Original Article

Evaluation of Immunohistochemical Profile of Breast Cancer for Prognostics and Therapeutic Use

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Abstract

Introduction: Breast cancer is leading cancer in women, and the incidence of breast cancer in India is on the rise. The most common histologic type of breast cancer is infiltrating ductal carcinoma. Prognostic and predictive factors are used in the management of breast cancer. Estrogen receptor (ER), progesterone receptor (PR), and human epidermal growth factor receptor-2 (HER2/neu) are immunohistochemical markers of prognosis as well as predictors of response to therapy. **Aims and Objectives:** The study was conducted to evaluate ER, PR, and HER2/neu expressions in invasive ductal carcinomas of the breast by immunohistochemistry, to explore the correlation of these markers to each other and to various clinicopathological parameters: age of the patient, histological grade, tumor size, and lymph node metastasis. **Materials and Methods:** This prospective study was conducted on 100 cases of infiltrating ductal carcinoma. Slides were prepared from blocks containing cancer tissue, and immunohistochemical staining was done for ER, PR, and HER2/neu expressions. Interpretation of expressions was done using Allred scoring system for ER/PR and the American Society of Clinical Oncology/College of American Pathologists guidelines for HER2/neu. Statistical analysis was performed to determine the statistical significance by applying Chi-square test. **Results:** Majority of tumors were ER and PR positive and HER2/neu negative. ER and PR correlated significantly with age, tumor size, and tumor grade; whereas, HER2/neu correlated significantly with tumor size only. No association was seen with axillary lymph node metastasis. ER and PR expression correlated with each other, but none correlated with HER2/neu. **Conclusions:** As the majority of the tumors are ER, PR positive and since ER and PR correlate with each other as well as with age, tumor size, and grade. Therefore, routine assessment of hormone receptors is recommended for prognostic and therapeutic information in breast cancer cases.

Keywords: *Allred scoring system, human epidermal growth factor, immunohistochemical markers, estrogen receptors, progesterone receptors*

Introduction

Breast carcinoma is the most common malignant tumor and the leading cause of deaths due to carcinoma in women. It is more common in developed countries.[1] There are so many types of breast carcinomas, but infiltrating ductal carcinoma is the most common histological type of breast cancer.[2] The mainstay of breast cancer treatment is surgery when the tumor is localized, followed by chemotherapy (when indicated), radiotherapy and for estrogen receptor (ER) and progesterone receptor (PR) positive tumors, adjuvant hormonal therapy.[3]

Breast cancer is curable if diagnosed at early stage. Traditional morphological prognostic factors include tumor size, tumor grade, and axillary lymph node

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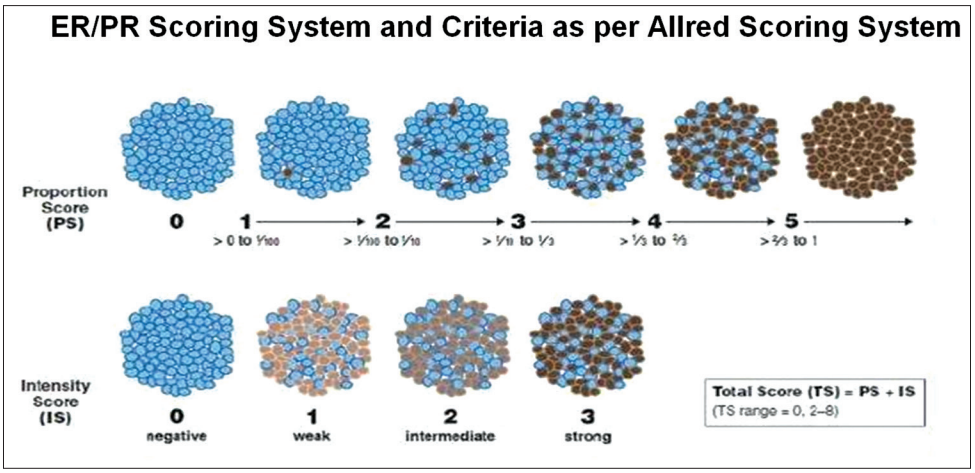

Figure 1: Allred scoring system for estrogen receptor/progesterone receptor scoring

metastasis. Nowadays, more importance is given to biological molecular prognostic factors because a significant number of patients with early-stage breast cancer harbor microscopic metastasis at the time of diagnosis.[4] Hormone receptors (ER and PR) and human epidermal growth factor receptor-2 (HER-2) are the most relevant clinical biomarkers that are widely used in stratifying breast cancer cases management.[5] Knowledge of hormone receptors and HER-2 expressions are vital for breast cancer management plans and decision-making.[5] Prognostic and predictive factors are used in the management of breast cancer. Prognostic factors are those which influences patient's overall outcome such as chances of recurrence after treatment. These factors help in the selection of patients for a specific treatment.[6] Predictive factors evaluate the likelihood of benefit from a specific treatment. ER, PR, and HER2/neu are prognostic as well as predictive factors.[6]

Oestrogen receptor

Breasts undergo important physiological changes during a woman's lifespan, and these changes are actively mediated by estrogen. ER is of two types – ERα and ERβ.[7] Receptor ERα is a well-established prognostic and predictive factor in breast cancer. The prognostic significance of ERβ is not well defined.[7,8] The majority of ER-positive breast cancers contain both ERα and ERβ subtypes; although, some cancers have only ERβ expression. This may lead to distinct clinical behaviors and responses. It is observed that in contrast to ERα, ERβ expression declines during breast carcinogenesis.[9]

Progesterone receptor

PR is of two types as follows: PR-A and PR-B. Progesterone acts as a modulator of estrogen function.[10]It is observed that ER-positive breast cancers which lack PR expression, are less responsive to hormonal treatment than those that are PR positive. It is also seen that ER and PR are not stable phenotypes. These can change over the natural history of the disease or as consequence of treatment.[11]

Human epidermal growth factor receptor-2/neu (c-erbB-2)

It is a member of the four-member family of closely related growth factor receptors, including epidermal growth factor receptor or HER1, HER2, HER3, and HER4. HER2/neu amplification or overexpression is involved in oncogenic transformation and tumorigenesis in breast cancer. Inappropriately increased signaling occurs as a result of receptor overexpression. It may lead to increased and uncontrolled cell proliferation, decreased apoptosis, increased cancer cell motility, and angiogenesis and hence worse prognosis.[12]

At present, determining ER, PR, and HER2/neu receptor status in breast cancer have become a common practice, as there is a survival advantage for patients with hormones receptor positive status by treatment with adjuvant hormonal or chemotherapeutic regimens. It is well known that strong ER-positive cases benefit from endocrine therapy alone, in contrast to those with low to moderate ER positivity. PR status is independently associated with disease-free and overall survival. Patients with ER, PR-positive tumors have a better prognosis than patients with ER, PR-negative tumors.[13]

The present study was conducted to correlate the expression of ER, PR, and HER2/neu with each other and to various clinicopathological parameters as follows: age of the patient, histological grade, tumor size, and lymph node metastasis.

Materials and Methods

Hundred patients with a diagnosis of infiltrating ductal carcinoma breast were enrolled for the study. Written

informed consent was obtained from all patients. We analyzed the expression of ER, PR, and HER2/neu by immunohistochemistry (IHC), with each other and to various clinicopathological parameters.

Inclusion criteria

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

Exclusion criteria

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

Procedure

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.[14]

Preparation of slides

Paraffin sections were cut and mounted on silanized 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 to room temperature after removing from the Decloaking chamber and by placing the slides in Tris-Saline buffer. 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 [Figure 1].

Reporting

Reporting done as per ER/PR scoring system and criteria as per Allred scoring system[15]

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[16]

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 is required 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 test was used to determine the statistical significance between ER/PR status and HER2/neu status along with their correlation with various clinicopathological parameters such as patient's age, axillary lymph node status, tumor size, and tumor grade with respect to infiltrating ductal carcinoma

breast. A value of $P < 0.05$ was considered as statistically significant.

Results

Age

Patients were in the age group between 24 and 80 years, with mean age 55.28 years. The majority (66%) were in the older age group >50 years. About 96%, ER, and PR positive cases were of age >40 years. Majority HER2/neu positive were of age <40 years [Table 1]. It was statistically concluded that ER, PR, and HER2/neu expression was significantly correlated with age [Table 1].

Tumour size

The average tumor size was 4.3 cm. Majority of ER/PR positive (46%–47%) tumors were of size between 2 and 5 cm, and majority of HER2/neu positive (71.43%) tumors were of size <2 cm. Correlation of expression of ER, PR, and HER2/neu compared to tumor size [Table 2], was statistically significant.

Tumour grade

In our study, according to Nottingham Modified Bloom–Richardson System score, majority tumors were in Grade II (43%) followed by Grade III (31%) and then Grade I (26%). Majority of ER/PR positive (48%–49%) tumors were of Grade II, and the majority of HER2/neu positive (57.14%) tumors were of Grade III. Correlation of expression of ER, PR, and HER2/neu compared to tumor grade is shown in Table 3. It was concluded that ER/PR expression compared to tumor grade was statistically significant and HER/neu was not significant.

Axillary lymphnode status

All cases were evaluated for axillary lymph nodes metastasis and found that 38 patients had lymph nodes metastasis. Out of 63 ER-positive cases,

Table 1: Estrogen receptors, progesterone receptor and human epidermal growth factor receptor expression compared to age

Age (years)	ER			PR			HER2/neu		
	Positive	Negative	Total	Positive	Negative	Total	Positive	Negative	Total
<40	2	13	15	2	13	15	5	10	15
41-50	9	10	19	9	10	19	1	18	19
51-60	24	8	32	22	10	32	1	31	32
>60	28	6	34	25	9	34	0	34	34
Total	63	37	100	58	42	100	7	93	100
χ^2, df, P	25.305, 3, 0.000			18.051, 3, 0.000			19.363, 3, 0.000		

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

Table 2: Estrogen receptors, progesterone receptor and human epidermal growth factor receptor expression compared to tumour size

Tumour size (cm)	ER			PR			HER2/neu		
	Positive	Negative	Total	Positive	Negative	Total	Positive	Negative	Total
<2	23	6	29	21	8	29	5	24	29
2-5	30	13	43	27	16	43	2	41	43
>5	10	18	28	10	18	28	0	28	28
Total	63	37	100	58	42	100	7	93	100
χ^2, df, P	13.098, 2, 0.001			8.587, 2, 0.014			7.144, 2, 0.028		

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

Table 3: Estrogen receptors, progesterone receptor and human epidermal growth factor receptor expression compared to tumour grade

Grade	ER			PR			HER2/neu		
	Positive	Negative	Total	Positive	Negative	Total	Positive	Negative	Total
I	20	6	26	18	8	26	1	25	26
II	31	12	43	28	15	43	2	41	43
III	12	19	31	12	19	31	4	27	31
Total	63	37	100	58	42	100	7	93	100
χ^2, df, P	11.534, 2, 0.003			6.976, 2, 0.031			2.421, 2, 0.298		

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

Table 4: Expression of estrogen receptors, progesterone receptor and human epidermal growth factor receptor compared to axillary lymph node status

Lymph node status	ER			PR			HER2/neu		
	Positive	Negative	Total	Positive	Negative	Total	Positive	Negative	Total
Positive	25	13	38	23	15	38	2	36	38
Negative	38	24	62	35	27	62	5	57	62
Total	63	37	100	58	42	100	7	93	100
χ^2, df, *P*	0.205, 1, 0.651			0.161, 1, 0.689			0.284, 1, 0.594		

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

Table 5: Estrogen receptors and progesterone receptor expression compared to human epidermal growth factor receptor expression

ER/HER2/neu	HER2/neu positive	HER2/neu negative	Total	PR/HER2/neu	HER2/neu positive	HER2/neu negative	Total
ER positive	2	61	63	PR positive	2	56	58
ER negative	5	32	37	PR negative	5	37	42
Total	7	93	100	Total	7	93	100
κ, ASE, *P*	0.079, 0.048, 0.058			−0.072, 0.048, 0.102			

ER: Estrogen receptors, PR: Progesterone receptor, HER2/neu: Human epidermal growth factor receptor, ASE: Asymptotic standard error

25 had positive axillary lymph nodes, whereas out of 58 PR positive cases 23 had positive axillary lymph nodes. Out of 7 HER2/neu positive cases, 2 had positive axillary lymph nodes. It was concluded that correlation of expression of ER, PR, and HER2/neu compared to axillary lymph node status [Table 4], was not significant.

Oestrogen receptor status

Sixty-three tumors were ER-positive and 37 were ER negative. ER-positive tumors showed weak, moderate to strong nuclear positivity in >1% of tumor cells.

Progesterone receptor status

Fifty-eight tumors were PR positive and 42 were PR negative. PR positive cases showed weak, moderate to strong nuclear positivity in >1% of tumor cells.

Out of 100 cases, 58 cases were ER and PR positive, 37 cases negative and 5 cases showed different expressions of ER and PR. On statistical analysis, it was observed that $\kappa = 0.854$; asymptotic standard error = 0.053; $P = 0.000$ and using kappa as a measure of agreement, it was concluded that expressions of ER and PR agree significantly to each other.

HER2/neu expression

Seven patients were HER2/neu positive, and 93 were HER2/neu negative. Only 2 cases were ER, PR, and HER2/neu positive. A total of 32 cases were both ER and HER2/neu negative. Sixty-Six cases showed different expressions of ER and HER2/neu [Table 5]. Out of 100 cases, only 2 cases were both PR as well as HER2/neu positive, 38 cases were both PR and HER2/neu negative, and 60 cases showed different expressions of PR and HER2/neu. On statistical analysis using kappa as measure of agreement, it is concluded that expressions of ER/PR and HER2/neu do not agree with each other.

Discussion

Breast cancer is leading cancer in women accounting for 25% of all cases worldwide and leading cause of death due to carcinoma in women. It is more common in developed countries.[1,2,5,17] Outcomes for breast cancer vary greatly depending on the cancer type, extent of disease and person's age. Five years survival rates in the developed world are high, 80% and 90%, in England and the United States, respectively.[18] In developing countries, survival rates are poor. This can be attributed to the lack of effective screening programmes and lack of awareness regarding signs and symptoms of breast lump, which leads to advanced disease with larger tumor size and nodal involvement at presentation.[19]

Data from India indicate that among females, the most common site of cancer is the cervix, with the second most common site being the breast. The mainstay of breast cancer treatment is surgery when a tumor is localized, followed by chemotherapy (when indicated), radiotherapy and for ER and PR positive tumors, adjuvant hormonal therapy.[4] ER, PR, and HER2/neu are immunohistochemical markers of prognosis as well as predictors of response to therapy. At present also, determining ER, PR, and Her2/neu receptor status in breast cancer have become common practice as there is a survival advantage for patients with hormones receptor positive status by treatment with adjuvant hormonal or

chemotherapeutic regimens. Patients with ER PR positive tumors have a better prognosis than patients with ER PR negative tumors.[13]

The present study was conducted to observe the correlation of expression of ER, PR, and HER2/neu with each other and to various clinicopathological parameters:-age of the patient, histological grade, tumor size, and lymph node metastasis.

Age

More than two-thirds of breast cancer cases are diagnosed in women aged 50 years and older; the majority of these cases are in developed countries. For women aged 15–49 years, twice as many breast cancer cases are diagnosed in developing countries than in developed countries.[20] In countries where mammography is available and affordable, adherence to recommendations for routine screening is associated with reduced mortality from breast cancer.[20]

In the present study, infiltrating ductal carcinoma seen in the age group between 24 and 80 years, with mean age 55.28 years is similar to study conducted by Sengal *et al*.[5] and Kaul *et al*.[21] Majority of ER and PR positive cases were of age >60 years, as seen in a study conducted by Alzaman *et al*.[22] about 71% HER2/neu positive were of age <40 years, similar to Alzaman *et al*.[22] observations. A significant correlation was observed between the age of the patient and ER (P = 0.000) and PR (P = 0.000) expression as shown in studies by Dodiya *et al*.,[23] and Ghosh *et al*.[24] Significant correlation was also observed between the age of the patient and HER2/neu expression (P = 0.000), similar to study conducted by Ramić *et al*.[25]

Tumor size

Tumor size was 0.1–12 cm, with average size 4.3 cm. Forty-three had sizes ranging from 2 to 5 cm. 47.61% of ER-positive and 46.55% of PR positive tumors were of size 2–5 cm. 71.43% of HER2/neu tumors were of size <2 cm. There was seen significant correlation between tumor size and ER (P = 0.001), PR (P = 0.014) expression in the present study. Similar to Almasri and Hamad[26] study, a significant correlation was seen between tumor size and HER2/neu expression (P = 0.028) in the present study.

Tumour grade

Forty-three tumors were Grade II, 31 Grade III and 26 Grade I. Majority of ER-positive (49.21%) and the majority of PR positive (31.03%) tumors were of Grade II, but the majority of HER2/neu positive (57.14%) tumors were of Grade III. A study conducted by Siadati *et al*.[27] showed similar results. There was seen significant correlation between tumor grade with ER (P = 0.003) and PR (P = 0.031). The study done by Dodiya *et al*.[23] showed similar results. No association was seen between tumor grade and HER2/neu expression (P = 0.298) similar to study done by Dodiya *et al*.[23]

Axillary lymph node status

Metastasis in axillary lymph nodes was seen in 38% of patients. Out of ER and PR positive cases about 39% had positive axillary lymph nodes positive for metastasis. About 28.57% of HER2/neu positive cases had positive axillary lymph nodes for metastasis. Study conducted by Ali *et al*.[28] showed similar results. As shown in Table 4, no significant correlation was observed between axillary lymph node status with ER (P = 0.651), PR (P = 0.689), and HER2/neu (P = 0.594) expression, similar to studies conducted by Azizun-Nisa *et al*.[29]

Receptor positivity

In the present study, ER positivity was 63%, closely matched the results of the study conducted by Idirisinghe *et al*.[30] and PR positivity was 58%, closely matched the results of the study conducted by Engstrøm *et al*.[31] HER2/neu positivity was only 7% was much lower as compared to other studies. The possible explanation for this is due to variations in different populations. In addition, HER2/neu assay results are influenced by multiple biologic, technical and performance factors. Since many aspects of HER2/neu assays have not been standardized, the effects of these disparate influences could not be isolated. ER and PR correlated with each other (P = 0.000), whereas expression of HER2/neu was inversely related to ER (P = 0.058) and PR expression (P = 0.102). Similar results were found in studies conducted by Siadati *et al*.,[27] Maha[32] etc.

Conclusions

Invasive ductal carcinomas of the breast was seen in the age of 24–80 years, with a mean age was 55.28 years. The maximum number of cases were seen in the age above >50 years (66%). Majority of tumors were ER and PR positive and HER2/neu negative. Majority of ER and PR positive tumors were of Grade II; whereas, the majority of HER2/neu positive tumors were of Grade III. The present study confirmed that ER and PR are correlated with age, tumor size, and tumor grade but not with lymph node status. HER2/neu expression correlated with age and tumor size but not with tumor grade and lymph node status. ER and PR expression correlated with each other, but none were correlated with HER2/neu. ER and PR positive cases may have a favorable outcome with adjuvant hormonal therapy.

Assessment of hormone receptors for clinical management of breast cancer patient is strongly recommended to provide prognostic information and therapeutic.

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Conflicts of interest

There are no conflicts of interest.

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