Original Research Article

Study of reactivity pattern of hormone receptors in patients with breast cancer at a tertiary care hospital, Aurangabad, India

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Background: Breast cancer is one of the most common malignancies affecting the female population worldwide. Prognosis and management of breast cancer are influenced by variables such as stage, grade, and hormone receptor status. Tumours that express ER and/or PR have a better prognosis and most of them respond well to hormonal therapy. In addition to hormone receptors, HER2 has emerged in recent years as an important independent predictive marker.

Methods: All surgically operated female cases of breast carcinoma which were submitted for immunohistochemistry test for estrogen, progesterone and human epidermal growth factor receptor-2 in the Department of Pathology, MGM Medical College, Aurangabad for the duration of December 2015 to October 2017 were included in this prospective study. A total of 50 patients were taken up for the study.

Results: The maximum age of the patients were in the 4th -5th decade and were mostly premenopausal. The tumour was maximum involving the right breast, upper outer quadrant and were BI-RADS 4. Majority were grade 2 and were invasive ductal carcinoma. Hormone receptor status showed ER positivity 48%, PR positivity 46% and HER2 positivity 28%.

Conclusions: So, to conclude, immunohistochemical analysis of ER, PR and HER2 receptors is widely available at a reasonable cost and provides valuable prognostic, predictive and therapeutic information. Although we could see different patterns of hormonal receptor status, irrespective of the histological grade, type and lymph node status in our study, HER2 testing along with ER/PR status should be performed routinely in all the patients diagnosed of breast cancer as this will help the clinicians to manage the patients further.

Keywords: Breast cancer, Estrogen receptor, Grade, Human epidermal growth factor receptor 2, Hormone receptor, Progesterone receptor

INTRODUCTION

Breast cancer is one of the most common malignancies affecting the female population worldwide. Breast cancer is a heterogeneous disease and is composed of recognized biological subtypes. Prognosis and management of breast cancer are influenced by variables such as stage, grade and hormone receptor status.

Hormone receptor studies such as estrogen receptor(ER), progesterone receptor (PR) and human epidermal growth factor receptor-2(HER-2/neu) are routinely done in breast carcinoma. It not only helps in the prognosis of the
tumour but also helps in deciding its treatment. The goal of doing this receptor status is to provide right treatment to the right patient. The role of the pathologist is to accurately assess these biomarkers, and the role of the oncologist is to treat the patient with one of the several established therapies, depending on the hormone status.

Majority of the carcinomas arise from the ER positive luminal cells. ER negative carcinomas may arise from ER negative myoepithelial cells or an ER positive precursor that has lost the expression in the process of evolution of cancer. Tumours that express ER and/or PR have a better prognosis and most of them respond well to hormonal therapy. In addition to hormone receptors, HER2 has emerged in recent years as an important independent predictive marker. HER2 proto-oncogene is amplified and/or over expressed in approximately 25% of invasive primary breast cancers. HER2 status has become important for prognostic implication and to assess the response of patients to Herceptin. Perou et al. describe four molecular subtypes: Luminal-like, HER2 enriched, Basal-like and Normal-like. The “basal-like” category of tumors is composed almost entirely of “triple-negative” breast cancers. Breast cancer negative for estrogen receptor (ER), progesterone receptor (PR), and human epidermal growth factor receptor 2 (HER2) the so-called triple receptor–negative cancer is a subtype of breast cancer that has a more aggressive clinical course than other forms of breast cancer and is associated with higher risk of relapse and high mortality rate when compared to other breast cancer subtypes. The present study was planned keeping in mind predictive importance of receptor status for the prognosis of illness and application of appropriate therapy. Hence the study is being undertaken to establish a correlation between ER and PR status, HER-2 overexpression, clinical features and tumour histopathology, and to effectively use these parameters to prognosticate and treat breast cancer patients.

METHODS

Population of study

All surgically operated female cases of breast carcinoma which were submitted for immunohistochemistry test for estrogen, progesterone and human epidermal growth factor receptor-2 in the Department of Pathology, MGM Medical College, Aurangabad, Maharashtra, India for the duration of December 2015 to October 2017 were included in this prospective study. Study sample size: 50.

Study Design

1. 10% neutral buffered formalin (NBF) fixed tissue received in Department of Pathology of suspected cases of carcinoma breast was processed routinely and paraffin blocks were prepared.

2. Routine sections were cut and stained with Haematoxylin & Eosin (H and E) stain and histological typing and grading of tumor was done according to Nottingham Richardson classification.
   - Grade 1: tubular pattern with small round nuclei, low proliferation
   - Grade 2: tubule formation with solid clusters, nuclear pleomorphism and mitotic figures are present.
   - Grade 3: solid sheets of cells with irregular nuclei, high proliferation and tumor necrosis are seen.

3. The paraffin block of tumor tissue was cut and three more sections was taken on three glass slides precoated with adhesive (silane) for immunohistochemistry to detect ER, PR and HER-2 overexpression.

4. Representative sections of tumour and adjacent uninvolved breast tissue (internal control) were further processed for immunohistochemistry using Peroxidase-antiperoxidase (PAP) echnique. Sections were taken on positively charged slides. Antigen retrieval was done using EDTA buffer solution at pH 9.0 and slides were stained with monoclonal antibodies obtained from ‘BIOGENEX’ company. ER (clone EP1), PR (clone EP2), HER2/neu (clone EP3) immunohistochemistry markers were used.

5. Immunohistochemistry staining was conducted by BIOGENEX reagents for ER, PR and HER-2. ER and PR positivity was interpreted and reported using Allred scoring system which takes into account both intensity of nuclear staining and proportion of immune-positive tumour cells. ER, PR positivity is considered to be positive if ≥ 1% of tumour cell nuclei are immune-reactive which was as per the American Society of Clinical Oncology/College of American Pathologists (ASCO-CAP 2010 guidelines-Table 2) which takes into account the cytoplasmic membrane staining and the proportion of immune-positive tumour cells.

6. The relationship between various clinical and histological findings with ER, PR and Her-2 status was studied. Clinical data was collected as per the Proforma.

Inclusion criteria

All suspected/confirmed female cases of breast cancer patients admitted in our hospital; willing to undergo this study.

Exclusion criteria

1. Histologically diagnosed cases of breast cancer patients admitted in our hospital; not willing for this study.
2. Male cases with breast carcinoma.
Table 1: Allred scoring system for ER PR reporting.

| Proportion Score (PS) | Intensity score (IS) |
|-----------------------|----------------------|
| % Positive Tumour Cells | Score | Intensity of Staining in Tumour Cells | Score |
| None | 0 | Weak | 1 |
| Less than 1% | 1 | Intermediate | 2 |
| 1 to 10% | 2 | Strong | 3 |
| 11 to 33% | 3 | | |
| 34 to 66% | 4 | Allred score= PS+IS | |
| 67 to 100% | 5 | Score 0-1 (Negative); Score 2-8 (Positive) | |

Table 2: ER PR Reporting guideline(ASCO-CAP guideline).

| % Tumor Cells | Result |
|---------------|--------|
| <1%ER PR tumor cells | Negative |
| >1%=ER PR tumor cells | Positive |

Table 3: HER-2 Reporting guideline(ASCO-CAP guidelines).

| Staining                                                                 | % Of Invasive tumour cells | Score | Result |
|-------------------------------------------------------------------------|---------------------------|-------|--------|
| No staining or incomplete faint / barely perceptible membrane staining in ≤10% of tumour cells | - | 0 | Negative |
| Incomplete faint / barely perceptible membrane staining >10% | 1+ | Negative |
| Incomplete and/or weak to moderate circumferential membrane staining >10% | 2+ | Equivocal |
| Circumferential intense complete membrane staining ≤10% | 2+ | Equivocal |
| Circumferential intense complete membrane staining >10% | 3+ | Positive |

Steps

1. Heat immobilizes slides at 60 degrees for 1 hour. Allow slides to cool briefly.
2. Deparaffinize slides by immersing in, 10 min Xylene, 10 min Xylene, 5 min 100% Ethanol, 5 min 90% Ethanol, 2-3 min under running tap water.
3. Antigen retrieval using E-Z Retrieval® system
4. Hydrogen peroxide block: By applying one drop of peroxide block solution on each section for 10 min at room temperature in humidity chamber.
5. Tissue block: By applying one of drop power blocks on each section for 10 min at room temperature in humidity chamber.
6. Primary block: Slide to drain off excess block solution and apply one drop of primary antibody on respective sections for 1 hour at room temperature in humidity chamber.
7. Antibody strengthen: By applying one drop of super enhancer on each section for 20 min at room temperature in humidity chamber. Rinse in wash buffer three times with 5 dips each.
8. Secondary method: Apply one drop of polymer-HRP (SS label) on sections for 30 min at room temperature in humidity chamber.
9. Detection: Apply few drops of freshly prepared DAB using dropper for 5-10 min at room temperature. Rinse slides in tap water (8-10 dips)
10. Counterstain with freshly filtered Harris hematoxylin with 2-3 dips. Rinse in running tap water (4-5 dips).
11. Dehydrate slides immersing in, 5 min 75% Ethanol, 5 min 90% Ethanol, 5 min 100% Ethanol, 10 min Xylene, 10 min Xylene.

12. Mount with permount and coverslip.

RESULTS

The present prospective study comprises of 50 cases with breast cancer referred by surgery/oncology department, which were subjected to hematoxylin and eosin staining for histological typing and grading and this was followed by processing and staining for immunohistochemistry which were processed at pathology department of MGM Medical College, Aurangabad during period of December 2015 to October 2017.

Table 4: Age distribution of the cases.

| Age Group (in years) | Cases (N=50) | Mean±SD |
|----------------------|--------------|---------|
| 31-39                | 06           | 12      |
| 40-49                | 18           | 36      |
| 50-59                | 08           | 16      |
| 60-69                | 13           | 26      |
| 70-79                | 05           | 10      |
| Total                | 50           | 100.0   |

Number of breast cancer cases were 50, out of these maximum cases were found in the 4th-5th decade i.e. 18 cases (36%) and minimum in 7th decade i.e. 5 cases (10%) (Table 4). From this data, it is clear that younger patients are coming with cancer breast in India.

Number of breast cancer cases were 50, out of these maximum cases were found to have premenopausal menstrual status, i.e 27 cases (54%) (Figure 1).

In our study of 50 cases, right breast was seen to be slightly more affected (50%) than the left breast and there was one case of bilateral breast lump (Figure 2).

In our study, out of 50 cases of breast cancer patients, majority of the patients had tumour located in the upper outer quadrant (17 cases) (Figure 3).

Out of 50 cases of breast cancer patients, majority of the tumour were radiologically graded as BI-RADS IV (35 cases) (Figure 4).
In our study, out of 50 cases of breast cancer patients, majority of the tumours were of stage T2, i.e. tumours were of size >2cm but <5cm. (Figure 5).

![Figure 5: Staging of tumour based on size.](image)

In our study, out of 50 cases of breast cancer patients, majority was modified radical mastectomy specimens (33 cases) and biopsy samples were 17 cases (Figure 6).

![Figure 6: Type of histopathology specimens.](image)

Out of the 50 cases, 35 cases of tumours were invasive carcinoma (no special type), followed by 6 cases of lobular carcinoma, one case each of medullary and mucinous carcinoma, 4 cases of Paget’s disease of nipple with three cases co-existing with invasive carcinoma and one case with only ductal carcinoma in situ.

There were 3 cases of mixed carcinoma which were one case each of invasive carcinoma with medullary differentiation, invasive carcinoma with apocrine differentiated and another case of papillary carcinoma with neuroendocrine differentiation. (Figure 8).

Out of 49 cases of breast cancer, majority of tumours were of grade 2, followed by grade 3 and grade 1. One of the breast cancer case could not be graded as the tumour was of histologic type Paget’s disease of the nipple with ductal carcinoma in situ only, no invasion was noted hence could not be graded. (Figure 9).

![Figure 8: Histologic type of breast cancer.](image)

![Figure 9: Histologic grade of tumour.](image)

Out of 50 cases, estrogen receptor negativity was seen in 26 cases and positivity was seen in 24 cases. (Figure 10)

Out of 50 cases, progesterone receptor negativity was seen in 23 cases and positivity was seen in 27 cases (Figure 11).
Out of 50 cases, HER-2 reactivity pattern was seen negative in 31 cases, positive in 14 cases and equivocal in 5 cases. The equivocal cases were advised for confirmation using fluorescent in-situ hybridization (Figure 12).

### Figure 12: HER-2 reactivity pattern of breast cancer.

Out of 50 cases, HER-2 reactivity pattern was seen negative in 31 cases, positive in 14 cases and equivocal in 5 cases. The equivocal cases were advised for confirmation using fluorescent in-situ hybridization (Figure 12).

### Figure 10: ER reactivity pattern of breast cancer.

![ER reactivity pattern](image)

### Figure 11: PR reactivity pattern of breast cancer.

![PR reactivity pattern](image)

### DISCUSSION

In our study of 50 patients of breast cancer, the mean age of the patients was seen to be 52 years and 36% of patients were in the 4th to 5th decade and were mostly perimenopausal. This was in concordance with the studies done by Urmila Devi et al, and Hussain et al, who had the mean ages of the patients as 51.5 and 46 years respectively.\textsuperscript{12,13} The study by Nidal M Almsari et al, also had the mean age of 47 years in their study with 37% patients within the age of 45 years.\textsuperscript{14}

Literature search reveals breast carcinomas are more common in the left breast than right. However side of breast involved has no clinical significance.\textsuperscript{15-17} In our study, right breast was marginally more affected than the left breast and a single case of bilateral breast carcinoma was noted. A study done by Fahad Pervaz showed a slight increase of cases affecting the right breast.\textsuperscript{18}

Majority of tumours (34%) were seen in the upper outer quadrant. Numerous clinical studies, dating back decades, have shown that the upper outer quadrant (UOQ) of the breast is the most frequent site of carcinoma, but an adequate explanation for this asymmetric occurrence of breast cancer within the breast has never been established. A study done by Philippa D. Darbre\textsuperscript{19} showed that 52.5% of tumours were seen in the upper outer quadrant. The accepted explanation for the maximum occurrence of breast cancers in the upper outer quadrant is that this region of the breast contains a greater proportion of the epithelial tissue, which is the target site for breast cancer. In our study majority of tumours were of BI-RADS of 4 and 66% of the cases were modified radical mastectomy specimens.

Sixty six per cent (66%) of tumours were of size >2 and <5 cm. This was in concordance with the study done by Fahad Pervaz\textsuperscript{19}, in which majority of the cases (44 cases) were of tumour size >2 and <5cm and also was in concordance with the study done by Hussain et al\textsuperscript{13} in which majority of the tumours (69.8%) were >2cm and <5cm in size.

Out of 33 cases of modified radical mastectomy specimens, 49% of the cases showed no lymph node involvement (16 cases) followed by 1 to 3 lymph node involvement in 13 cases. This was in concordance with the study done by Sumita et al, in which around 50% of cases had no lymph nodes involved.\textsuperscript{19}

Out of the 50 cases, majority of our cases (35 cases) of tumours were invasive carcinoma (no special type). This was in concordance with the study by Sumita et al, in which 91.1% showed invasive carcinoma of no special type and was also in concordance with the study done by Hussain in which 90.5% cases were of invasive carcinoma.\textsuperscript{13,19}

In our study, out of 50 cases of breast cancer patients, majority of the tumours were of histological grade 2. This was in concordance with studies done by Azizun-Nisa et al, and Ambroise et al.\textsuperscript{15,20} But it was in discordance with a study done by Ghosh et al, in which majority of tumours were of grade 3 tumour.\textsuperscript{21}

In our study the proportion of ER positive and ER negative cases did not differ much. Also PR positive and PR negative cases also did not differ much, although both
ER and PR negative cases were slightly higher than ER/PR positive cases. Majority of tumours were HER2 negative tumours (62%). And this was in concordance with the study done by Sumita et al, in which there were 65% of HER2 negative tumours.19

CONCLUSION

So, to conclude, immunohistochemical analysis of ER, PR and HER2 receptors is widely available at a reasonable cost and provides valuable prognostic, predictive and therapeutic information. Although we could see different patterns of hormonal receptor status, irrespective of the histological grade, type and lymph node status in our study, HER2 testing along with ER/PR status should be performed routinely in all the patients diagnosed of breast cancer as this will help the clinicians to manage the patients further. Further research should be carried out to understand the various trends and relationships between different variables associated with breast cancer for better prognosis of breast cancer patients in order to obtain better clinical outcomes with improved survival.

ACKNOWLEDGEMENTS

Author would like to acknowledge all the staffs and friends from Pathology Department for their sincere help and a special thanks to my husband Dr. Philips Antony.

Funding: No funding sources

Conflict of interest: None declared

Ethical approval: The study was approved by the Institutional Ethics Committee

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Cite this article as: George GA, SS Mulay, Borde N, Antony P. Study of reactivity pattern of hormone receptors in patients with breast cancer at a tertiary care hospital, Aurangabad, India. Int J Res Med Sci 2019;7:3444-51.