Original article:

The incidenceof ER, PR and HER2/neuin breast cancer cases in ruralThiruporur, Kancheepuram district - Tamilnadu

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Abstract

Aim: Receptor status study in breast cancer is uncommon in rural population in India. Our aim in this study is to evaluate the expression of hormone receptors - Estrogen receptor (ER), Progesterone receptor (PR) along with Human Epidermal growth receptor 2 (HER2/neu) expression in breast cancer cells and distribution with regards to age, menopausal status, tumor size, histological grading and lymph node status.

Materials and Method: This is a six-year retrospective study from 2011-2016. The sample consisted of 27 patients(26-female and 1-male) who underwent treatment in the town of Thiruporur, Kancheepuram district, Tamilnadu. In routine H&E sections diagnosed as breast cancer IHC was used to evaluate the expression of ER, PR and HER2/neu.

Results: The proportion of each type in our patient population was ER/PR 15 cases (56%) and HER2/neu was 7 cases (26%). 8 cases (30%) were negative for all the 3 receptors and distribution with regards to age, menopausal status, tumor size, histological grading and lymph node status was also studied.

Conclusions:Hormone receptors were inversely related to HER2/neu. ER and PR were directly related. Breast cancer patients should be subjected to immunohistochemistry to find out the status of hormone receptors for recommending hormone therapy and Herceptin for overexpression of HER2/neu.

Keywords: Breast Cancer, Estrogen receptor, Progesterone receptor, HER2/neu Immunohistochemistry.

Introduction

Breast cancer is the second common cancer in India next to carcinoma cervix[1]. In India, every year nearly 80000 cases of breast cancer are detected[2]. Breast cancer is mainly classified as three types based on receptor expression by neoplastic cells in breast cancer as Estrogen receptor (ER), Progesterone receptor (PR) and HER2/neu (Human Epidermal Growth Factor receptor). A sub-group of tumors with ER, PR and HER2 negative are called Triple negative breast cancer. Immunohistochemistry (IHC) plays an important role in differentiating various types of breast cancer.

Estrogen receptor, Progesterone receptor expression is a predictor of response to hormone therapy and prognosis of the patient[3]. Hormone receptor status by IHC technique is useful because of addition of selective Estrogen receptor modulator to the conventional surgery or chemotherapy, improves the disease-free survival period in women with hormone receptor positivity [4].

Some breast cancer cases are positive for HER2/neu. It is a proto-oncogene amplified in some human

breast cancers. It encodes a tyrosine kinase receptor - epidermal growth factor receptor (erbB2), present in cell membrane. Breast cancer cases with HER2 over expression have bad prognosis. They respond well to monoclonal antibody Herceptin/Trastuzamab. HER2 is prognostic and predictor of therapeutic response in breast carcinoma[5].

Triple Negative tumors lacking the expression of ER, PR and HER2/neu, respond only to chemotherapy. They have more aggressive clinical behavior[6].

The aim of the study is to find out incidences of ER, PR and HER2 expression and correlate them with the distribution of age, menopausal status, tumor size, histological grading and lymph node status in breast cancer cases. The study was a retrospective analysis of patient specimens who underwent treatment in rural Thiruporur, Kanchipuram District of Tamil Nadu.

Material and Methods

Study Subjects and Protocol

This study was conducted in Department of Pathology of our Institute after approval from the Institutional ethical committee.The sample set consists of 27 breast cancer cases referred from the surgical department from 2011 to 2016. The paraffin blocks of histopathologicallyproven invasive breast cancer were subjected for immunohistochemical expression study.

IHC Procedure

The kit used for immunohistochemistry was a polymer based detection system (Envision, Dako) using monoclonal antibodies for ER and PR; Rabbit anti-human ER alpha clone EPI, PgR 636 mouse monoclonal antibody, and Herceptin kit (HercepTest, Dako) containing polyclonal Antihuman CerbB2 oncoprotein per the manufacturer's instructions.

Control sample

Tumor tissue with normal breast tissue or non-neoplastic mammary epithelium was taken for internal positive control for ER/PR. External positive control for HER2/neu were cases of known invasive breast carcinoma with known HER2/neu over expression.Evaluation of staining and scoring was done by the author. Quick score system was used for ER and PR (Barns et al 1998)[7]. Nuclear staining was accessed for ER and PR. Membrane staining was assessed for HER2/neu. Dark brown colour of nuclei was interpreted as positive score for Estrogen and Progesterone receptor.

Scoring System for Hormonal receptors

Table 1 and table 2 shows the scoring system used to score the proportion and intensity of the ER&PR staining. The individual proportion and intensity scoring was summed up to calculate the overall ER&PR scoring. The maximum overall ER&PR score is 8. An overall score of greater than 2 is considered ER&PR positive.

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Table 1: ER & PR Scoring (Proportion of nuclear staining)

Scoring	Proportion of Staining
0	No nuclear staining
1	<1% nuclear staining
2	1-10% nuclear staining
3	11-33% nuclear staining
4	34-66% nuclear staining
5	67-100% nuclear staining

Table 2: ER & PR SCORING (Intensity of nuclear staining)

Scoring	Intensity of staining
0	No staining
1	Weak staining
2	Moderate staining
3	Strong staining

Scoring System for HER2/neuReceptor

Her2/neu was scored as positive if greater than 30% of tumor cells showed complete membrane staining[8] (3+). 0 to +1 are considered negative. 2+ is considered negative unless verified by fluorescent in situ hybridization (FISH).

Results

All 27 specimens were subject for immunohistochemistry analysis and examined under light microscope. The dark brown color of the nuclei was interpreted as positive for ER&PR as per the quick scoring system. A total score of more than 2 was considered as positive. 26 of the sample cases were from female. One was from a male. The histological type of breast carcinoma was Infiltrating Ductal Carcinoma 'Not Otherwise Specified (NOS)' in 26 cases. One was mucinous carcinoma. Table 3 shows the ER&PR and Her2/neu scoring results for all 27 samples. ER+ staining was observed in 15 cases. ER+/PR+staining was seen in 8 cases. ER+/PR+ along with HER2/neu+ was seen in 3 cases. HER2/neu+ expression was seen in a total of 7 cases. Triple negative staining was seen in a total of 8 cases. Figure 1 shows infiltrating ductal breast carcinoma Hematoxylin & Eosin Stain. Figure 2, 3 and 4 illustrates the ER+, PR+ and HER2/neu positive staining of malignant cells of breast carcinoma by IHC.

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Table 3: ER&PR and Her2/neuScoring Results

S.no	ER			ER+/ER-	PR			PR+/PR-	HER2/neu
	Proportion Score	Intensity Score	Total Score		Proportion Score	Intensity Score	Total Score		
1.	0	0	0	ER-	0	0	0	PR-	Negative
2.	2	2	4	ER+	0	0	0	PR-	Negative
3.	0	0	0	ER-	0	0	0	PR-	Negative
4.	5	3	8	ER+	1	2	3	PR+	3+
5.	0	0	0	ER-	0	0	0	PR-	3+
6.	1	2	3	ER+	0	0	0	PR-	Negative
7.	0	0	0	ER-	0	0	0	PR-	Negative
8.	2	3	5	ER+	0	0	0	PR-	Negative
9.	0	0	0	ER-	0	0	0	PR-	Negative
10.	1	2	3	ER+	1	2	3	PR+	Negative
11.	3	3	6	ER+	1	2	3	PR+	3+
12.	2	3	5	ER+	0	0	0	PR-	Negative
13.	0	0	0	ER-	0	0	0	PR-	Negative
14.	0	0	0	ER-	0	0	0	PR-	3+
15.	0	0	0	ER-	0	0	0	PR-	3+
16.	0	0	0	ER-	0	0	0	PR-	3+
17.	5	3	8	ER+	0	0	0	PR-	Negative
18.	0	0	0	ER-	0	0	0	PR-	Negative
19.	5	3	8	ER+	0	0	0	PR-	Negative

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S.no	ER			ER+/ER-	PR			PR+/PR-	HER2/neu
	Proportion Score	Intensity Score	Total Score		Proportion Score	Intensity Score	Total Score		
20.	3	3	6	ER+	2	2	4	PR+	Negative
21.	0	0	0	ER-	0	0	0	PR-	Negative
22.	5	3	8	ER+	5	3	8	PR+	Negative
23.	2	3	5	ER+	1	2	3	PR+	Negative
24.	2	3	5	ER+	2	3	5	PR+	Negative
25.	1	2	3	ER+	0	0	0	PR-	Negative
26.	0	0	0	ER-	0	0	0	PR-	Negative
27.	1	2	3	ER+	5	3	8	PR+	3+

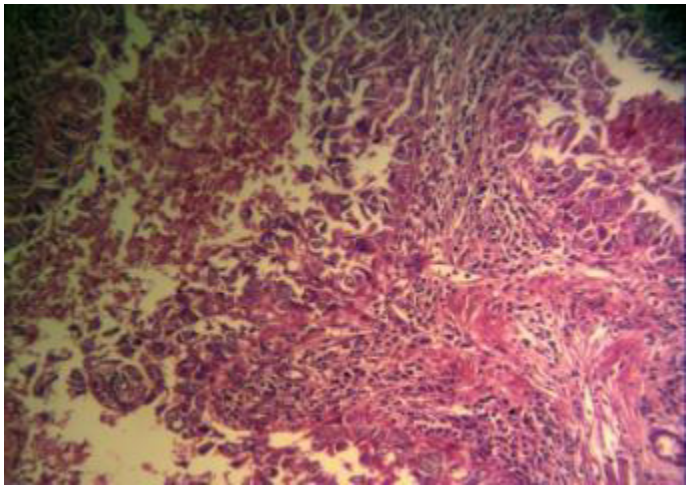

Figure 1 Photomicrograph of breast carcinoma Hematoxylin&Eosin Stain (10X magnification)

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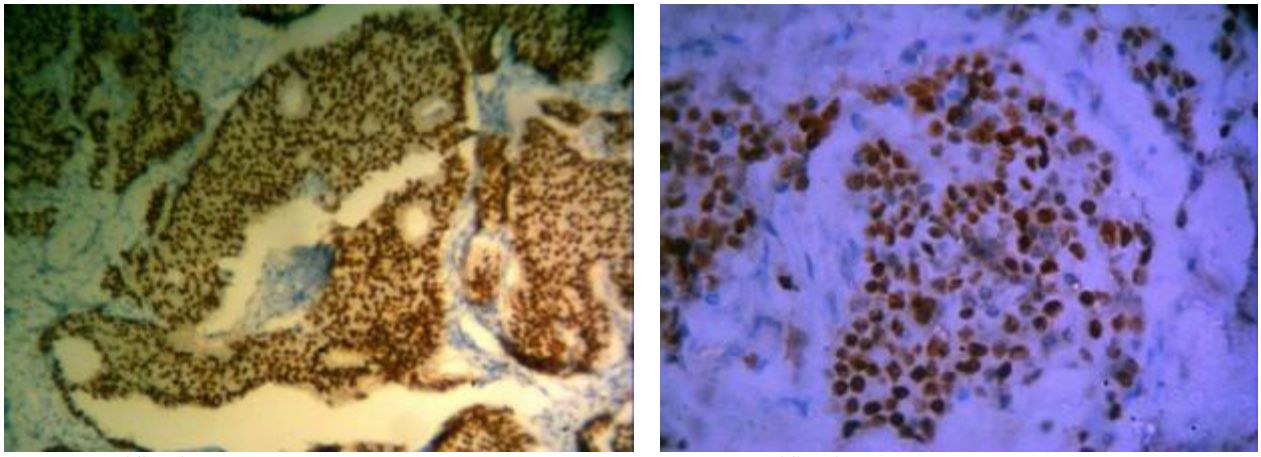
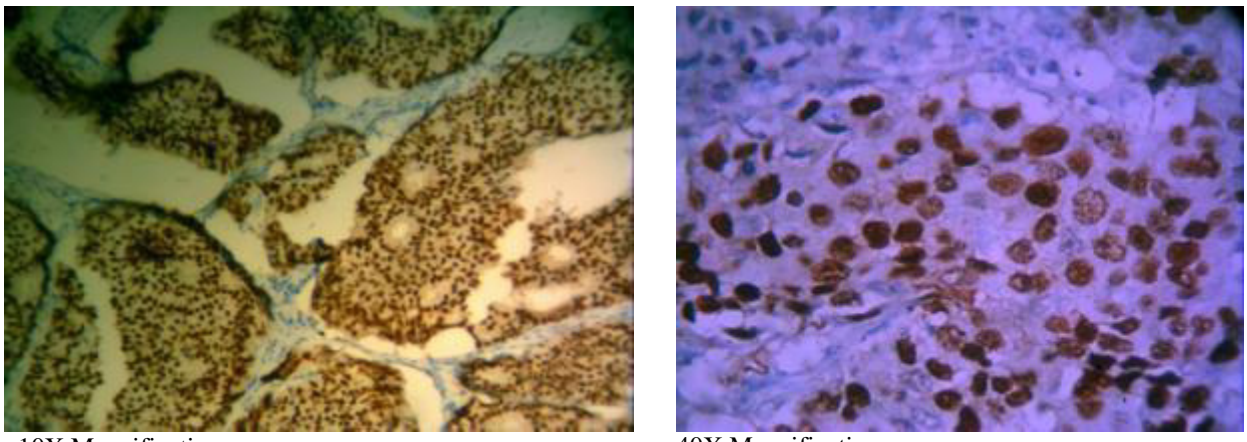


10X Magnification 40X Magnification

Figure 2 Photomicrograph illustrates the pattern of ER+ nuclei of malignant cells of breast cancer by IHC



10X Magnification 40X Magnification

Figure 3 Photomicrograph illustrates the pattern of PR+ nuclei of malignant cells of breast carcinoma by IHC (10X magnification)

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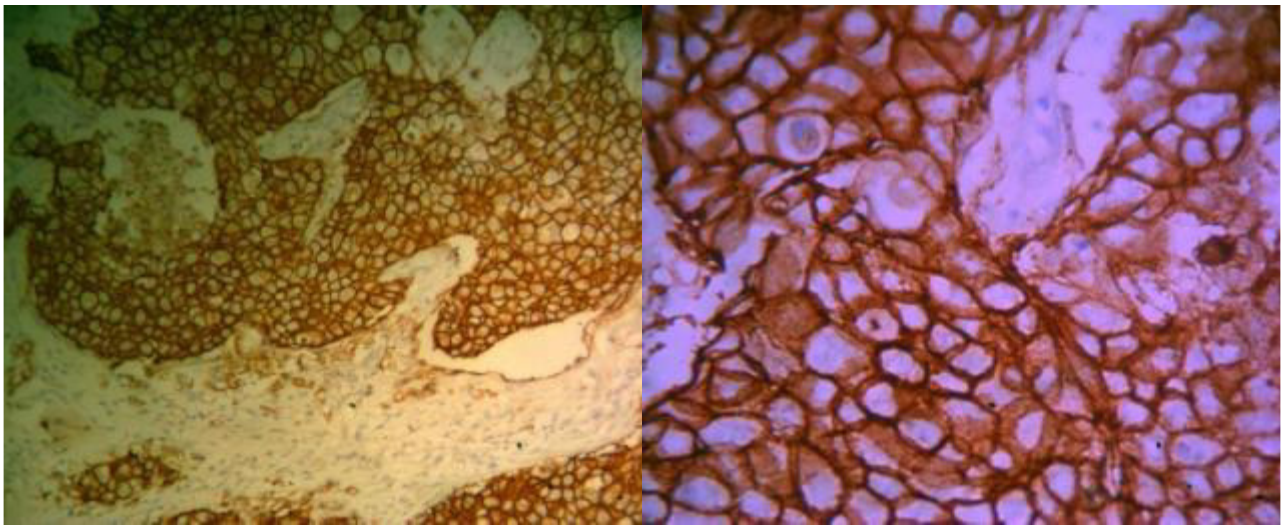

10X Magnification 40X Magnification

Figure 4Photomicrograph illustrates the pattern HER2/neu + of cell membrane of malignant tumor cells of breast carcinoma IHC (10X magnification)

Table 4 presents the age-wise distribution of the tumor. The mean age of all the cases was 54.1 years ranging from 30 to 77 years. The mean age of the patients with HER2/neu expression was 43.1 years which is 8.7 years younger than the mean age of HER2/neu negative cases (51.8). The mean age of patients with positive ER/PR expression was 51.8 as opposed to 43.1 among patients lacking ER expression.Tumors with strong HER2/neu expression tend to be larger than that which lack expression with mean sizes of 4.5cm and 3cm respectively. Among patients with tumor size more than 5cm, 57.1 % were HER2/neu compared to 42.8% with tumor size between 2-5 cm. The mean size of ER/PR tumors was 2.6 cm versus 4.8 cm for those lacking ER.Histological grading was done in 22 out of 27 cases. HER2/neu expression was seen in similar proportion of grade 2 and grade 3 cases. No correlation was detected between grade of tumors and expression of ER and PR.Lymph nodes were involved in 17 cases. They were not involved in 6 cases. Details were not available in 4 cases. The number of premenopausal woman in HER2/neu cases was 5 (71.4%). Postmenopausal cases were 2 (28.5%). In ER/PR case premenopausal woman were 5 (33.3%), Postmenopausal were 9 (64.28%). One was male.

Table 4: Age wise distribution of the tumor

S. No	Age group (years)	Carcinoma breast
1.	< 40	7
2.	41 - 60	15
3.	> 60	5
	Total number of cases	27

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Table 5 shows distribution between Estrogen receptors and Progesterone receptors. A total of 7 cases was positive for ER alone. 8 cases were positive for both ER and PR. Both receptors were negative in total of 8 cases.

Table 5: Distribution of Estrogen Receptors with Progesterone Receptors

S. No	Group	No of cases
1	ER+ Alone	7
2.	ER+/PR+	8
3.	ER-/PR-	8

Table 6 shows HER2/neu overexpression in 7 cases while 20 cases were negative. Out of 7 cases, 3 cases were positive for ER+/PR+.

Table 6: Expression of HER2/neu in breast carcinoma

S.no	HER2/neu expression	No of cases	Percentage
1.	Positive	7	25.9
2.	Negative	20	70.1

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Table 7 shows an inverse relationship of ER, PR with HER2/neu. A total of 15 hormone receptor positive cases were observed. All of the hormone receptor positive cases typically had a tumor size of <3cm with mean age of 51 years at the time of diagnosis. A total of 12 hormone receptor negative cases were observed. HER2/neu positive cases had an average tumor size of 4.5 cm and mean age of 43.1 years at the time of diagnosis.

Table 7: Distribution of hormonal receptors with respect to HER2/neu

S.NO	Hormone Receptor status	HER2/neu	
		Positive	Negative
1.	Positive (15)	3	12
2.	Negative (12)	4	8

Discussion

This study was done to find out the incidence of ER, PR and HER2/neu overexpression in breast cancer cases visiting our Institute in rural Thiruporur, Kanchipuram District of Tamil Nadu. Distribution of different factors like age, menopausal status, tumor size, histological grading was done in hormone receptor (ER, PR) positiveand HER2/neu overexpression cases. Clinical and pathological variables may help in predicting prognosis and need for adjuvant therapy [9]. In this study 7 cases were positive for HER2/neu out of 27 cases (25.9%). Our data appears to be within the accepted rates of 20% to 30%[10,11,12,13,14]seen in previous studies. HER2/neuoverexpression incidence was higher in patients of lower age than the ER/PR positive group. The mean age of HER2/neu patients was 8 years less than those lacking HER2/neu expression. Patientspositive for breast carcinoma and are younger than 50 years of age are more likely to express HER/neu than patients older than 50[15]. Higher rate of HER2/neu overexpression was documented in

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older studies. Typically, tumors expressing HER2/neu were on average 1.5 cm larger than HER2/neu negative tumors. The number of lymph node involvement was not available in all patients, so it was impossible to correlate with lymph node status. No correlation was seen in histological grading.

Her2/neu patients were mostly premenopausal while in ER+/, PR +ve patients were mostly postmenopausal patients as reported in the literature [16]. Hormonal receptoroverexpression was seen in 55.5% cases. This value is less than the number reported in literature 60-70%[17]. This difference is likely attributed to the smaller number of patients (27 cases) and a higher percentage of patients less than 40 years of age (26%) evaluated in this study. These findings agree with other reports in the literature, which show an association between ER expression in breast carcinomas patients and age at the time of diagnosis [18, 19].In our study, we also found that ER/PR positive breast carcinoma patients were on average 1.5cm smaller than cases lacking ER expression. In ER positive tumors 2-5 cm size tumors were 80 %as opposed to tumors > 5 cm (20 %). These data are also in accordance with data reported [20].

In our study distribution of ER/PR with HER2/neu showed inverse relation in accordance with literature [21]. Out of total 7HER2/neu positive cases,20.0%of ER/PR positive were HER2/Neu positive (3 out of 15 cases) and 33.0% of ER/PR negative cases were HER2/neu positive (4 out of 12 cases)[21].As reported in the literature,higher number of ER and PR patients were postmenopausal, whereas over expression of HER2/neu was high in premenopausal women[16].

Conclusion

This retrospective analysis shows the importance of identifying the nature of breast carcinoma (Hormonal receptors vs. HER2/neu overexpression) in rural patients in recommending the appropriate treatment. Breast cancer patients should be subjected to immunohistochemistry to find out the status of hormone receptors for recommending hormone therapy. It is also important to identify the group of patients with HER2/neu overexpression so that they can avail benefit from therapy targeted against HER2/neu overexpression such as Herceptin/Trastuzumab.

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