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Original Research Article

Role of IHC on Cell Blocks from FNAC of Neoplastic Breast Lesions

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Abstract:

Introduction: Breast cancer is one of the emerging cancers of women. Most of the women in India present with breast lumps. FNAC is usually done in palpable breast lumps; it is a rapid, cheap, and less invasive procedure. Cell block can be prepared from aspirated material, fixed and processed as histopathology specimens. Cell blocks are also called micro biopsies provides large battery of sections for IHC.

Aims and Objectives: The present study was undertaken to evaluate the efficacy of cell block in comparison of FNAC in various breast neoplasm.

Materials and Methods: A prospective study over a period of 18 months from July 2021 to January 2023. 168 patients were referred to cytopathology unit and FNAC was done, remaining material in the aspirating syringe was kept for cell block. The findings of cell blocks were studied in detail to arrive at diagnosis and correlated with FNAC findings. Cell blocks of malignant breast lumps were processed for IHC status.

Results: Out of total 168 cases, most of cases were benign lesions (76.78%), with fibroadenoma being most common. Among malignant tumours, invasive carcinoma of no other special type was the most common type. Cell blocks are more accurate (96%) compared to FNAC (88%) in diagnosis of both benign and malignant lesions.

Conclusion: FNAC smears and cell block combined the diagnostic accuracy can be around 90%. The principal advantage of cell block is that of large battery stains can be easily done. Cell blocks are useful assessor hormone expression markers ER, PR and HER 2 neu to know the prognosis of tumour for target therapy.

Keywords: Cell block, FNAC, Breast lump.

Introduction

Breast cancer is one of the emerging cancers of women. Most of the women in India present with breast lumps. Periodical check-up helps in early detection of breast cancer and decrease the mortality and morbidity.[1,2] Triple assessment which includes clinical evaluation, mammography/USG and FNAC helps medical professional in the provisional diagnosis. FNAC is usually done in palpable breast lumps, as it is a rapid, cheap, and less invasive procedure.[3] Core needle biopsy provides more accurate diagnosis but it needs admission of patients, anaesthesia, skilful staff and more time.

Cell block can be prepared from aspirated material, fixed and processed as histopathology specimens.[4] So cell blocks are also called micro biopsies alternative need for core needle biopsies providing battery of sections for IHC.[2] Nuclear and cytoplasmic markers like ER,PR,HER 2 neu, ki 67 can be done on cytological smears.[5] Mini-blocks can be preserved for ancillary studies FISH/CISH and PCR. Combination of FNAC, cell blocks IHC helps in more accurate diagnosis and reduces false negative cases. Misdiagnosis of breast cancer is more physical and psychological burden for patient.

The present study was undertaken to evaluate the efficacy of cell block in comparison of FNAC in various breast neoplasms.

Materials and Methods

The present study is a prospective study done over a period of 18 months from July 2021 to January 2023 in 168 patients referred to cytopathology unit who was complaining of breast lump in our hospital (Government Medical College, Srikakulam). Informed consent was obtained from all patients in local language. Clinical history was taken and local examination was done. FNAC was performed following standard procedure using 5 to 10ml syringe with 22 to 24G needle.[6] Smears were obtained and stained by Haematoxylin and Eosin (H&E) stain. Remaining material in the aspirating syringe was kept for cell block 9 parts of ethyl alcohol and 1 part of 10% formalin for 12 hrs [7].

The cell button was removed and wrapped in a Whatman filter and placed in cassette.

This sample was processed in automated tissue processor. About 3 to 5 microns thick paraffin embedded tissue were cut and stained with H and E, studied under microscope. The findings of cell blocks were studied in detail to arrive at diagnosis and correlate with FNAC findings. Suspicious of malignancy or positive for malignancy in cell blocks of breast lump were sent for IHC status. In our study ER, PR, HER2 neu IHC studies were included helpful for prognosis and treatment.

The sections were deparaffinized in xylene first and hydrated in gradual manner through graded alcohol to water. Secondly, the sections were immersed in H2O2 and heated in a microwave oven, washed with phosphate-buffered saline (PBS; pH 7.4), and immersed in citrate buffer solution (pH 6.0). Thirdly, the sections were blocked with nonimmune serum, stained with the primary antibody, and then with the secondary rabbit anti-mouse (HRP) IgG antibody (DAKO).

The sections were incubated with SP (streptavidin-peroxidase) and then freshly prepared DAB solution for color development. Lastly, the sections were counterstained with Haematoxylin, cleared in water, mounted with neutral balsam, and observed under microscopy (MLX: Magnus).

Interpretation of the results: The results of pathological and immuno histochemical examinations were evaluated by 2 senior pathologists, who were not aware of the lymph nodes and the patients' diagnosis before the examination [8].

Inclusion Criteria:

Patients presenting with breast lump irrespective of sex, caste, religion, socio-economic status and severity of illness.

Exclusion Criteria:

- Patient not willing for FNAC
- Patients on adjuvant or neoadjuvant chemotherapy
- Patients who are in radiotherapy or post radiotherapy.

Results

In our study out of 168 cases, 164 cases are breast neoplasm, 4 cases were inadequate for opinion. (Table 1)

Table 1: Age wise distribution (n=164)

Age	No of cases	Percentage
15 – 25	13	7.73
25 – 35	31	18.45
35 – 45	48	26.78
45 – 55	42	25
55 – 65	21	12.5
65 -75	9	5.35
Total	164	100%

Total 164 cases are categorised into benign, borderline and malignant lesions in FNAC, cell block separately and correlated with histopathology taking as gold standard. (Table 2,3)

Table 2: Categorization of breast lesion on histopathology

Category	No of cases	Percentage
Benign	129	78.65
Borderline	3	1.82
Malignant	32	19.51

Table 3: Correlation of FNAC and cell block with histopathology findings in breast

Category	Diagnosis	FNAC	Cell blocks	Histopathology
Benign lesions	Fibroadenoma	47	51	52
	Fibrocystic disease	26	28	29
	Phyllodes	1	1	1
	Galactocele	3	3	3
	Gynaecomastia	12	12	12
	Fibroadenosis	20	23	25
	Benign epithelial hyperplasia	4	5	7
Borderline lesions	Phyllodes	0	1	1
	Atypical ductal hyperplasia	2	2	2
Malignant lesions	Invasive carcinoma NOS	28	30	31
	Mucinous carcinoma	1	1	1

With the help of statistician we calculated sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV), diagnostic accuracy. (Table 4)

Table 4: Sensitivity and Specificity for FNAC and cell block diagnostic accuracy parameters:

	FNAC (%)	Cell Block (%)
Sensitivity	96	96
Specificity	22	66
Accuracy	88	96
PPV	91	98
NPV	40	44

With the help of cell block sections we did immunohistochemistry for 31 cases of malignant cases diagnosed on cell block. (Table 5)

Table 5: ER, PR, HER2 neu expressions in cell block preparation of breast carcinoma (31 cases):

IHC markers	ER – PR Positive	ER – PR Negative	ER – positive PR - Negative	HER-2 neu positive in 13 ER, PR negative cases	HER-2 neu Negative in 13 ER,PR negative cases
Number of malignancy	11 cases	13 cases	07 cases	07 cases	06 cases
Prevalence (%)	35.4%	41.9%	22.5%	53.8%	38.4%

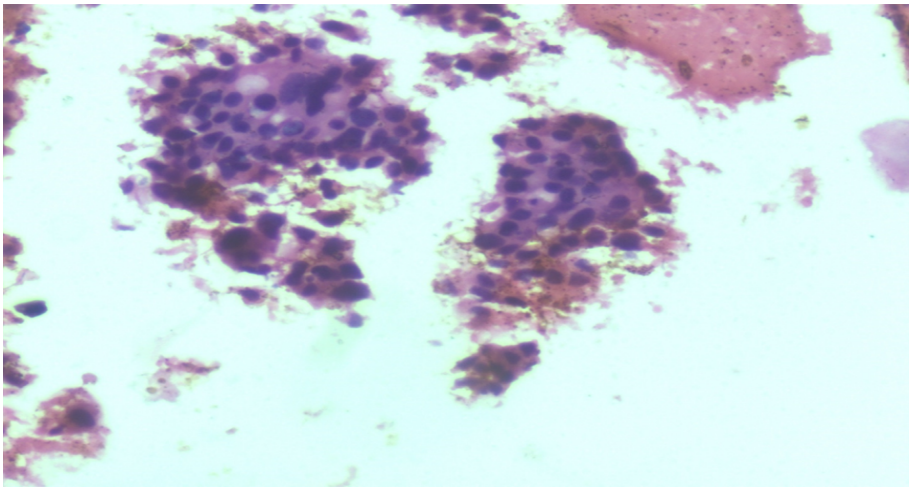

Figure 1: Low power view of Ductal carcinoma of breast showing pleomorphic ductal epithelial cells.

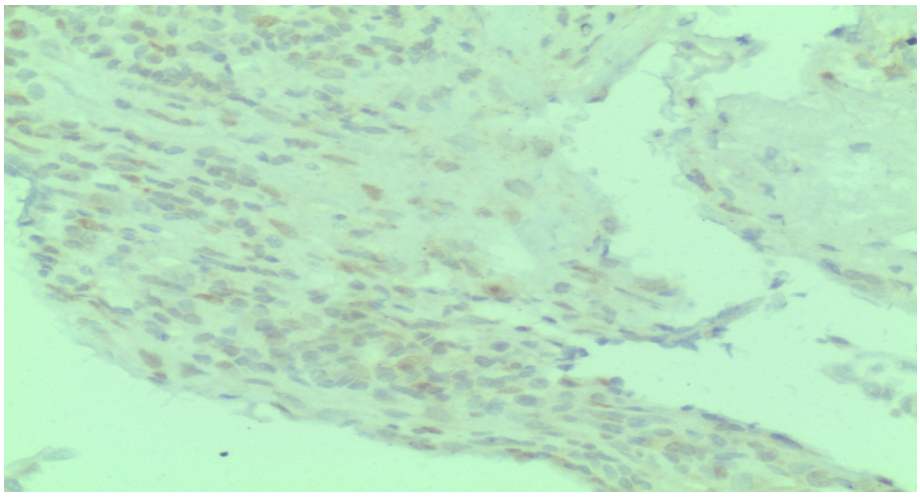

Figure 2: Low power view of Ductal carcinoma of breast showing estrogen receptor staining with strong nuclear +ve proportion score 4, intensive score 3

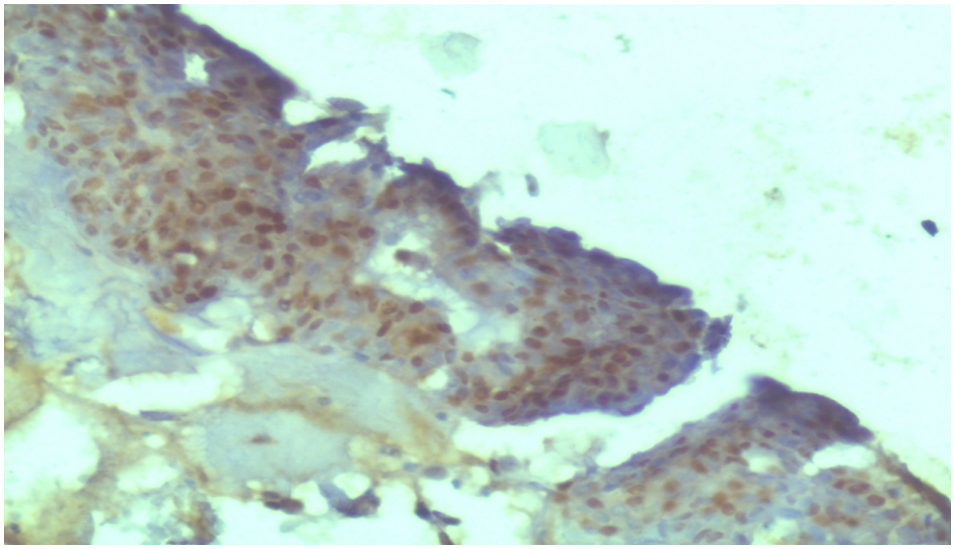

Figure 3: Low power view of Ductal carcinoma of breast showing PR showing nuclear +ve, p score 4; I score 2

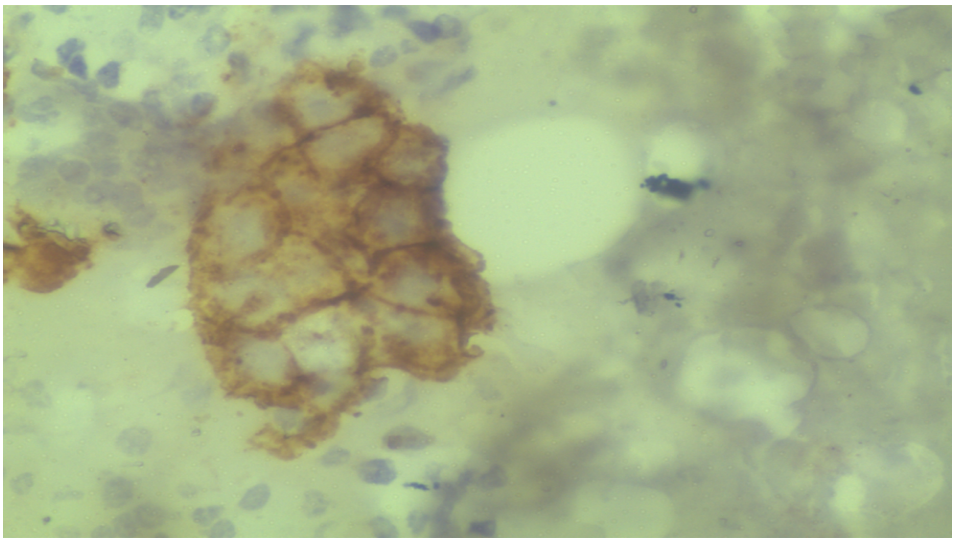

Figure 4: Ductal carcinoma with Her2 neu strong nuclear membrane (+ve)

IHC in breast carcinoma provides both therapeutic and prognostic information not achievable alone. ER, PR positive, HER2neu positive with statistically equivalent ER/PR positive HER2 neu negative subtype both types have better prognostic and therapeutic convection. ER/PR negative and HER2neu negative have worse prognosis.

Discussion

In our study out of 168 cases, 164 cases are breast neoplasms, 4 cases were inadequate for opinion. Among the neoplastic lesions, maximum cases were benign breast lesions (129 cases), followed by borderline lesions (3 cases) and malignant lesions (32cases) which was seen in similar studies by kawatra et al[1], patel et al[3], yalavarthi et al.[9] In our study most common age group of presentation was 35 to 55 years. Most common quadrant involved by both benign and malignant breast lesions in upper outer quadrant seen in 74 cases. Least common is lower inner quadrant.

Benign lesions were most common and borderline lesions were least common. Fibroadenoma is the most common breast lesion followed by fibrocystic disease, fibroadenosis. Invasive carcinoma – no special types was the most common malignancy. Other malignancy is papillary cystic carcinoma and mucinous carcinoma.

In our study out of 164 cases cell block was performed in all the cases. FNAC showed correct diagnosis in 144 cases. Cell block showed correct diagnosis for 157 cases. Cell block yielded good material and was helpful in identifying borderline phylloides, malignant lesions avoiding the need of biopsy by giving architectural information. (Figure 1) Air drying artifacts, poor spreading and thick tissue fragments are the pitfalls in FNAC.

The diagnosis of fibroadenoma was made in case of fibrocystic disease in cell block possibly due to overlapping features. Combined cell block and FNAC given more accurate results for diagnosis of breast lumps.

FNAC smears show some features of malignancy even in benign lesions like high cellularity, less cohesion, nuclear anisocytosis. Cell block solved this problem in cellular fibroadenoma [2]. Some cases which showed borderline and benign in FNAC were proved as malignant in cell block and histopathology. Cell block reveals the architectural pattern of cells, individual cell morphology clearly without any artifact.

Immunohistochemistry ER, PR and HER2 neu was done in cell block for 31 cases out of which ER, PR negative cases were more (41.9%) which correlated with Tripathy et al[10], Hanley et al[11] and William et al[12]. (Figure 2,3,4) Cell block provides ribbon of sections which can be used to access hormone expression markers ER, PR and HER 2neu to prognosticate the tumor and targeted therapy. Cell blocks can be shared for research and second opinion without the risk of losing the original diagnostic material. In our study we got significant difference in specificity and diagnostic accuracy for cell block than FNAC as it reveals the correct diagnosis comparable with histopathology and avoids the trucut biopsy in breast lumps.

The sensitivity, specificity, diagnostic accuracy values in our study are comparable with sakshikawara et al[1]. In contrast to our study, study done by ashwinikumar et al[13], the accuracy of FNAC is bit more than cell block.

The diagnostic accuracy in Raafat et al[2] and patel et al[3] showed that combination of FNAC and cell block yields good results in diagnosis of breast lumps. (Table 6)

Table 6: Comparison with other studies of diagnostic Accuracy:

Studies	Total no of cases	FNAC	Cell block
Present study	168	88	96
Raafot et al	310	88	90
Sakshi kowari et al	130	69.2	88.8
Ashwini kumar et al	270	81.85	79.25
Patel et al	33	80	94

Conclusion

In our study, aspirated material in FNA processed for cytology and also for cell block combined, the diagnostic accuracy can be around 90%. The principal advantage of immunohistochemistry cell block is that of large battery stains can be easily done useful for ancillary studies. Immunocytochemistry and IHC helpful in providing accurate diagnosis. FISH/CISH and PCR are helpful in confirmation of diagnosis.

In breast lesions complementary cell block preparations helps categorise suspicious lesions especially when there is low grade carcinoma and pleomorphism is not very obvious.

Cell blocks are useful assessor hormone expression markers ER, PRand HER 2 neu to know the prognosis of tumor for target therapy.

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