

Comparative Evaluation of Immunohistochemical Expression of Estrogen Receptor, Progesterone Receptor and HER2 in Fine Needle Aspiration Cell Blocks and Surgical Biopsies in Primary Breast Carcinoma

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ABSTRACT

Introduction: Breast carcinoma is the leading cause of cancer mortality among females. Estrogen and Progesterone receptors (ER, PR) and HER2 have been used for theranostics in breast cancer. Fine needle aspiration cytology (FNAC) can yield highly cellular material for cytological diagnosis. Predictive biomarker assessment can be done on cytologic specimens to know the patient's eligibility for endocrine therapy and anti-HER2- targeted therapy. Cell blocks further can increase the diagnostic accuracy through morphology and the use of immunohistochemistry (IHC). The present study was undertaken to evaluate immunohistochemical expression of estrogen receptor, progesterone receptor and HER2 in fine needle aspiration cell blocks and surgical biopsies in primary breast carcinoma cases.

Material and methods: IHC for ER, PR and HER2 was assessed on 50 pre chemotherapy breast carcinoma cell blocks (fixed in 10% formalin) and subsequent tissue sections. The scoring for ER/ PR was done according to ASCO/CAP guidelines. Strong circumferential membrane staining in greater than 10% of tumor cells was considered positive for HER2.

Results: Immunostaining assessment on cell block and their corresponding tumor tissues showed a good concordance: ER (92%), PR (92%) and HER2 (93.75%). Taking histology as the final outcome, the sensitivity of ER, PR and HER2 on cell block was 92.30%, 86.36% and 91.67%, respectively, while specificity was 92.85%, 96.43% and 94.44%, respectively.

Conclusion: IHC on cell blocks from breast carcinoma cases is useful especially when planning neoadjuvant chemotherapy. The pre analytic and analytic variables should be validated to optimize the diagnostic utility of cell blocks.

Keywords: Breast, Cytology, Immunohistochemistry, Estrogen, Progesterone, HER2.

morphology and the use of ancillary techniques such as immunohistochemistry. The upcoming role of IHC is in the field of "Theranostics" for oncology patients through hormone receptor testing for breast cancer and HER2 analysis. The present study was conducted to evaluate the assessment of the different immunostains on cell blocks of the primary breast carcinoma in comparison with surgical biopsy specimens using a panel of three IHC markers as ER, PR and HER2.

MATERIAL AND METHODS

The present prospective study was done over a period of 2 years from the year 2015-2017, in the Department of Pathology of our institute which is a tertiary care centre of Amritsar, Punjab, India. The study included 50 females diagnosed as cases of primary breast carcinoma on FNAC. Those females in whom FNAC was done but subsequent histopathological examination was not available, mesenchymal breast tumors, metastatic breast carcinomas, patients with recurrent malignancy, past or current chemo-therapeutic patients were excluded from the study. Informed consent was taken from all the patients prior to enrolment in the study. The procedures followed were in accordance with the Ethical Standards of the Institutional Research Committee. FNAC was sampled from palpable lesions by a standard technique under aseptic conditions. Wherever necessary, a second pass was taken. Smears were prepared, air dried for May-Grunwald-Giemsa (MGG) stain and immediately alcohol fixed for haematoxylin and eosin (H&E) stain for routine diagnosis. For cell block

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INTRODUCTION

Breast carcinoma is the leading cause of cancer mortality amongst females. To improve breast cancer outcomes, pathologic evaluation should not be bypassed even when clinical/ imaging studies are highly suggestive of breast cancer. Breast FNAC is safe, simple, fast, and cost effective if properly performed and provides "one stop" diagnosis by yielding highly cellular material representative of the lesions.¹⁻³ Cell block technique helps in making the best use of the available material and complements FNAC smears by increasing diagnostic sensitivity and specificity through

analysis, a separate dedicated pass was given. After letting the aspirated material to clot in the syringe for additional 5-7 minutes, 10% buffered formalin was aspirated in the syringe so as to dislodge the clot from the syringe wall. The aspirated formalin along with the dislodged clot was transferred into the specimen container with 10% formalin fixative for 6-48 hours fixation. The formed cell button was processed as routine biopsy specimen and stained with H&E staining. Cell blocks containing atleast 100 cells per cell block were included in the study for IHC.⁷ Cold ischaemia time varied from 30- 60 min. Representative sections were prepared from the cut surface of the corresponding surgical biopsy specimens, such as core needle biopsies, lumpectomies and mastectomy samples. Tissues were fixed in 10% buffered formalin for 6-48 hours in all samples and embedded in paraffin according to standard histologic techniques.

Sections of suitable thickness (3µm) were cut from both the cell blocks and histological sections. Immunohistochemistry was done, after epitope removal with Heat antigen retrieval method, with a polymer- based detection system (Envision+; Dako) using mouse monoclonal antibodies for estrogen receptor (ER, monoclonal, Clone 1D5; procured from DAKO [Dako Denmark A/S, Glostrup, Denmark], dilution 1:20), progesterone receptor (PR, monoclonal, Clone PgR636; from DAKO, dilution 1:50) and HER2 (polyclonal; Hercep test procured from DAKO, dilution 1:250) on the cell blocks and the subsequent histological sections in all the 50 cases. IHC staining was done using automated system (DAKO A utostainer). The IHC score was calculated using ASCO/ CAP guidelines for ER/PR⁷ that ER and PR assays were considered positive if there were at least 1% positive tumor nuclei in the sample on testing. The intensity of the staining was reported as weak, moderate or strong. When present, normal breast tissue was used as a positive internal control in tissue block specimens. External controls were used in the absence of normal breast tissue and in all cell block specimens. HER2 results were interpreted based on the maximum area of staining intensity and was recorded as 1+, 2+ or 3+. Only strong, circumferential membranous, staining in >10% of cells (3+) was considered as positive.¹⁹

Cases with Her2 scores of 2+ were not reflexed to FISH.

The data obtained was analyzed using Statistical Package for the Social Sciences software version 24.0 to determine concordance, sensitivity, specificity and statistical significance (using the Fisher exact test, wherever appropriate). p value of ≤ 0.05 was used to establish statistical significance.

RESULTS

The age of the patients (n=50) varied from 32 to 75 years. 33/50 (66%) were between 31 to 50 years of age group, with mean age as 48.74 years. Of 50 pre- chemotherapy cases, 44 (88%) were mastectomy specimens and 6 (12%) were core needle biopsies. On gross examination, the largest dimension of the tumor varied from 2-11 cm. 30/44 (68%) were having tumor size between 2-5 cm while only 2/44 (5%) had tumor size <2 cm. 12/44 patients (27%) had tumor size >5 cm, out of which, 8 showed lymph node metastasis (67%). The most common histological subtype was Infiltrating Ductal Carcinoma- NST (82%). This was followed by Invasive Lobular Carcinoma (ILC) (10%), Metaplastic carcinoma (4%) and a single case each (2%) of Mucinous carcinoma and Cribriform carcinoma. IHC for ER, PR and HER2 was performed in all 50 cases (Table 1) and Figures 1,2,3,4.

In comparison with surgical biopsies, 11 cases (ER, 4/50; PR, 4/50; HER2, 3/48) were found to be discordant on cell block. Histopathology being the gold standard, the sensitivity for ER, PR and HER2 on cell block was 92.30%, 86.36% and 91.67%, respectively, while specificity was 92.85%, 96.43% and 94.44%, respectively. The data was found to be statistically significant.

DISCUSSION

The decision regarding neoadjuvant chemotherapy, especially in locally advanced breast cancer, depends upon the hormonal receptor (ER and PR) and HER2 status of the breast cancer. The availability of HER2 targeted therapy has markedly improve the outcome of the patients. Therefore, in addition to IHC being used for diagnostic problems with breast biopsies, the latter lend themselves to the frequent use of IHC for prognostic and predictive tests,

Histology	Cell block	Number of cases	Correlation	
ER+	ER+	36	Concordant- 46/50 (92%)	p= 0.000 Sensitivity- 92.30%
ER-	ER-	13		
ER+	ER-	3	Discordant- 4/50 (8%)	Specificity- 92.85%
ER-	ER+	1		
PR+	PR+	19	Concordant- 46/50 (92%)	p= 0.000 Sensitivity- 86.36%
PR-	PR-	27		
PR+	PR-	3	Discordant- 4/50 (8%)	Specificity- 96.43%
PR-	PR+	1		
HER2+	HER2+	11	Concordant- 45/48 (93.75%)	p= 0.000 Sensitivity- 91.67%
HER2-	HER2-	34		
HER2+	HER2-	1	Discordant- 3/48 (6.25%)	Specificity- 94.44%

Table-1: Comparison of ER, PR and HER 2 staining on cell block versus histopathology (n=50)

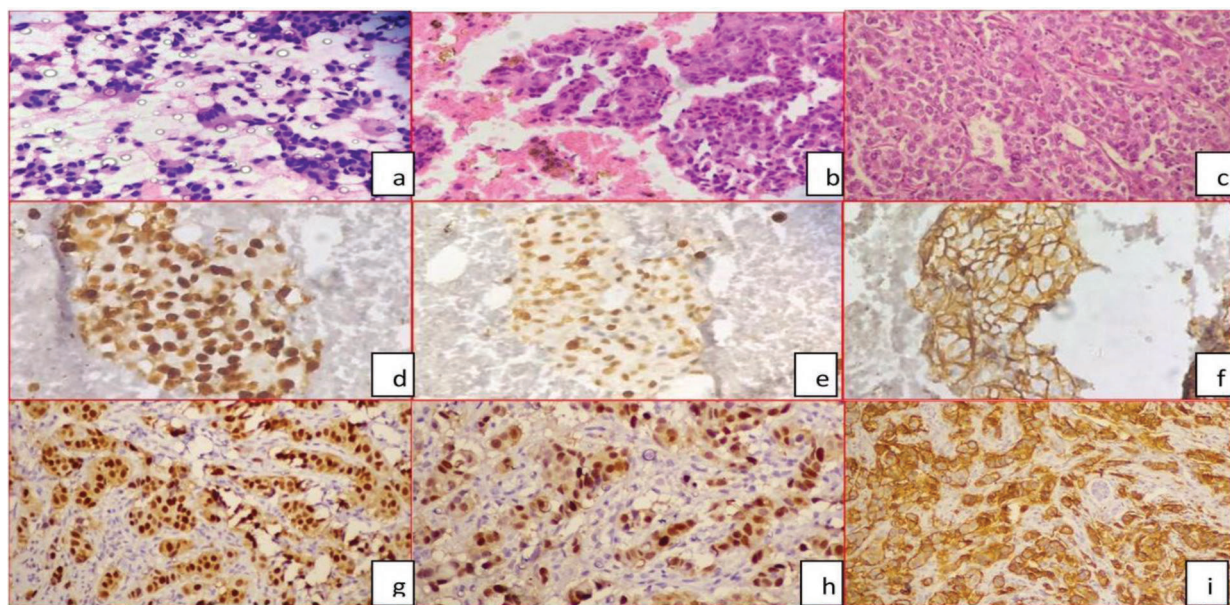


Figure-1: Infiltrating Ductal Carcinoma- NST; a, FNAC,MGG x400; b, cell block, H&E x400; c, tissue block, H&E, x200; d-f, cell block(ER+/PR+/HER2+); d, ER (IHC x400); e, PR (IHC x400); f, HER2 (IHC x400); g-i, tissue block (ER+/PR+/HER2+); g, ER (IHC x200); h, PR (IHC x200); i, HER2 (IHC x100).

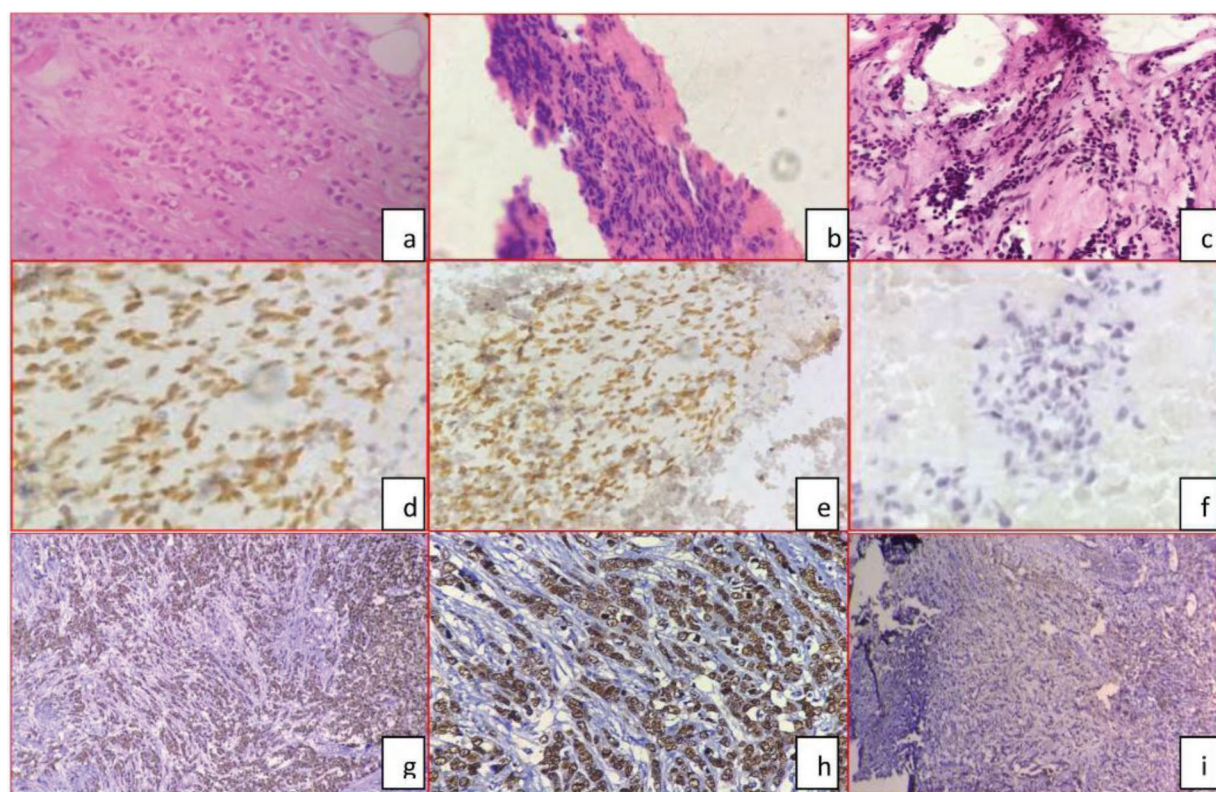


Figure-2: Invasive Lobular Carcinoma-; a, FNAC,MGG x400; b, cell block, H&E x400; c, tissue block, H&E, x400; d-f, cell block(ER+/PR+/HER2-); d, ER (IHC x400); e, PR (IHC x400); f, HER2 (IHC x400); g-i, tissue block (ER+/PR+/HER2-); g, ER (IHC x100); h, PR (IHC x400); i, HER2 (IHC x100).

thus enabling clinicians planning the extent of the surgery. Core needle biopsy allows for biomarker studies, similar to other histopathology specimens. FNA samples both direct smear and liquid- based preparation can also be used for immunoperoxidase stains but cell block is the preferred sample type being analogous to surgical pathology material. IHC for ER/ PR on cell blocks is considered better than

immunocytochemistry (ICC) on FNAC smears.⁴ Besides, cell blocks can be used for second opinion or tissue recuts and immunohistochemical analysis of cases as in formalin-fixed histological sections. The importance of this technique was first studied in 1985 for analyzing multiple prognostic markers by means of immunohistochemistry in breast cancer.⁵ The use of non- formalin based fixatives has been

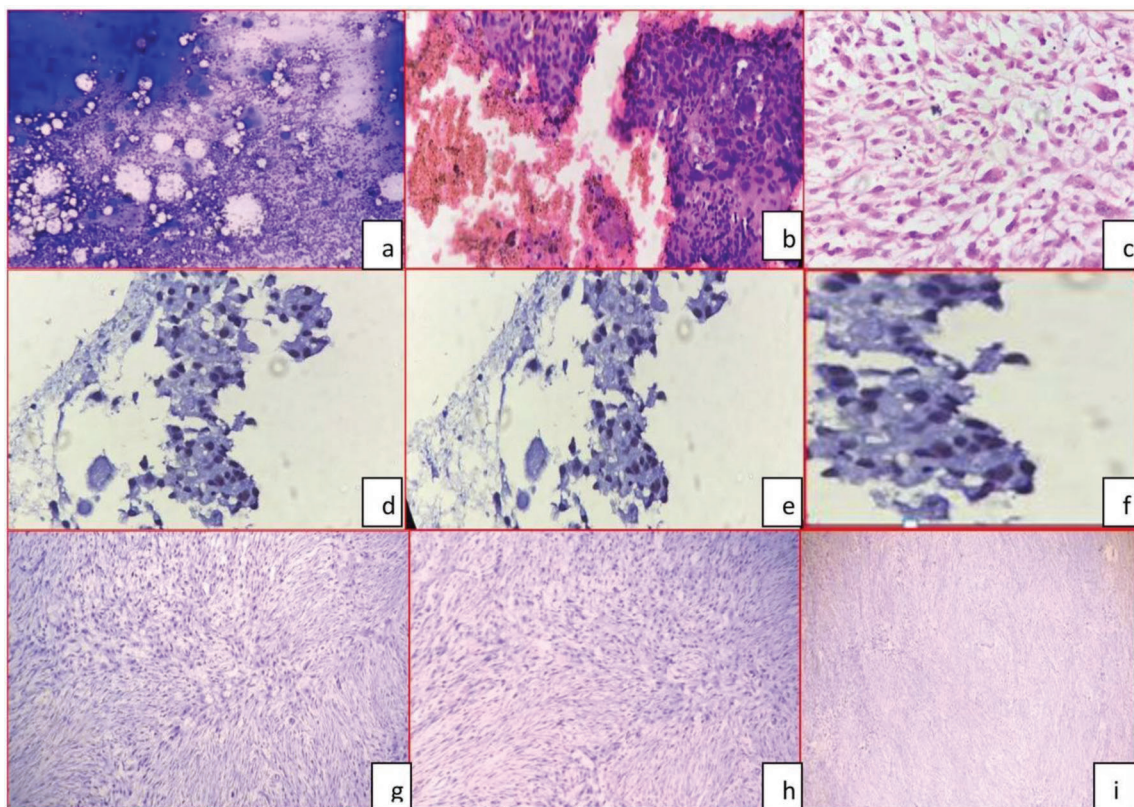


Figure-3: Metaplastic Carcinoma; a, FNAC,MGG x400; b, cell block, H&E x400; c, tissue block, H&E, x400; d-f, cell block(ER-/PR-/HER2-); d, ER (IHC x400); e, PR (IHC x400); f, HER2 (IHC x400); g-i, tissue block (ER-/PR-/HER2-); g, ER (IHC x100); h, PR (IHC x100); i, HER2 (IHC x400).

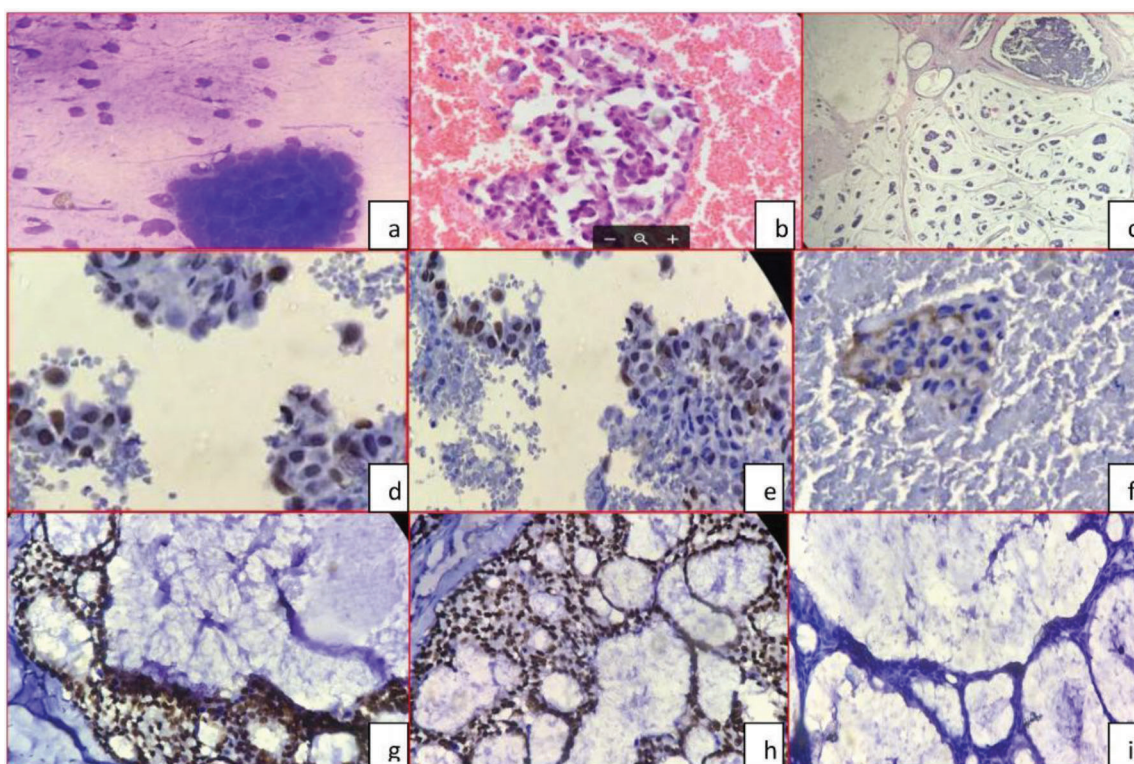


Figure-4: Mucinous Carcinoma; a, FNAC,MGG x400; b, cell block, H&E x400; c, tissue block, H&E, x40; d-f, cell block(ER+/PR+/HER2-); d, ER (IHC x400); e, PR (IHC x400); f, HER2 (IHC x400); g-i, tissue block (ER+/PR+/HER2-); g, ER (IHC x400); h, PR (IHC x400); i, HER2 (IHC x400).

discouraged due to them being unreliable for IHC assay.⁶ As per ASCO/CAP guidelines, for optimal staining results, a minimum of 6 hours of formalin fixation and not more than 72 hours for ER and PR and for HER2 are recommended.^{7,19} Our immunostaining data showed very good concordance rates for ER(92%) and PR (92%) in agreement with concordance rates of 84% to 98% reported in the literature for ER/PR.^{8,12,21} The findings are in moderate agreement between PR results on cytology samples and tissue samples.¹² The exact reason for these findings is unknown. In the present study, ER positivity of 72% was observed which is in agreement with the positivity rate of 75% for ER in breast carcinoma reported in western literature.⁹ In the present study, PR positivity of 44% was observed. A study from Bengaluru found ER and PR positive rates of 65.74% and 59.72%, respectively.¹¹ Our evaluation results were basically at the same reliable level as tissue sections. On analyzing HER2 amplification, our result (24% HER2 positive on tissue blocks) is in accordance with the reported rate of 25-30% in a western study but higher than the commonly accepted rate of 15-20%.^{10,19} A study observed 38% positive tumors on cell blocks which is in higher discrepancy with our findings (26% positivity on cell blocks).⁵ In our study, the concordance rate between the HER2 overexpression on cell blocks and tissue blocks was 93.75%, the sensitivity was 91.67% and specificity was 94.44%. Similar results have been observed by other investigators.^{8,12} However, a study by Nishimura et al and Dong et al has reported concordance rate of 77% and 83.1% respectively between the cytological and histological samples.^{13,21}

In the present study, discordance observed between the IHC results of cell blocks and tissue blocks is mainly due to technical errors (sampling error and staining error). Technical errors include suboptimal manual assays and inefficient antigen retrieval.^{14,15} Low cellularity on cell blocks with suboptimal preservation of morphology due to pre-fixation time lag could be the reason for ER/ PR discordance. Interpretational error, with a lot of cytoplasmic staining could be the reason for HER2 discordance on cell blocks. Various scoring systems used for ER and PR positivity also add to the difficulty.¹⁶ For HER2, immunostaining is associated with high variability in sample preparation, fixation, staining, and interpretation.^{8,17,18} Only strong complete membrane staining in >10% tumour cells is considered positive on IHC.¹⁹ The main problems in interpretation arise from cases that are at the 1+ / 2+ and 2+ / 3+ borderlines. Tumors that have equivocal (2+) staining for HER2 should be evaluated further by FISH, because 8% to 25% or even up to 48% of tumors can reveal HER2 amplification with this method.²⁰ In our study, due to non availability of ISH, all equivocal cases (2/50) were not evaluated further. Significantly more tumors were stained positive (3+) for HER2 by IHC on cell block preparations (26%) than on tissue block preparations (24%). Histopathology being the final outcome, this discrepant case was considered to be negative in final evaluation. On the basis of our data, we conclude that in the setting of limited resources without access to ISH, the cell block/ surgical

specimen biomarker results correlation is reasonable. The present study is however limited by small sample size, less cellularity on cell blocks, suboptimal preservation of morphology due to pre fixation time lag and lack of ISH for HER2 equivocal cases.

CONCLUSION

Cell blocks are highly useful in the assessment of hormone receptors and HER2 status by IHC, especially when the patient requires neo adjuvant chemotherapy. It is highly recommended to have good quality cell blocks. All aspects of the FNA process including proper specimen collection, fixation, cell block preparation and immunohistochemistry techniques need to be assessed and continually monitored to ensure optimal results.

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