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Original Research Article

The diagnostic utility of cell block as an adjunct to routine cytology

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ABSTRACT

Introduction: Despite the diagnostic utility of cell block in FNAC and fluid cytology, known for better cellular yield and improved diagnostic accuracy, the technique remains to be underutilized for diagnosing neoplastic lesions.

Materials and Methods: In this paper, a hospital based prospective study of 195 samples was conducted on cytological samples with the aim (a) to evaluate the diagnostic utility of cell block as an adjunct to routine cytological evaluation of aspiration and effusion fluid specimens, (b) To correlate the findings of routine cytology, cell block and routine histopathological examination wherever possible and (c) to explore the possibility of using ancillary techniques such as immunohistochemistry on cell blocks.

Results and Discussion: The study revealed, most of the patients were between 51- 60 years with female preponderance. Among 195 samples, 79 (40.5%) were peritoneal fluids and 85 (43.5%) were lymph node. The diagnosis on conventional smear and cell block showed 71 (36.5%) cases and 68 (35%) cases were negative for malignancy respectively. 6 (3%) cases and 4 (2%) cases were suspicious for malignancy respectively. 118 (60.5%) cases and 123 (63%) cases were positive for malignancy respectively. Amongst the peritoneal effusion the most common primary site was ovary whereas majority of the primary site was unknown in lymph node FNAC. Peritoneal effusion showed 8 additional cases as positive for malignancy in CB preparation which were negative or suspicious for malignancy on CS. In FNAC, two additional positive case was found in CB preparation of lymph node. A Kappa value of 89.5 % for statistical correlation between Conventional smear and cell block preparation was calculated. The use of Cell block technique increases the detection of malignancy when used as an adjunct to conventional smears. Cell block technique is simple, inexpensive and reliable adjuvant to smears and it is recommended for routine cytological diagnosis and for application of immunomarkers and molecular studies.

1. Introduction

The cell block (CB) technique has been in use for more than a century. The first report of this technique was made by Bahrenburg in 1896.[1] Following this report, various Cell Block techniques have been developed over the years that vary in scope and the type of fixatives, processing, and embedding techniques used. Conventional smear preparation from centrifuged serous fluid and from FNAC of superficial or deep lesion is an increasingly common procedure in diagnosis of neoplastic as well as non-neoplastic lesions. Sometimes conventional smear does not yield sufficient information for precise diagnosis and the risk of false negative or intermediate diagnoses always exists. In order to overcome these problems, cell block technique has been resorted to make the best use of the available material.

Cell Block is known to increase cellular yield and improve diagnostic accuracy. It allows the identification of architectural patterns similar to those observed in

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histological sections, which, associated with morphological cellular details present in the other cytological preparations, enable a definitive diagnosis, with neoplasm classification similar or identical to histological classification. Besides, it also allows additional studies, such as histochemical staining and immunohistochemical analysis as well as molecular tests.[2]

Despite the above diagnostic utility of cell block in FNAC and fluid cytology, the technique remains to be underutilized. Hence, this study will be undertaken to determine the utility of cell block method as an adjunct to conventional smear preparation in the diagnosis of various lesions in our setup. Also, the possibility of using ancillary techniques such as immunohistochemistry on cell blocks and the use of cell block as a routine diagnostic tool will be studied.

2. Materials and Methods

A hospital based prospective study of 195 consecutive cases of all cytological specimens (FNAC and body fluids) fulfilling the inclusion criteria was included in the study. Cell block was prepared using AAF fixative comprising of 95% ethyl alcohol 34 ml, Glacial acetic acid 2 ml and formalin 4 ml.

2.1. Inclusion criteria

1. All cases of Peritoneal and pleural fluid.
2. All cases of palpable lymph node, breast and other superficial palpable masses suspected or diagnosed as malignancy.

2.2. Exclusion criteria

1. Swellings other than lymph node, breast and other superficial palpable masses suspected or diagnosed as malignancy.
2. All cases of thyroid and salivary gland swelling.
3. All other fluids except pleural and peritoneal.
4. Inadequate smears (acellular or bloody samples with poorly preserved cells).

FNAC is performed using 23 gauge needle attached to the 10 ml disposable syringe under aseptic condition. For routine cytological examination, FNA conventional smears are made, air dried for Giemsa stain or immediately alcohol fixed in 95% ethyl alcohol for Papanicolaou stain wherever needed. For cell block analysis, the material in the aspirating syringe is pushed in the test tube containing 5 ml isotonic saline and centrifuged at 2500 rpm for 10 minutes. The supernatant is poured off and cell sediment is mixed with thrice the volume of AAF fixative, and the mixture fluid is centrifuged for 10 minutes at 2000 rpm. Again, the supernatant is poured off and the cell button is re-suspended in AAF fixative and centrifuged for 10 minutes at 3000 rpm. The centrifuged tube is set aside and left undisturbed overnight.

For cytological smear of body fluids, specimens are centrifuged at 2500 rpm for 10 min and four conventional smears are made from the sediment in each case. Half of the smears are air dried for Giemsa staining, and the remaining are fixed by 95% ethanol for Pap staining. For cell block preparation, fluids are centrifuged at 2500 rpm for 10 min. The supernatant is poured off and cell sediment is mixed with thrice the volume of AAF fixative, and is centrifuged for 10 minutes at 2000 rpm. Again, the supernatant is poured off and the cell button is re-suspended in AAF fixative and centrifuged for 10 minutes at 3000 rpm. The centrifuged tube is set aside and left undisturbed overnight.

The cell button from both the body fluids and FNAC is then put in a filter paper, wrapped and is processed as routine biopsy specimen. The cell blocks are then embedded in paraffin and sectioned at 4 μm thickness and stained with hematoxylin and eosin (H&E) staining. Immunohistochemical staining is done wherever applicable.

2.3. Interpretation of conventional smears and cell block

Every conventional smear and cell block slide were analyzed for cellularity, background, cytoplasmic and nuclear details, tissue architecture. On the basis of following criteria, the conventional smear and cell block slides were diagnosed:

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

2.4. Statistical analysis

Frequency tables of demographic and clinicopathologic parameters were established. A comparison will be done between cytological smear, cell block and histology wherever possible. Kappa value for statistical correlation between Conventional smear and cell block preparation was calculated.

3. Results

As represented in Figure 1, both Males and Female were predominantly from the age group of 51-60 years with 30.3% and 27.1% respectively. Out of the 195 samples, 96 (49.2%) samples were male and 99 (50.8%) samples female.

As represented in Figure 2, the samples were divided into peritoneal fluids, pleural fluid, lymph node and other FNAC sites. Among 195 samples, 79 (40.5%) were peritoneal fluid, 14 (7.2%) were pleural fluid, 85 (43.5%) were lymph node and other FNA sites were 17 (8.7%). Out of which were 4 (2%) from breast, 4 (2%) from scalp, 5 (2.7%) from skin (2 from cheek and 3 abdominal wall), 3 (1.5%) from soft

tissue (1 each from arm, foot and sternum) and 1 (0.5%) from cystic swelling in mandible bone.

The diagnosis on conventional smear (Table 1) showed 71 (36.5%) cases were negative for malignancy. 6 (3%) cases were suspicious for malignancy and 118 (60.5%) cases were positive for malignancy.

The diagnosis of the cell block (Table 2) showed 68 (35%) cases were negative for malignancy, 4 (2%) cases were suspicious for malignancy and 123 (63%) cases were positive for malignancy.

By conventional smear, the diagnosis of Negative for malignancy was made in 71 cases (36.5%), positive for malignancy was made in 118 cases (60.5%) and suspicious of malignancy was made in 6 cases (3%). In cell block, Negative for malignancy was made in 68 cases (35%) and positive for malignancy was made in 123 cases (63%) and suspicious for malignancy was 4 (2%). (Table 3)

Of the 79 cases of peritoneal effusion the most common primary site was ovary in 35 cases and stomach in 18 cases. Of the 14 cases of pleural effusion the most common primary site was lung and breast with 4 cases each and followed by ovary and oesophagus with 2 cases each. Of the 85 cases of lymph node FNAC the primary site was unknown in 17 cases while 13 cases of primary in larynx, 11 cases in oesophagus and 10 cases in oral cavity. Among the 17 cases of FNA from other sites, the most common primary was breast with 6 cases followed by oral cavity and lung with 2 cases each. (Table 4)

In peritoneal effusion maximum positive for malignancy cases were from ovarian primary followed by GIT on both CS and CB. Of the 7 cases of pleural effusion positive for malignancy the most common primary site was lung with 4 cases followed by breast and ovary with 2 cases each on both CS and CB. In lymph node FNAC positive for malignancy in both CS and CB were maximum in cases with unknown primary with 14 cases each followed by larynx and oesophagus, while in other FNAC sites it was oral cavity and lung with 2 cases each followed by breast. (Table 4)

Eleven cases of peritoneal effusion were subjected to immunohistochemistry on CB preparation. Out of which, 2 cases of endometrial carcinoma and one case of breast carcinoma were stained for ER and PR for confirmation while one case of colon cancer was stained for CD 20 and CDX-2 and five cases of ovarian cancer were stained for CK, WNT and calretinin for confirmation as positive for malignancy.

Two cases of CB preparations of pleural effusion were also subjected to ER, PR and Her2neu IHC which confirmed the cases as positive for metastasis from primary breast which also correlated with the IHC status of primary breast cancer.

Four cases of MUO on lymph node CB were subjected to p16 immunostaining to rule out associated EBV infection. The four cases of suspicious for malignancy on cell block could not be subjected to IHC for confirmation due to insufficient material for IHC.

Peritoneal effusion showed 8 additional cases as positive for malignancy in CB preparation which were negative or suspicious for malignancy on CS. Out of these 8 cases, the primary sites were from ovary (5 cases), breast (1 case), colon (1 case) and peritoneum (1 case). Of these, 3 cases were suspicious of malignancy on conventional smear with 1 case each with ovary, colon and peritoneum.

There were 3 cases of peritoneal effusion diagnosed as positive for malignancy on CS but on CB, 1 case each with breast and ovarian primary was found to be negative while 1 case was suspicious for malignancy with ovarian primary. (Table 5)

There was no discrepancy between CS and CB among the 7 cases of pleural effusion which were positive for malignancy.

In FNAC, two additional positive case was found in CB preparation of lymph node FNAC, with 1 case each with larynx and breast as primary. One case positive for malignancy on CS of lymph node FNAC, was suspicious for malignancy on CB, with oral cavity as primary. One case of breast FNA was found to be negative on CB which was positive for malignancy on CS. (Table 5).

By using the above data, we calculated a Kappa value of 89.5 % for statistical correlation between Conventional smear and cell block preparation.

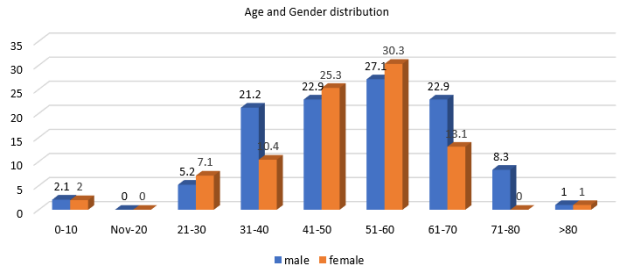

Figure 1: Age and gender distribution in the sample

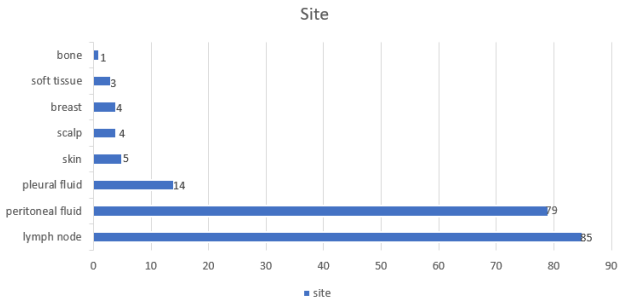

Figure 2: Chart of different samples sites received

The diagnosis on conventional smear (Table 1) showed 71 (36.5%) cases were negative for malignancy. 6 (3%)

Table 1: Samples and diagnosis on Conventional smear

Sample	Negative for malignancy	Suspicious for malignancy	Positive for malignancy
Peritoneal fluid	46	5	28
Pleural fluid	7	0	7
Lymph node	12	1	72
Others	6	0	11
Total	71 (36.5%)	6 (3%)	118 (60.5%)

Table 2: Samples and Diagnosis on Cell block

Sample	Negative for malignancy	Suspicious for malignancy	Positive for malignancy
Peritoneal fluid	44	2	33
Pleural fluid	7	0	7
Lymph node	10	2	73
Others	7	0	10
Total	68 (35%)	4 (2%)	123 (63%)

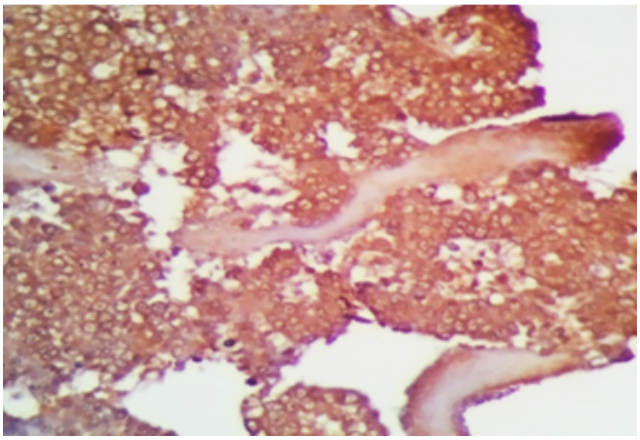

Figure 3: CB Peritoneal fluid IHC: Cytokeratin (40x).

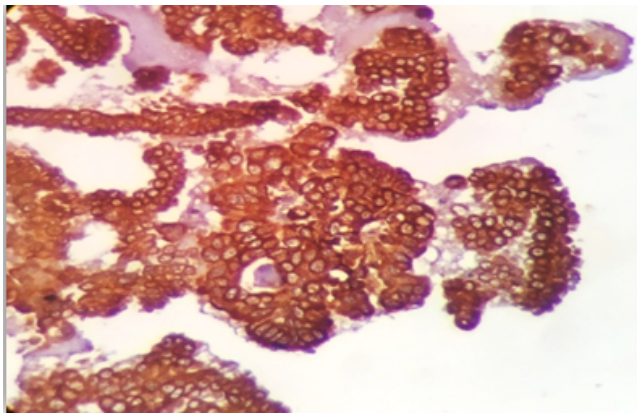

Figure 4: CB Peritoneal fluid IHC: Calretinin (40x)

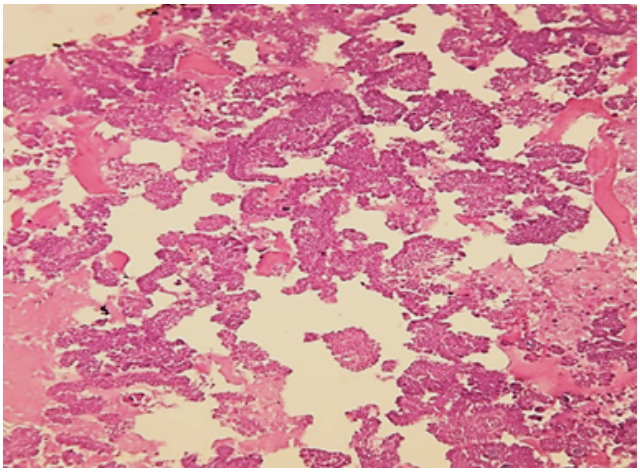

Figure 5: CB Pleural fluid H&E (40x)

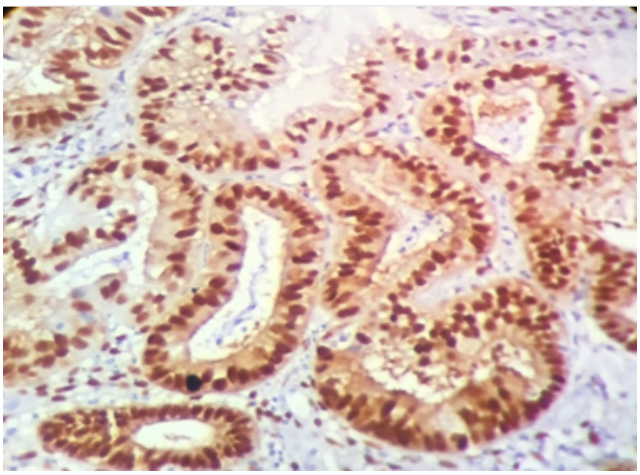

Figure 6: CB Pleural fluid IHC: ER positive (40x)

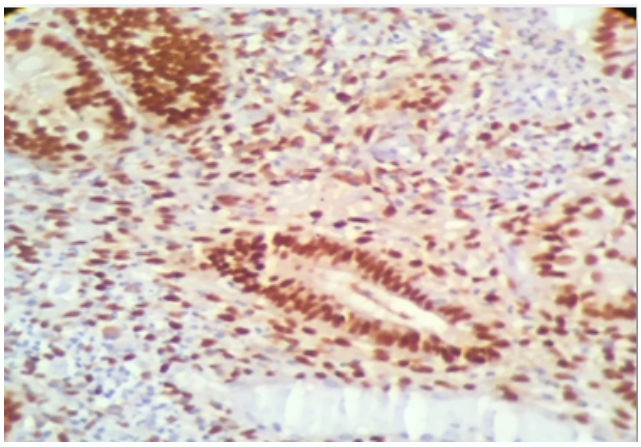

Figure 7: CB Pleural fluid IHC: PR positive (40x)

cases were suspicious for malignancy and 118 (60.5%) cases were positive for malignancy.

4. Discussion

In the present study, most cases were in the age group of 51-60 years (29%) followed by 41-50 years (24%) and 61-70 years (18%). Both effusion and FNAC cases showed higher incidence in the same age groups. These findings were similar to study done by Bansode et al[3] and Padmavathi et al[4] who have reported higher number of cases in the age group 41–60 years as 54% and 69.3%, respectively. This was in contrasts with findings by Matreja et al[5] with most cases 26.1% in the age group 21–30 years. No patients in the age group of 11–20 years was found. Mean age of patients was 50.68 years.

No significant difference was found in gender-wise distribution of cases with Male: Female ratio of 1: 0.98. Similar findings on effusions study were observed by Matreja et al, Dey S et al and Khan et al.[5–7]

Greater frequency of effusion in females (34.3% vs 13.2%) and conversely greater number of fine needle aspirates from males (36% vs 16%) was recorded.

Majority of the sample received was from lymph node (43.7%) followed by peritoneal fluid (40.4%), superficial sites (8.8%) and then pleural fluid (7.1%).

Overall, it was found that the greater numbers of positive for malignancy both on CS (60.5%) and CB (63%) which may be attributable to the fact that our pathology department is based in a tertiary cancer hospital and majority of our patients are suspected or confirmed cases of malignancy.

In the present study, it was found that cases positive for malignancy was greater on FNAC with relatively fewer effusions positive for malignancy. In other words, majority of the effusion were negative for malignancy which is comparable to other studies by Bansode et al.[3] and Padmavathi et al.[4] and Thapar M et al.[8]

On CS, out of the total 195 cases, positive for malignancy was 17.9% on effusion and 42.6% on FNAC, negative for malignancy was 27.2% on effusion and 9.3% on FNAC while suspicious for malignancy was 2.5% on effusion and 0.5% on FNAC. Similarly, on CB, out of the total 195 cases, positive for malignancy was 20.5% on effusion and 42.5% on FNAC, negative for malignancy was 26.2% on effusion and 8.8% on FNAC while suspicious for malignancy was 1 % both on effusion and FNAC. The greater number of positive cases in FNAC could be attributable to the larger sample size and early presentation of lymph node metastases while malignant effusions are more commonly present at advanced stage of disease.

Similar to our study, Matreja et al[5] and Nair et al[9] have reported most common primary neoplasm in cases of malignant peritoneal effusions were carcinoma of the ovary followed by adenocarcinoma of gastrointestinal tract (GIT), whereas the most common primary malignancy causing pleural effusion was carcinoma of the lung, followed by carcinoma of the breast. Sears et al[10] have reported most common primary neoplasm causing peritoneal effusions were carcinoma of the ovary (32%), carcinoma of the breast (15%), and lymphoreticular malignancies (7%), whereas common primary malignancies in cases of pleural effusions were carcinoma of the breast (24%), followed by carcinoma of the lung (19%), and malignancies of lymphoreticular system (16%) in their study. Shivakumarswamy et al[11] have reported that common primary lesions in their study on pleural effusion were lung and then in GIT.

Amongst the Fine needle aspirates maximum cases were from lymph nodes (43.5%) followed by 4 (2%) from breast, 4 (2%) from scalp, 5 (2.7%) from skin (2 from cheek and 3 abdominal wall), 3 (1.5%) from soft tissue (1 each from arm, foot and sternum) and 1 (0.5%) from mandible bone. Sharma R et al[12] also reported a greater frequency of FNA from lymph node followed by breast. Out of the 85 cases of lymph node FNAC, majority of the positive FNAC cases were performed on cervical neck nodes and metastatic squamous cell carcinoma comprised majority of the diagnosis. In FNAC of lymph node, 17 cases had unknown primary site, out of which 14 cases were positive for malignancy. In FNAC of lymph node with known primary site, majority cases were from larynx, followed by oesophagus, oral cavity and oropharynx.

Eleven cases of peritoneal effusion CB preparation were subjected to immunohistochemistry using cytokeratin, calretinin, WT-1, TTF-1, CD20, CDX2, Oestrogen and Progesterone receptor to confirm the adenocarcinoma cells. Two cases of endometrial carcinoma and one case of breast carcinoma were positive for ER and PR while one case of colon cancer was positive for CD20 and CDX-2 and five cases of serous ovarian cancer were positive for WT-1.

Two cases of CB preparations of pleural effusion were subjected to ER, PR and Her2neu IHC which confirmed

Table 3: Comparison of Diagnosis of samples

Diagnosis	Conventional smear		Cell block	
Negative for malignancy	71	36.5%	68	35%
Suspicious for malignancy	6	3%	4	2%
Positive for malignancy	118	60.5%	123	63%

Table 4: Primary sites of positive for malignancy cases

Peritoneal fluid				Pleural fluid			
Primary	No. of cases	No of positive cases		Primary	No. of cases	No of positive cases	
		CS	CB			CS	CB
Ovary	35	15+2	15+5	Lungs	4	3	3
Stomach	18	2+1	2	Breast	4	2	2
Breast	2	0	1	Oesophagus	2	0	0
Gall Bladder	6	2	2	Stomach	1	0	0
Colon	3	1	1+1	Ovary	2	2	2
Rectum	5	1	1	Lymphoma	1	0	0
Endometrium	5	3	3	**Total**	**14**	**7**	**7**
Liver	2	1	1				
Pancreas	2	0	0				
Peritoneum	1	0	1				
Total	**79**	**28**	**33**				
	Lymph node				**FNA Others**		
Primary	No of cases	No of positive cases		**Primary**	**No of cases**	**No of positive cases**	
		CS	CB			CS	CB
MUO	17	14	14	Breast	6	1+1	1
Larynx	13	12	12+1	Oral cavity	2	2	2
Oesophagus	11	11	11	Lung	2	2	2
Oral cavity	10	9+1	9	Arm ES	1	1	1
Oropharynx	9	8	8	Bone (mandible)	1	0	0
Orbit	2	1	1	Thyroid	1	1	1
Pharynx	1	1	1	Larynx	1	1	1
Gall bladder	1	1	1	GB	1	1	1
Nasopharynx	1	1	1	Lymphoma	1	0	0
Pancreas	1	1	1	Skin (abd wall)	1	1	1
Stomach	2	2	2	**Total**	**17**	**11**	**10**
Colon	2	1	1				
Rectum	2	2	2				
Ovary	2	2	2				
Breast	1	**0**	1				
Thyroid	2	1	1				
Bladder	1	1	1				
Skin	3	2	2				
Lymphoma	3	0	0				
Testis	1	1	1				
Total	**85**	**72**	**73**				

Table 5: Discordant results between conventional smear and cell block.

Sample	Smear positive with negative cell block for malignancy	Cell block positive with negative smear for malignancy
Effusion	3	8
FNAC	2	2
Total	5	10

the cases as positive for metastasis from primary breast cancer, which also correlated with the IHC status of primary breast cancer. Knowledge on the hormonal and Her2neu status in breast cancer was valuable in prognostication and predicting patient's response to therapy. Briffod M et al[13] observed an excellent correlation between cell-block results for primary tumors and node metastases and also concluded that cell blocks prepared from FNA specimens of breast carcinomas and their node metastases were useful when planning neoadjuvant treatment.

A basic panel of immunohistochemical markers (CK, CD45, synaptophysin, chromogranin, Vimentin and S-100) was applied on 7 cell blocks of FNAC from lymph node where primary was unknown. Four cases of MUO on lymph node CB were subjected to p16 immunostaining to rule out associated EBV infection.

By the combined use of smears and cell blocks, positivity for malignancy increased by 15 cases. In the present study, 8 additional cases positive for malignancy on CB preparation of peritoneal effusion were found, out of which 5 cases of ovarian malignancy and one case each of breast, colon and peritoneal carcinomatosis was reported. Of these, 3 cases of ovarian malignancy were suspicious on conventional smear while the rest were negative for malignancy on CS. Conventional smear showed 3 cases positive for malignancy, which include one positive case of breast primary and one positive case of ovarian primary which were negative on cell block and 1 case on CB as suspicious for malignancy with ovarian primary. There was no discordance in the findings of 7 cases of pleural fluid on cell block and conventional smear preparation.

Bhansode et al[3] and Bodele et al[14]in their study on effusion also identified additional 9 cases and 10 cases of malignant lesions respectively by cell block method when compared to conventional smear. A study by Dey S et al[6] concluded that CB techniques definitively increased detection of malignancy in body cavity effusion when used as an adjunct to conventional smears.

In FNAC of lymph node, two additional positive for malignancy case was found in CB preparation with primary from larynx and breast which were negative on CS. Lymph node FNA of one case positive for malignancy on CS showed to be suspicious for malignancy on CB, which had oral cavity as primary. One case of breast FNA positive for malignancy on CS was found to be negative on CB. This could be attributable to lower cellular yield and technical issues. We did not find any significant difference between CS and CB among FNA cases positive for malignancy. However, cases with cystic lesions provided better yield in cell block preparations as compared to solid and fibrous lesion on FNA.[15–21]

In our experience, we found architectural features and morphology were better appreciated on CB and application of immunohistochemistry was possible on Cell block preparations which provided additional advantage over conventional smear preparations. We also found that effusions and metastatic or primary cystic lesions on FNAC yielded better material for cell block in comparison to conventional smear preparations, whereas no significant advantage was observed in the cell block and conventional smear preparation of FNAC on solid superficial lesions.[22–24]

5. Conclusion

In the present study, the use of Cell block technique increases the detection of malignancy when used as an adjunct to conventional smears, especially when applied in effusion and cystic nodal and superficial lesions. It was observed that morphological and architectural features are better identified in Cell Block technique thus improving sensitivity. The application of IHC in CB in doubtful cases, help in confirming the diagnosis of primary site of the malignancy, thus improving specificity. The role of IHC as a prognostic and predictive marker can also be exploited in cell block preparations in confirmed cases of malignancy. Cell block technique is simple, inexpensive and reliable adjuvant to smears and it is recommended for routine cytologic diagnosis and for application of immunomarkers.

6. Data and Material Availability

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7. Authors' Contributions

All the authors including corresponding author have contributed equally towards data collection, data analysis, preparation of the draft and approval of the final manuscript of this article. All the authors to confirm that they have met the ICMJE's requirements for authorship.

8. Source of Funding

None to disclose.

9. Conflict of Interest

The authors declare no competing or conflicts of interest

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