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Significance of Immunohistochemical Biomarker in Breast Carcinoma from a Single Tertiary Care Hospital in India

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Abstract

Background: Breast carcinoma is the most common malignancy in female worldwide, leading cause of death in women. Immunohistochemistry plays a very important role in the prognostication and treatment determination of breast carcinoma patients. **Objective:** The objective of the study was to analyze the immunohistochemical markers in invasive carcinoma of breast and to correlate the expression of hormonal receptors with age of the patient, tumor size, histological grade, and lymph node metastasis. **Materials and Methods:** The study was conducted on 88 infiltrating ductal breast carcinoma sample in a tertiary care hospital of Southern Assam for a period of 2 year (January2018-December 2019). Data including age, tumor size, and histologic grade and lymph node status retrieved from pathology department. Chi-square was used to determine the statistical significance between estrogen receptors and progesterone receptors (ER/PR) status human epidermal receptor growth factor 2 (HER2/neu) status along with their correlation with various clinicopathological parameters with respect to infiltrating ductal breast carcinoma. **Result:** The mean age of the patients was 56.6 years. We observed correlation between ER and PR expression with age, tumor size, and tumor grade. There was correlation between HER2/neu expression and age only. None of the markers showed correlation with lymph node involvement ($P > 0.05$). **Conclusion:** Our findings showed the importance of biomarkers (ER, PR, and HER2/neu) expression as prognostic factors for therapeutic decision.

Keywords: Estrogen receptor, progesterone receptor, human epidermal receptor growth factor 2, infiltrating ductal carcinoma, breast

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Introduction

Breast cancer is a major concern and one of the leading causes of cancer related death throughout the world. Breast cancer like many other types of cancer is a complex heterogeneous disease controlled by a multitude of genetic and epigenetic alterations.[1] During the past two decades, the mortality rate has declined significantly, primarily due to the early use of adjuvant chemotherapy as well as detection of earlier stage tumors due to increased screening.[2] Prognosis and management of breast cancer are influenced by the classical variables such as histological type and grade, tumor size, lymph node status, and status of hormonal receptors, estrogen receptors (ER) and progesterone receptors (PR) of the tumor, and more recently human epidermal receptor growth factor 2 (HER2/neu) oncoprotein status.[3] ER expression is undoubtedly the most important biomarker in breast cancer, because it provides the index for sensitivity to endocrine treatment. ER positive tumors (80% of breast cancer) use the steroid hormone estradiol as their main growth stimulus; ER is therefore direct target of endocrine therapies. PR expression is strongly dependent on the presence of ER. Tumors expressing PR but not ER are uncommon and represent <1% of all breast cancer.

The tumors that are ER positive and/or PR positive have lower risks of mortality after their diagnosis compared to women with ER and/or PR negative disease. Clinical trials have also shown that the survival advantage for women with hormone receptor-positive tumors is enhanced by treatment with adjuvant hormonal and/or chemotherapeutic regimens.[4] In breast cancer, the average incidence of ER and PR positivity is 57% and 43%, respectively, as shown in the studies. However, lower rates of positive estrogen and PR breast cancers are found in Indian population from the western literature. The frequency of negative ER and PR is much more common in India (46.5%) than in the West (10%). Breast cancer patients of Indian origin tend to be younger, tumors are often large when first diagnosed, and of a high grade as compared to western series.[5]

The purpose of this study was to analyze the immunohistochemical markers in invasive carcinoma of breast and to correlate the expression of hormonal receptors with age of the patient, tumor size, histological grade, and lymph node metastasis.

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Materials and Methods

Study Design

Eighty eight patients with a diagnosis of infiltrating ductal carcinoma of breast in a tertiary care hospital of Southern

Assam over a period of 2 year (January 2018-December 2019) were included in this study. Data including age, tumor size, and histologic grade and lymph node status retrieved from pathology department. We analyzed the expression of ER, PR, and HER2/neu by immunohistochemistry his too chemistry (IHC), with each other and to various clinicopathological parameters. Institutional ethical committee approval was taken.

Inclusion Criteria

All patients with histologically confirmed infiltrating ductal carcinoma of the breast were included in the study.

Exclusion Criteria

Patients with inflammatory breast lesions, post-traumatic breast lesions, benign breast diseases, and patients with breast cancer who received neoadjuvant chemotherapy were excluded from the study.

Paraffin blocks containing cancer tissue were selected from histopathologically confirmed cases of infiltrating ductal carcinoma. After preparing slides from blocks, immunohistochemical staining was done for ER, PR, and HER2/neu by standard procedure.[6]

Preparation of Slides

Paraffin sections were cut and mounted on salinized slides. Slides were melted at 65°C and then dipped into xylene to remove the paraffin. After rehydrating tissues, slides were washed with distilled water. Then, slides were dipped into a fresh aqueous solution of 3% peroxide for 3 min and rinsed with Tris buffer.

Antigen Retrieval and Detection of Antigens

Heat retrieval was done with citrate buffer in the Decloaking chamber for 40 min at 95°C and then brought at room temperature after removing from the Decloaking chamber and by placing the slides in Tris-Saline buffer. About 1% mouse serum was added to the tissue section to block nonspecific immunostaining. The sections were exposed to the primary antibody for about 1 h, and then primary antibody was washed with Tris buffer.

Secondary Detection of the Primary Antibody

Sections were incubated with biotinylated mouse anti-species antibody for 10 min, and then rinsed in Tris buffer. A solution of chromogen, 3, 3'-diaminobenzidine (DAB) at 1 mg/ml in Tris buffer with 0.016% fresh H_2O_2 was prepared and added to the slides. DAB from the slides was washed with tap water.

Counterstaining

A solution of hematoxylin diluted 1:1 with distilled water was made slides were dipped into hematoxylin solution for staining. Then, slides were washed in distilled water and dehydrated by dipping in ethanol. Washed in xylene and coverslip was applied for viewing and reporting.

Reporting

Reporting done as per ER/PR scoring system and criteria as per Allred scoring system.[7]

Proportion Score

- 0 – No cells are ER +ve.
- 1 – ≤1% of cells are ER +ve.
- 2 – 1%–10% of cells are ER +ve.
- 3 – 11%–33% of cells are ER +ve.
- 4 – 34%–66% of cells are ER +ve.
- 5 – 67%–100% of cells are ER +ve.

Intensity Score

- 0 – Negative.
- 1 – Weak.
- 2 – Intermediate.
- 3 – Strong.

Interpretation

Total (proportion score + intensity score).

0–2 = Negative; 3–8 = Positive

Human epidermal growth factor receptor-2/neu scoring system and criteria according to the American Society of Clinical Oncology College of American Pathologists guidelines[8]

0 = no staining or incomplete faint and barely perceptible in <10% of tumor cells.

1+ = incomplete membrane staining which is faint and barely perceptible and within >10% of tumor cells.

2+ = circumferential membrane staining that is incomplete and/or weak/moderate and within >10% of the invasive tumor cells; or complete and circumferential membrane staining that is intense and within ≤10% of the invasive tumor cells.

3+ = circumferential, complete, and intense staining and within >10% of tumor cells.

FISH will be done for equivocal HER2/neu positivity. Hence, HER2/neu 2+ was taken as negative along with HER2/neu 0 and 1+. Only 3+ on IHC was taken as positive.

Statistical Analysis

Chi-square was used to determine the statistical significance between ER/PR status HER2/neu status along with their correlation with various clinicopathological parameters such as patient's age, tumor size, tumor grade, and axillary lymph node status with respect to infiltrating ductal carcinoma breast. A value of $P < 0.05$ was considered as statistically significant.

Results

Receptor Status

Fifty tumors were ER-positive and 38 were ER-negative. ER-positive tumors showed weak, moderate to strong nuclear positivity in >1% of tumor cells [Figure 1].

Forty six tumors were PR positive and forty two were PR negative. PR positive cases showed weak, moderate to strong nuclear positivity in >1% of tumor cells [Figure 2]. Seven tumors that were positive for HER2/neu showed complete and intense staining and within >10% of tumors cells [Figure 3].

Out of 88 cases, 46 cases were ER and PR positive, 38 cases were negative for both ER and PR. Four cases showed different expressions of ER and PR.

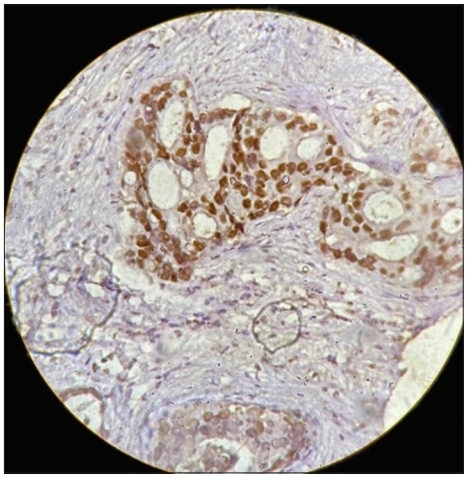

Figure 1: Immunohistochemical staining showing positive for ER

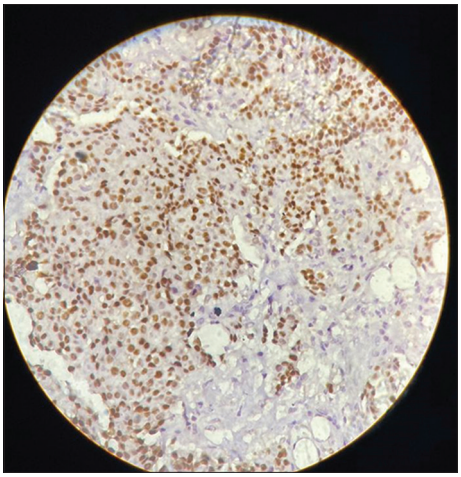

Figure 2: Immunohistochemical staining showing positive for PR

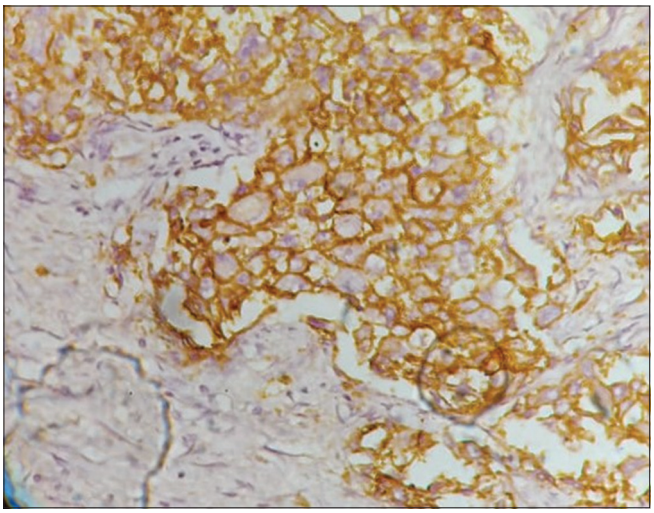

Figure 3: Immunohistochemical staining showing positive for HER2/neu

Age

Patients were in the age group between 24 and 80 years, with mean age 56.6 years. The majority 35.22%, 29.54% were in the age group 51–60 and >60 years, respectively. About 82% ER positive and 80.43% PR positive cases were of age group >50 years whereas 57.14% HER2/neu positive were in age <40 years [Table 1]. It was statistically concluded that ER, PR, and HER2/neu expression shows significant correlation with age.

Tumor Size

The average tumor size was 4.1 cm. About 48% ER positive and 56% PR positive tumors were of size between 2 and 5 cm whereas approx. 71% of HER2/neu tumors were of size <2 cm. Correlation of expression of ER, PR, and HER2/neu compared to tumor size is shown in Table 2. Hence, there exist statistically significance between tumor size and correlation of expression of ER, PR, and HER/2neu.

Tumor Grade

In this study, according to Nottingham Modified Bloom–Richardson System score, 42% of the tumors were in Grade II followed by Grade III (32%) and then Grade I (26%). Correlation of expression of ER, PR, and HER2/neu compared to tumor grade is shown in Table 3. There is statistically significance between tumor grade and ER, PR expression, whereas there exists no significance between tumor grade and HER/2 neu expression.

Axillary Lymph Node Status

All the infiltrating ductal carcinoma cases were evaluated for association of axillary lymph nodes metastasis and observed that out of 50 ER positive cases, 18 were axillary lymph nodes positive and 19 out of 46 positive PR cases had positive axillary lymph nodes. Out of seven HER2/neu positive cases, two have axillary lymph node metastasis. Correlation of expression of ER, PR, and HER2/neu compared to axillary lymph node status is shown in Table 4. It was concluded that correlation of expression of ER, PR, and HER2/neu compared to axillary lymph node status was not significant.

Discussion

Female breast cancer is the most common malignancy worldwide, with over 2 million cases diagnosed in 2018.[9]

Breast cancer is the most common cancer in female, representing approximately 25% of all cancers. It is also ranked number one cancer among Indian females with age adjusted incidence rate of 25.8/1 00,000 women and mortality 12.7/100,000 women.[10] Treatment of breast cancer includes combined therapy; surgery, radiotherapy, chemotherapy, endocrine therapy, and targeted therapy and so forth. Hormone therapy can be started before surgery (as neoadjuvant therapy) or used after surgery (as adjuvant therapy) or as a prophylactic treatment of high risk populations as in BRCA mutation carriers. Evaluation of hormone receptor on surgically resected specimen or core biopsy material is essential to assess the utility of hormone therapy and thus the College of American Pathologists and American Society of Clinical Oncology recommend ER and PR testing for all newly diagnosed cases of invasive breast cancer and breast cancer recurrences.[11]

Various biomarkers such as hormone receptors, vascular endothelial growth factors, epithermal growth factor, tumor suppressor genes, multidrug resistant genes, and adhesion

Table 1: Age versus ER, PR and HER2/neu expression

Age (years)	*ER*			*PR*			*HER2/neu*		
	Positive	*Negative*	*Total*	*Positive*	*Negative*	*Total*	*Positive*	*Negative*	*Total*
<40	3	12	15	3	12	15	4	11	15
41–50	6	10	16	6	10	16	2	14	16
51–60	21	10	31	18	13	31	1	30	31
>60	20	6	26	19	7	26	0	26	26
Total	50	38	88	46	42	88	7	81	88
χ^2, df, P	16.51, 3, 0.0008			21.95, 3, 0.00006			10.84, 3, 0.0126		

ER: Estrogen receptor, PR: Progesterone receptor, HER2/neu: Human epidermal growth factor receptor

Table 2: Tumor size versus ER, PR, and HER2/neu expression

Tumor size (mm)	*ER*			*PR*			*HER2/neu*		
	Positive	*Negative*	*Total*	*Positive*	*Negative*	*Total*	*Positive*	*Negative*	*Total*
<2	20	8	28	15	13	28	5	23	28
2–5	24	16	40	26	14	40	2	38	40
>5	6	14	20	5	15	20	0	20	20
Total	50	38	88	46	42	88	7	81	88
χ^2, df, P	8.36, 2, 0.0153			12.41, 2, 0.0021			5.95, 2, 0.5104		

ER: Esterogen receptor, PR: Progesterone receptor, HER2/neu: Human epidermal growth factor receptor

Table 3: Tumor grade versus ER, PR, and HER2/neu expression

Grade	*ER*			*PR*			*HER2/neu*		
	Positive	*Negative*	*Total*	*Positive*	*Negative*	*Total*	*Positive*	*Negative*	*Total*
I	15	8	23	13	10	23	1	22	23
II	27	10	37	24	13	37	2	35	37
III	8	20	28	9	19	28	4	24	28
Total	50	38	88	46	42	88	7	81	88
χ^2, df, P	13.71, 2, 0.0010			7.064, 2, 0.0292			2.25, 2, 0.1336		

ER: Esterogen receptor, PR: Progesterone receptor, HER2/neu: Human epidermal growth factor receptor

Table 4: Lymph node status versus ER, PR and HER2/neu expression

Lymph node status	*ER*			*PR*			*HER2/neu*		
	Positive	*Negative*	*Total*	*Positive*	*Negative*	*Total*	*Positive*	*Negative*	*Total*
Positive	18	15	33	19	14	33	2	31	33
Negative	32	23	55	27	28	55	5	50	55
Total	50	38	88	46	42	88	7	81	88
χ^2, df, P	0.105, 1, 0.74591			0.595, 1, 0.44049			0.26, 1, 0.61012		

ER: Esterogen receptor, PR: Progesterone receptor, HER2/neu: Human epidermal growth factor receptor

molecules have been identified.[12] At present, determination of ER, PR, and HER2/neu receptor is routine in the diagnosis of breast cancer[13] with at least 1% positivity is necessary for commencement for hormone therapy.

ER is a biomarker found in over 56.82% of infiltrating breast cancer in this study and contributes significantly to its pathobiology. ER positivity makes it responsive to hormonal therapy, resulting in a more favorable outcome. PR, like ER, is also a transcription factor, which is largely controlled by ER and to a lesser extent by growth factors. About 43.8% of infiltrating breast cancers show PR positivity. PR commonly coexists with ER. Studies from other region have documented lower positivity for ER and PR receptors. Desai *et al*.[14] from India have reported 32.6% and 46.1% positivity for ER and PR, respectively. Another study by Suvarchala *et al*.[15] from South India showed 46.87% ER positivity and 43.75% PR positivity Similarly, a study from Sri Lanka by Mudduwa[16] have reported 45.7% ER positive and 48.3% PR positive tumors. Another study from Western India has also reported 44.6% ER positive and 40.4% PR positive tumors.[17] In contrast, study from Bahrain reported high positivity for ER (72.6%), PR (71%), and HER2/neu (51%).[18]

HER2/neu has the potential of enhancing proliferation and survival of tumor cells. In this study, its overexpression occurs in about 7.95% of infiltrating breast cancer, results in a more aggressive growth and poor response to treatment. In this study, the sample showing equivocal HER2/neu expression will be evaluated by fluorescence *in situ* hybridization. Unlike our study, Ranvijay *et al*.[19] and Rashmi *et al*.[20] reported 34.2% and 69.2% HER2/neu expression.

The mean age of breast cancer is 56.6 years in our study which is much lower than the mean age of 62 year reported in UK[21] whereas in the US,[22] peak is observed at the age 75 years. Our study corroborates with the study done by Elsayed *et al*.[23] at Egypt where the mean age is 50.4 years. In India, the incidence rates begin to rise in the early thirties and peak at ages 50–64 years. Although the reason entirely is still not clear, a major factor could be ignorance, lack of awareness, and under reporting among the elderly population in India. Majority of ER and PR positive cases were of age >60 years and HER2/neu positive were of age <40 years, as seen in the study conducted by Alzaman *et al*.[18] A significant correlation was observed between age of the patient and ER (0.000), PR (0.000), and Her2/neu (0.012) expression as shown in studies by Dodiya *et al*.[24]

Tumor size was 1–8.9 cm, with average size 4.1 cm. In this study, significant correlation expression was seen between tumor size and ER ($P = 0.015$) and PR ($P = 0.002$). In contrary to the study by Ariga *et al*.,[25] we found correlation between tumor size and ER, PR expressions. However, our result was similar with Bhatavdekar *et al*.[17] findings.

Thirty-seven tumors were of Grade II followed by 28 and 23 tumors were of Grade III and Grade I, respectively. In contrary to other studies from developed country where well differentiated breast cancers are more common than poorly differentiated because of the availability of routine screening and awareness which has led to the detection at the early stage.[26] The majority of ER positive cases (54%) were observed in Grade II carcinomas. Most PR positive cases (52.17%) were also seen in Grade II, whereas most HER2/neu positive cases (57.14%) were seen in Grade III. There was seen significant correlation between tumor grade and ER and PR expression whereas no significant correlation was observed between tumor grade and HER2/neu expression. Our study corroborates with the study done by Saptarishi *et al*.[27] where ER and PR status significantly correlated with the stage of the disease. Similar correlation was found in other studies.[28]

Metastasis in axillary lymph nodes was seen in 37.5% of patients. About 54.55% ER 57.58% PR and %HER2/neu positive cases had positive axillary lymph nodes positive for metastasis. In our study, we found that ER/PR expression had no significant correlation with lymph node metastasis. Furthermore, HER2/neu overexpression showed no significant association with lymph node metastasis and this result is in agreement with Almasri *et al*.[29] Unlike our study, Siadati *et al*., 2015, showed significant association between HER2/neu overexpression and lymph node status.[28]

Conclusion

Invasive ductal carcinoma of the breast cancer was seen in the age of 24 and 80 years, with a mean age of 56.6 years. The maximum number of cases was seen in the age above 50 years (64.77%). Majority of the tumors that were ER and PR positive were of Grade II, whereas majority of HER2/neu positive tumors were of Grade III. This study showed that ER and PR are correlated with age, tumor size, and tumor grade but not with lymph node status. HER2/neu expression is correlated with age only but not with tumor size, tumor grade, and lymph node status. Therefore, it is strongly recommended to assess the hormone receptors for clinical management of a breast cancer patient to provide prognostic information and therapeutic measurement.

Compliance with Ethical Standards

This work is approved by institutional ethical committee.

Declaration

Consent for Publication

All the authors have given their consent to publish the article.

Availability of Data and Material

All the data generated and analyzed during this study are included in this published article.

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Authors Contribution

The authors MN, BC, BB, and BD contributed in analyzing and interpreting the result. JS contributed in performing the experiment, ND contributed in performing the experiment, analyzing the data, and writing the manuscript.

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