cases) malignancies by cell block method when compared to conventional smear

method. Additional diagnostic yield of cell block was noted in various other studies. It was 6.33% in the study by Shubada et al⁽⁸⁾. In the study done by Bodele et al, additional 7% of malignant lesions were identified by cellblock method⁽⁹⁾. Khan et al in a study titled as usefulness of cellblock versus smears in malignant effusion cases reported that the recovery rate for malignant lesions by cellblock preparation is 20% greater than that obtained for specimen examined in smear only⁽¹⁰⁾. Richa Nathani et al reported 5% increase in the diagnosis of malignancies⁽¹¹⁾. According to the study by Shivakumarswamy, the increase in diagnostic yield by the cell block method was 15%⁽⁷⁾.

Table 1: Additional yield of malignancy in various studies by cell block

SL No.	Study	%
1	Shubada et al	6.33
2	Bodele et al	7
3	RichaNathani et al	5
4	Shivakumarswamy	15
5	Khan et al	20
6	Present study	6.67

By the conventional smear method, 24/30 (80%) cases were positive for malignancy. This is higher than the study by Bhanvadia et al where 18/34 (53%) of the malignant cases could be identified by CS alone⁽¹²⁾. Other studies by Chandler⁽¹³⁾ have shown 65% accuracy and by Ceelen⁽¹⁴⁾ proved 71% accuracy in the result by conventional smear method.

Table 2: Diagnostic accuracy of conventional smear method in various studies

SL No.	Study	%
1	Bhanvadia et al	53
2	Chandler	65
3	Ceelen	71
4	Present study	80

By the cell block method, 26/30 (86.7%) cases were positive for malignancy. Ceelen has shown a diagnostic efficiency of 89% by the cell block method⁽¹⁴⁾. The diagnostic accuracy reported in

various other studies are as follows; Thapar et al 86%⁽¹⁵⁾, Bhanvadia et al 97%⁽¹²⁾ and Zemansky AP 90%⁽¹⁶⁾.

Table 3: Diagnostic accuracy of cell block method in various studies

SL No.	Study	%
1	Ceelen	89
2	Thapar et al	86
3	Bhanvadia et al	97
4	Zemansky AP	90
5	Present study	86.7

By the cell block method, the number of cases that were suspicious for malignancy were 4/30 (13.3%) cases and by conventional smear analysis, 6/30 (20%) cases. But according to the study by Bhanvadia et al, it was 0% by cell block method and 11% by conventional smear analysis⁽¹²⁾.

Diagnostic efficiency of cell block and conventional smear together was found to be 96.7% which was more than that of conventional smear (80%) or cell block (86.7%) alone. Dekker A, Bupp PA in their study also reported similar findings⁽¹⁷⁾.

The sensitivity of cell block method was found to be 86.7%. This is in accordance with the study by Nityananda where the sensitivity was found to be 89.4%⁽¹⁸⁾. Other studies by Wojcik and Selvaggi have shown a sensitivity of 84%⁽¹⁹⁾. Leung and Bedard have reported a sensitivity of 86% by the cell block method⁽²⁰⁾.

Table 4: Sensitivity of cell block method in various studies

SL No.	Study	%
1	Nityananda	89.4
2	Wojcik&Selvaggi	84
3	Leung &Bedard	86
5	Present study	86.7

In this study, the sensitivity of conventional smear analysis was found to be 80%. Bhanvadia et al reported a sensitivity of 70% by conventional smear method⁽¹²⁾. Other studies by Oyafuso et al⁽²¹⁾ & GG Nair et al⁽²²⁾ have shown a much lower sensitivity of 44.5% and 32.3% respectively.

Table 5: Sensitivity of conventional smear method in various studies

SL No.	Study	%
1	Bhanvadia et al	70
2	Oyafuso	44.5
3	GG Nair	32.3
5	Present study	80

The positive predictive value of cell block method was found to 86.7% & that of conventional smear analysis 80%. Both these values were higher than that obtained by G.G Nair which was 33.56% for cell block method & 14.48% for conventional smear analysis⁽²²⁾.

The most common primary malignancy in the fluid samples was Serous Carcinoma Ovary (33.33%) in the present study; similar to the studies by Karki S et al⁽²³⁾, Bjorn et al⁽²⁴⁾ and Zachon et al⁽²⁵⁾. Other malignancies included Lung Adenocarcinoma (26.67%), Carcinoma Stomach (13.33%), Carcinoma Pancreas (10%), Mucinous Carcinoma Ovary (6.67%), Carcinoma Breast (3.33%), Seromucinous Carcinoma (3.33%) and Carcinoma Rectum (3.33%). The most common cause for malignant pleural effusion was Adenocarcinoma Lung and that of malignant ascites, Serous Ovarian Carcinoma. This is concordance with the studies by Ghosh et al⁽²⁶⁾ & Bhanvadia et al⁽¹²⁾.

Immunohistochemistry was done on the cell block of all 30 cases to find the primary malignancy. In 19/30 (63.33%) cases, it was possible to narrow down to a single primary malignancy. In 11/30 (36.67%) cases, immunohistochemistry could help in suggesting 2 or 3 possible primary malignancies. There were no cases (0%) where primary malignancy could not be identified. The best results for identifying the primary malignancy was for metastatic Serous Carcinoma Ovary and Adenocarcinoma Lung. Pomjanski N et al in his study also reported similar results in identifying primary malignancies⁽²⁷⁾.

Conclusion

Diagnostic efficiency of the cell block method is superior than that of conventional smear analysis.

When cell block & conventional smear analysis were combined together, the diagnostic efficiency is more than that of either method done alone. Immunohistochemistry performed on cell blocks can help in identifying the primary site of origin in carcinoma of unknown primary.

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