Retrospective Analysis of Immunohistochemical Expression of Breast Cancer in North West India

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Abstract

Background: Breast cancer remains the leading cancer in women worldwide with increasing incidence in India. The common histologic type is Infiltrating Ductal Carcinoma of Breast. Estrogen receptor (ER), progesterone receptor (PR), and human epidermal growth factor receptor-2 (HER2/neu) are Immunohistochemical markers of prognosis and predictors of response to therapy useful in the management of breast cancer.

Aims and Objectives: This study evaluates the ER, PR, and HER2/neu expressions in invasive ductal carcinomas of the breast by immunohistochemistry and explores the correlation of these markers to each other and to various Clinicopathological parameters like age of the patient, histological grade, tumor size, and lymph node metastasis.

Materials and Methods: This study was conducted on all the cases of infiltrating ductal carcinoma diagnosed from January 2016 upto January 2021 at a tertiary care hospital. Immunohistochemical staining was done for ER, PR, and HER2/neu expressions and interpretation done using Allred scoring system for ER/PR and the American Society of Clinical Oncology/College of American Pathologists guidelines for HER2/neu. The correlation of these markers with age, tumor size, axillary lymph node status and tumor grade was done.

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

Results: The present study confirmed that the majority of the tumors were ER, PR positive and that ER correlated with the tumor size, but not with age, tumor grade and lymph node status. HER2/neu expression didn't show any correlation with various clinicopathological parameters.

Conclusion: ER and PR positive cases usually have a favorable outcome with adjuvant hormonal therapy.

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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

One of the major causes of mortality and morbidity in the women worldwide is breast carcinoma. [1] Amongst the various types of breast carcinomas, infiltrating ductal carcinoma is the most common histological type .[2] A lot of work is being done for better treatment and improving the quality of life of patients. Surgery followed by chemotherapy and radiotherapy forms the mainstay of breast cancer treatment when the tumor is localized. In case of estrogen receptor (ER) and progesterone receptor (PR) positive tumors, the treatment is adjuvant hormonal therapy.[3] If diagnosed at an early stage breast cancer is curable. The various morphological prognostic factors include tumor size, grade, and axillary lymph node metastasis. More importance is given to the molecular prognostic factors because many patients with early-stage breast cancer have micro-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 molecular biomarkers that are used in the management of cases breast cancer. These are most commonly used for determining the response to treatment & outcome. [5] Both predictive and prognostic markers are used in the management of breast cancer. Prognostic markers are those which influence the overall outcome such as chances of recurrence. Predictive markers determine the chances of benefit from a particular treatment. ER, PR, and HER2/neu are both predictive and prognostic markers. [6]

Nowadays, determining ER, PR, and HER2/neu receptor status in breast cancer is a common practice especially for patients with hormones receptor positive status. Such patients respond well to treatment with adjuvant hormonal or chemotherapy regimens. The cases with strong ER-positivity benefit from hormonal 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 are known to have a better prognosis. [13]

The aim of this study is to assess the breast carcinomas for expression of estrogen receptor, progesterone receptor and HER-2/neu reactivity by immunohistochemistry and correlate their reactivity pattern with each other and to parameters like age of the patient, tumor size, grade and lymph node metastasis.

Materials and methods

This study was done over a time period of five years from January 2016 to January 2021. Only mastectomy excision specimens with the diagnosis of infiltrating ductal carcinoma breast confirmed on histopathology were included in the study. Core biopsies were excluded from the study. Patients with benign, inflammatory breast lesions, who received neoadjuvant chemotherapy and histological types other than infiltrating ductal carcinoma were also excluded.

So eventually a total of 35 cases with a diagnosis of infiltrating ductal carcinoma breast were enrolled for the study. The expression of ER, PR, and HER2/neu was analysed by immunohistochemistry (IHC), with each other and to various clinicopathological parameters like age of the patient, tumor size, grade and lymph node metastasis.

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 the standard procedure.[14]

The slides were dipped into a solution of hematoxylin diluted 1:1 with distilled water for staining. The slides were then washed in distilled water and dehydrated by dipping in ethanol. Lastly the slides were washed in xylene and coverslip was applied for viewing and reporting under light microscopy.

Method of reporting by IHC:

PR and ER positivity is assessed using Allred Score system. The Allred score combines the proportion of positive cells and the intensity of the reaction produced in most of the specimen. The proportion score and intensity score are added together for a total score. [15]

Proportion score (ER/PR)

0 – No cell +ve.

1 – ≤1% of cells +ve.

2 – 1%–10% of cells +ve.

3 – 11%–33% of cells +ve.

4 – 34%–66% of cells +ve.

5 – 67%–100% of cells +ve.

Intensity score

0 – Negative.

1 – Weak.

2 – Intermediate.

3 – Strong

Total score: proportion score + intensity score

Interpretation: Score of 0-2 is taken as Negative and 3-8 as Positive.

HER2/neu scoring was done as follows-

Score 0 = No membranous staining or membranous staining that is incomplete within < 10% of tumor cells.

Score +1 = Weak and incomplete membranous staining is faint and within more than 10% tumor cells.

Score +2 = Circumferential membrane staining that is incomplete and or weak/moderate and in more than 10% of the tumor cells Or complete and circumferential membrane staining that is intense and within 10% of tumor cells.

Score+3 = Circumferential membrane staining that is complete, intense and in more than 10% of tumor cells.

Score 0 and 1 are negative. Score 2 is equivocal which has to be confirmed by FISH.As we found in earlier studies that majority of equivocal cases came out to be negative we considered them negative, as FISH was not available at our centre. Only 3+ on IHC was taken as positive. [16]

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 30 and 70 years, with mean age 55 years. The majority (83%) were in the older age group >41 years. About 75%, ER positive cases and 66 % PR positive cases were of age > 41 years. Majority HER2/neu positive were of age < 51 years. It was statistically concluded that ER, PR, and HER2/neu expression was not significantly correlated with age [Table 1].

Tumour Size

The average tumor size was 4.5 cm. Majority of ER positive tumors (88%) and PR positive tumors (78%) were of size between 2 and 5 cm, and majority of HER2/neu positive (57.14%) tumors were of size between 2 cm and 5 cm. Correlation of expression of ER compared to tumor size was statistically significant. [Table 2]

Tumour Grade

In our study, according to Nottingham Modified Bloom–Richardson System score, majority tumors were in Grade II (77.14%) followed by equal number of Grade III & Grade I (11.42%). Majority of ER positive tumors (94%) & all of PR positive tumors (100%) were of Grade II, and the majority of HER2/neu positive (86%) tumors were also of Grade II. 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 and HER/neu compared to tumor grade was statistically not significant.

Axillary Lymph Node Status

All cases were evaluated for axillary lymph nodes metastasis and it was found that all 35 patients had lymph node metastasis. Out of 16 ER-positive cases, 9 had positive axillary lymph nodes, whereas out of 9 PR positive cases 5 had positive axillary lymph nodes. Out of 14 HER2/neu positive cases, 10 had positive axillary lymph nodes. It was concluded that correlation of expression of ER, PR, and HER2/neu compared to axillary lymph node status, was not significant. [Table 4]

Oestrogen Receptor Status

Sixteen tumors were ER-positive and 19 were ER negative. ER-positive tumors showed weak, moderate to strong nuclear positivity in >1% of tumor cells. [Figure-1]

Progesterone Receptor Status

Nine tumors were PR positive and 26 were PR negative. PR positive cases showed weak, moderate to strong nuclear positivity in >1% of tumor cells.

Out of 35 cases, 16 cases were ER positive, 9 cases were PR positive and 10 cases showed different expressions of ER and PR. On statistical analysis, it was observed that P = 0.003 and hence, it was concluded that expressions of ER correlated significantly with tumor size. However the expressions of ER, PR and HER-2 neu didn't show any significant correlation with each other. [Figure-2]

Her2-neu Expression

Fourteen patients were HER2/neu positive, and 21 were HER2/neu negative. Only 6 cases were ER and HER2/neu positive. A total of 11 cases were both ER and HER2/neu negative. Eighteen cases showed different expressions of ER and HER2/neu [Table 5]. Out of 35 cases, only 4 cases were both PR as well as HER2/neu positive, 16 cases were both PR and HER2/neu negative, and 15 cases showed different expressions of PR and HER2/neu. [Table 6] [Figure-3]

On statistical analysis, it was therefore 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]

In India, among females, the most common site of cancer is cervix, while breast cancer being the second most common. 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 chemotherapeutic regimen. 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. [Tables1-6] [Figures 1-3]

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. 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 is seen in the age group between 24 and 80 years, with mean age 55 years is similar to study conducted by Sengal et al. and Kaul et al. [5] [21] Majority of ER and PR positive cases were of age >40 years and about 71% HER2/neu positive were of age <40 years, similar to Alzaman et al. observations. [22] A non-significant correlation was observed between the age of the patient and ER (P = 0.951) and PR (P = 0.293) expression which was in contrast to the studies by Dodiya et al. and Ghosh et al. [23] [24] A non-significant correlation was also observed between the age of the patient and HER2/neu expression(P = 0.059), which was dissimilar to study conducted by Ramić et al.[25]

Tumor Size

Tumor size was 1–10 cm, with average size 4.5 cm. Twenty two had sizes ranging from 2 to 5 cm. 84% of ER-positive and PR positive tumors were of size 2–5 cm. 57.14% of HER2/neu tumors were of size 2–5 cm. There was seen significant correlation between tumor size and ER (P = 0.003). However a non-significant correlation was seen between tumor size and HER2/neu expression (P = 0.597) and PR (P = 0.155) expression in the present study. These findings were similar to Almasri and Hamad study.[26]

Tumour Grade

Twenty seven tumors were Grade II, four were Grade III and four were Grade I. Majority of ER-positive (15) and the majority of PR positive (09) tumors were of Grade II, but the majority of HER2/neu positive (12) tumors were of Grade II. A study conducted by Siadati et al. showed somewhat similar results.[27] However there was seen a nonsignificant correlation between tumor grade with ER (P = 0.078) and PR (P = 0.166). The study done by Dodiya et al. showed contrasting results.[23] No association was seen between tumor

grade and HER2/neu expression (P = 0.615) similar to study done by Dodiya et al.[23]

Axillary Lymph Node Status

Metastasis in axillary lymph nodes was seen in all the 35 patients. Out of ER positive cases and PR positive cases about 56% had positive axillary lymph nodes positive for metastasis. About 71.42% of HER2/neu positive cases had positive axillary lymph nodes for metastasis. Study conducted by Ali et al. showed somewhat similar results. [28] As shown in Table 4, no significant correlation was observed between axillary lymph node status with ER (P = 0.739), PR (P = 1.000), and HER2/neu (P = 0.311) expression, similar to studies conducted by Azizun-Nisa et al.[29]

Receptor Positivity

In the present study, ER positivity was 46% and PR positivity was 26%, closely matched the results of the study conducted by Idirisinghe et al. [30] HER2/neu positivity was 40% which was higher as compared to other studies.[31-34] The possible explanation for this is due to variations in different populations and small sample size. 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.

On statistical analysis, it was observed that expressions of ER correlated significantly with tumor size(P = 0.003). However the expressions of ER/PR and HER-2 neu didn't show any significant correlation with each other (P = 1.000) Similar results were found in studies conducted by Siadati et al. [27]

Conclusion

Invasive ductal carcinomas of the breast was seen in the age of 24–80 years, with a mean age was 55 years. The maximum number of cases were seen in the age above >50 years (66%). Majority of tumors were ER/PR positive and HER2/neu positive. Majority of ER/PR and HER2/neu positive tumors were of Grade II. The present study confirmed that ER correlated with tumor size, but not with age, tumor grade and lymph node status. HER2/neu expression also didn't show correlation with age, tumor size, 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 and therapeutic information.

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Legend Tables and Figures

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

AGE	ER		TOTAL	PR		TOTAL	HER2-NEU		TOTAL
	+	_		+	_		+	_	
<40 YRS	04	02	06	03	03	06	03	03	06
41-50 YRS	05	07	12	04	08	12	08	04	12
51-60 YRS	05	08	13	01	12	13	03	10	13
>61 YRS	02	02	04	01	03	04	00	04	04
TOTAL	16	19	35	09	26	35	14	21	35
P Value	0.951 (NS)			0.293 (NS)			0.059 (NS)		

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

TUMOR SIZE	ER		TOTAL	PR		TOTAL	HER2-NEU		TOTAL
	+	_		+	_		+	_	
< 2 CM	02	03	05	02	03	05	03	02	05
2-5 CM	14	07	21	07	15	22	08	13	21
>5 CM	00	09	09	00	08	08	03	06	09
TOTAL	16	19	35	09	26	35	14	21	35
P Value	0.003 (S)			0.155(NS)			0.597(NS)		

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

TUMOR GRADE	ER		TOTAL	PR		TOTAL	HER2-NEU		TOTAL
	+	_		+	_		+	_	
GRADE I	01	03	04	00	04	04	01	03	04
GRADE II	15	12	27	09	18	27	12	15	27
GRADE III	00	04	04	00	04	04	01	03	04
TOTAL	16	19	35	09	26	35	14	21	35
P Value	0.078(NS)			0.166(NS)			0.615(NS)		

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

LYMPH NODE STATUS	ER		TOTAL	PR		TOTAL	HER2-NEU		TOTAL
	+	_		+	_		+	_	
POSITIVE	09	12	21	05	16	21	10	11	21
NEGATIVE	07	07	14	04	10	14	04	10	14

TOTAL	16	19	35	09	26	35	14	21	35
P value	0.739(NS)			1.000(NS)			0.311(NS)		

Table 5: RELATION B/W ER & HER 2-NEU-Estrogen receptors expression compared to human epidermal growth factor receptor expression

ER / HER 2-NEU	HER 2-NEU +	HER 2-NEU _	TOTAL
ER +	6	10	16
ER _	8	11	19
TOTAL	14	21	35
P value	1.000(NS)		

Table 6: RELATION B/W PR & HER 2-NEU-Progesterone receptor expression compared to human epidermal growth factor receptor expression

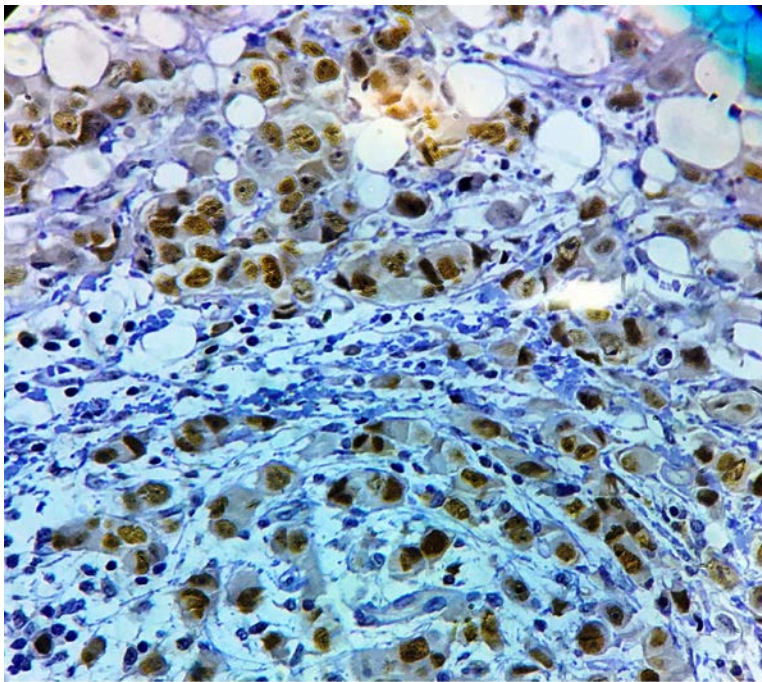| PR / HER 2-NEU	HER 2-NEU +	HER 2-NEU _	TOTAL
PR +	4	5	9
PR _	10	16	26
TOTAL	14	21	35
P value	1.000 (NS)		

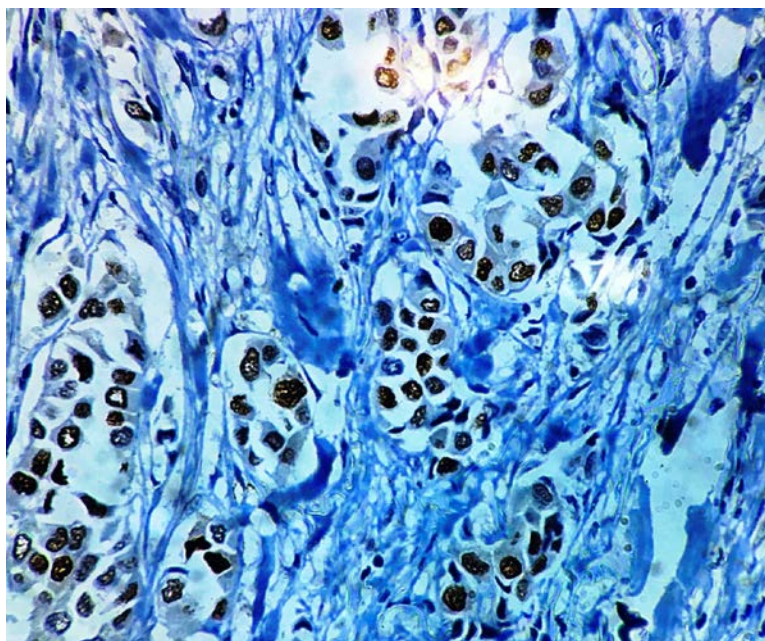Figure 1: Invasive ductal carcinoma of breast with ER positive nuclear staining.(40x)

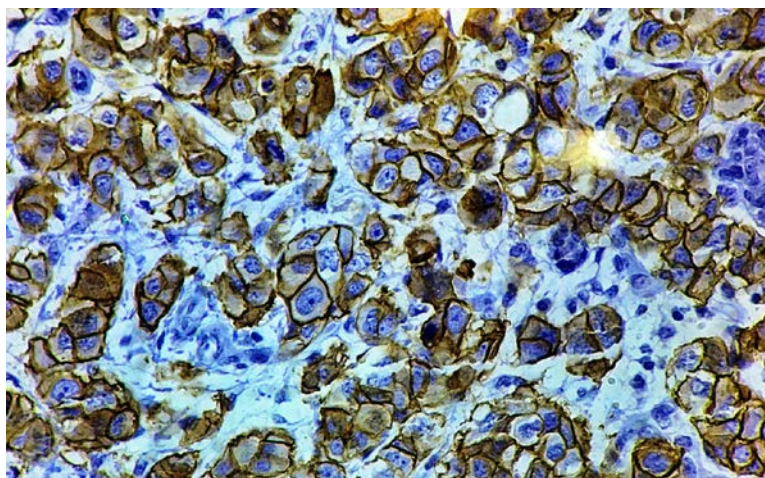Figure 2: Invasive ductal carcinoma of breast with PR positive nuclear staining.(40x)

Figure 3: Invasive ductal carcinoma of breast with strong circumferential membrane staining in more than 10% tumor cells. (40x)