Study of Hormone Receptors and Epidermal Growth Factor Expression in Invasive Breast Cancers in a Cohort of Western India

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Abstract The objective of study was to evaluate and correlate the pathological characteristics of breast cancer patients with estrogen receptor (ER), progesterone receptor (PR) and human epidermal growth factor receptor 2 (Her2/neu) detected by immunohistochemistry and/or fluorescent in situ hybridization method. We have conducted 2 year study of 204 cases of breast cancer at HCG-Medisurge Hospitals, Ahmedabad from 2009 to 2011. Significant correlation was found in ER and PR expression whereas no correlation was found in hormonal receptors and Her2/neu expression. ER and PR positivity increased with advancing age in breast carcinoma patients while not affecting Her2/neu expression. The expression of hormone receptors were higher in infiltrating lobular carcinoma and infiltrating duct carcinoma subtypes of breast carcinoma as compared to other subtypes such medullary and in situ carcinoma. High-grade carcinoma patients were predominantly ER/PR negative and Her2/neu positive as compared to lower grade breast carcinoma whereas high-stage carcinoma patients were ER/PR positive and Her2/neu positive as compared to lower stage breast carcinoma.

Keywords Estrogen receptors · Progesterone receptors · Human epidermal growth factor receptors

Introduction

Breast cancer is the commonest carcinoma of females all over the world and second leading cause of death.

Histological type, grade and stage, status of estrogen receptor (ER), progesterone receptor (PR) and human epidermal growth factor receptor type 2 (Her2/neu) are affecting the management and prognosis of breast cancer. ER, PR and Her2/neu are predictive and prognostic biomarkers of breast cancer, determining overall prognosis and predictive response to hormonal therapy (ER/PR) and trastuzumab (Her2/neu) [1]. ER and PR have been accepted as established procedure in the routine management of primary breast cancer patients along with Her2/neu [2]. Her2/neu is also called as c-erbB-2. Her2/neu is overexpressed in high-grade cancers and display lower responsiveness to hormone receptor modulators. Immunohistochemistry (IHC) and fluorescent in situ hybridization (FISH) are reliable ways to identify over expression or amplification of the ER/PR and Her2/neu gene. Hormone receptors (ER/PR) test is done routinely since hormone treatment (tamoxifene) has less side effects and it prevents recurrence in 25 % of cases [3]. The Her2/neu oncoprotein continues to be an important target in the development of variety of new cancer therapies, which include mAb-based therapy, small molecule drugs targeted at the internal tyrosine kinase protein of the Her2/neu oncoprotein, and vaccines (E75, GP2, AE37 vaccines). The most widely known Her2/neu directed therapy is trastuzumab [4]. Recently, US Food and Drug Administration (FDA) approved lapatinib for clinical use. Therefore, accurate determination of the ER/PR and Her2/neu status are extremely important in guiding therapy, and the reliability of diagnostic method used to determine ER/PR and Her2/neu status is critical in selecting the most appropriate patients for hormone and Her2/neu directed therapy. This study was designed to determine the hormone and epidermal growth receptors status and its correlation with histological type, grade, stage and age in breast cancer patients of Western region of India.

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Materials and Methods

Patients Selection

This study included 204 cases of operated breast cancer accessioned at the HCG-Medisurge Hospitals in the department of clinical research and development over a period of 24 months, from May 2009 to May 2011. ER/PR and Her2/neu receptors status was evaluated by the immunohistochemistry method. Her2/neu was also analyzed by FISH method only in cases in which receptor status was equivocal.

Histologic Examination

Histologic assessment of the tumor type, grade, stage and axillary node status was performed on routinely on 4–5 μ m thick haematoxylin and eosin stained sections of formalin-fixed, paraffin embedded tumors according to criteria outlined the World Health Organization classification of tumors.

Immunohistochemical Analysis (IHC) [5–7]

A single section showing both tumor and preferably with the adjacent uninvolved breast tissue was routinely submitted for immunohistochemistry, and stained with antibodies against ER (Immunotech, clone1D5) and PR (Immunotech, clone PR636). At molecular medicine, RLS, LSAB peroxidase based detection system is used for IHC. Tumor ER and PR status is reported using the Quick Scoring Method. Sections were interpreted positive if at least 1 % of tumor nuclei stained positive. Her2/neu IHC was performed using the antibodies (Immunotech, CB11) and results were interpreted according to ASCO/CAP (American Society of Clinical Oncology–College of American Pathology) guidelines.

Fluorescence In Situ Hybridization (FISH) [8]

The FISH technique is a morphology-driven slide-based deoxyribonucleic acid (DNA) hybridization assay using fluorescent-labeled probes. Both the hybridization steps and the slide scoring can be automated. The Path Vysion Her2/neu probe kit (Vysis) was used for FISH analysis. Tumors were interpreted as positive for Her2/neu gene amplification when the ratio of Her2/neu signals divided by chromosome 17 centromere signals was equal to or greater than 2.2. Although controversial, a group of investigators strongly favors FISH as being more accurate and reliable than IHC in the classification of Her2/neu status for breast cancer. Equivocal cases of Her2/neu expression by IHC were resolved by FISH analysis.

Statistical Methods

Statistical significance was set to at $P < 0.05$ and $P < 0.01$. Data were analyzed by Chi square test. Statistical analysis was performed using Graph Pad Prism Version 5.00.

Based on the hormone receptors status and human epidermal growth factor receptor (Her2/neu) status, the tumors were stratified into eight groups as ER+, PR+, Her2/neu+ (group I), ER+, PR+, Her2/neu– (group II), ER+, PR–, Her2/neu+ (group III), ER+, PR–, Her2/neu– (group IV), ER–, PR+, Her2/neu+ (group V), ER–, PR+, Her2/neu– (group VI) ER–, PR–, Her2/neu+ (group VII) and ER–, PR–, Her2/neu– (group VIII).

Results

Receptors Status and Its Relationship Between Receptors Expression

Patients were divided into eight groups according to Table 1. Overall, the analyses of ER, PR and Her2/neu receptors showed that majority of the breast cancer patients have the ER+, PR–, Her2/neu+ (26.0 %) receptor expression (Figs. 1, 2, 3). Further, the detailed category wise receptors status is shown in Table 1. A statistically significant correlation was found between the expression of ER and PR ($P < 0.0001$, Table 2). However, no statistically significant correlation was observed between the expression of hormone receptors and Her2/neu expression (Table 3). Negative correlation was found in co-expression between hormonal receptors and Her2/neu. Fig 2.

Age

The prevalence of breast carcinoma is highest (63.2 %) in ≥ 50 years age. ER and PR status revealed a higher reactivity with advancing age ($P < 0.05$) whereas no statistical significant correlation was found in reactivity of Her2/neu with respect to advancing age. (Table 4).

Table 1 Estrogen receptor, progesterone receptor and Her2/neu receptor status

Groups	Name	No. of patients (%)
I	ER+, PR+, Her2/neu+	45 (22.0)
II	ER+, PR+, Her2/neu–	53 (26)
III	ER+, PR–, Her2/neu+	10 (4.9)
IV	ER+, PR–, Her2/neu–	16 (7.8)
V	ER–, PR+, Her2/neu+	08 (3.9)
VI	ER–, PR+, Her2/neu–	06 (2.9)
VII	ER–, PR–, Her2/neu+	32 (15.8)
VIII	ER–, PR–, Her2/neu–	34 (16.7)

Fig. 1 IHC showing positive estrogen receptor tumour cells in breast cancer

Fig. 2 IHC showing positive progesterone receptor tumour cells in breast cancer

Fig. 3 IHC showing positive Her2/neu receptor tumour cells in breast cancer

Tumor Size

In study, majority of patients had (54.9 %) had T2 range tumors, means tumors size 2–5 cm. Tumor size did not correlate with hormonal and Her2/neu expression.

Histology Subtypes

The histologic examination of breast carcinoma revealed that 86 % patients were suffering from infiltrating duct carcinoma (IDC). However, 14 % patients were suffering from ILC, in situ carcinoma and medullary carcinoma. In IDC category, 67.4 % of ER+ and 61.7 % of PR+ tumors were present whereas 53.1 % of tumors were Her2/neu negative. The lobular carcinoma had expressed 81.8 and 63.6 % of ER and PR positivity respectively and only 27.3 % of tumors were Her2/neu negative. The relationship data between hormonal receptor and Her2/neu receptor with respect to histologic subtypes are shown in Table 5.

Table 2 Relationship between estrogen receptor and progesterone receptor

	PR+	PR–	Total	<i>P</i> value
ER+	98	26	124	<0.0001
ER–	14	66	80	
Total	112	92	204	

Table 3 Relationship between Her2/neu receptor and ER/PR receptor

	Her/neu+	Her2/neu–	Total	<i>P</i> value
ER+, PR+	45	53	98	0.70
ER+, PR–	10	16	26	
ER–, PR+	08	06	14	
ER–, PR–	32	34	66	
Total	95	109	204	

Table 4 Association of ER, PR and Her2/neu receptors with age

Groups/age (years)	<50 years (N = 75)	≥50 years (N = 129)	P value
ER			
Positive (%)	46 (61.3)	100 (77.5)	0.016
Negative (%)	29 (38.7)	29 (22.5)	
PR			
Positive (%)	34 (45.3)	91 (70.5)	0.0006
Negative (%)	41 (54.7)	38 (29.5)	
Her2/neu			
Positive (%)	24 (32)	36 (27.9)	0.632
Negative (%)	51 (68)	93 (72.1)	

Table 5 Association of ER, PR and Her2/neu receptors with different histological subtypes of carcinoma

Groups/ histology	IDC (<i>N</i> = 175)	ILC (<i>N</i> = 11)	In situ carcinoma (<i>N</i> = 14)	Medullary carcinoma (<i>N</i> = 4)	<i>P</i> value
ER					
Positive (%)	118 (67.4)	9 (81.8)	10 (71.4)	1 (25)	0.21
Negative (%)	57 (32.6)	2 (18.2)	4 (28.6)	3 (75)	
PR					
Positive (%)	108 (61.7)	7 (63.6)	10 (71.4)	1 (25)	0.41
Negative (%)	67 (38.3)	4 (36.4)	4 (28.6)	3 (75)	
Her2/neu					
Positive (%)	82 (46.9)	3 (27.3)	6 (42.9)	3 (75)	0.39
Negative (%)	93 (53.1)	8 (72.7)	8 (57.1)	1 (25)	

Tumor Grade

Out of 204 cases of breast carcinoma patients, 16 cases were grade I, 39 cases were grade II and 149 cases were grade III (Table 6). The reactivity of hormonal receptors was observed to decrease with increasing grade. Thus, the grade of tumor was significantly correlated to the expression of ER ($P < 0.05$) and PR ($P < 0.001$). The association between Her2/neu expression and grade of tumor was found to be not significant ($P > 0.25$).

Tumor Stage

Most of our patients (49.0 %) had stage II tumors. The correlation between hormonal receptors and Her2/neu expression with respect to stage of tumor were summarized in Table 7.

Axillary Node Status

On an average, 57.9 % patients with primary breast cancer presented with axillary nodal metastasis, and only 42.1 % were node negative.

Table 6 Association of ER, PR and Her2/neu receptors with different grade of carcinoma

Groups/grade	I (N = 16)	II (N = 39)	III (N = 149)	P value
ER				
Positive (%)	15 (93.7)	30 (76.9)	98 (65.8)	0.03
Negative (%)	01 (6.3)	09 (23.1)	51 (34.2)	
PR				
Positive (%)	16 (100)	26 (66.7)	78 (52.3)	0.0006
Negative (%)	00 (0)	13 (33.3)	71 (47.7)	
Her2/neu				
Positive (%)	05 (31.2)	15 (38.5)	70 (47)	0.35
Negative (%)	11 (68.3)	24 (61.5)	79 (53)	

Discussion

ER, PR and Her2/neu determination are established procedures in the routine management of breast cancer patients, primarily as a predictive factors for response to therapeutic and adjuvant hormonal (tamoxifen) or biological target therapy (trastuzumab). We have performed a retrospective study to correlate hormonal receptors with Her2/neu expression and also their correlation with other routinely used characteristics such as grade, histology, stage and patient's age in 204 primary operated breast cancer patients. In a recent Indian study, tumors expressed 59 % positive estrogen receptor, 51 % positive progesterone receptor and 27.1 % positive Her2/neu receptor [9]. Shet et al. [10] and Munjal et al. [11] had also reported same results for receptor expression. Similarly, our study also reports 64.2 % positive ER, 54.9 % positive PR but higher proportion of (46.7 %) positive Her2/neu receptor cases. This ER and PR expression is lower and Her2/neu expression is higher when compared with the Western studies which have shown 73 % ER positivity and 58 % PR positivity and 17–30 % Her2/neu positivity [12]. The frequency of Her2/neu positivity varies among Indian studies such as 43.2 % in Vaidyanathan et al. [13] study, 40.2 % in Munjal et al. [11] study and 46.3 % in Kumar et al. [14] study. This variation in Her2/neu positivity may be due to method of detection of Her2/neu in those studies. The frequency of Her2/neu expression may change if we detect Her2/neu by FISH analysis [9]. Previous published studies showed significant positive correlation between the ER and PR expression. Our study results for ER and PR positive correlation, also supports the previous study reports [3, 15, 16]. This correlation may be due to theory of ER-dependent PR synthesis [17].

The frequency of co-expression of ER and PR receptors with that of Her2/neu is variable as reported by earlier studies [18]. In present study, Her2/neu expression revealed a negative correlation with hormonal receptors status. ER and PR expression was increased in Her2/neu

Table 7 Association of ER, PR and Her2/neu status with different stage of carcinoma

Groups/stage	I (N = 42)	II (N = 100)	III (N = 46)	IV(N = 16)	P value
ER					
Positive (%)	31 (73.8)	64 (64)	35 (76.1)	9 (56.2)	0.28
Negative (%)	11 (26.2)	36 (36)	11 (23.9)	7 (43.8)	
PR					
Positive (%)	31 (73.8)	56 (56)	34 (73.9)	6 (37.5)	0.012
Negative (%)	11 (26.2)	44 (44)	12 (26.1)	10 (62.5)	
Her2/neu					
Positive (%)	20 (47.6)	43 (43)	24 (52.2)	5 (31.3)	0.48
Negative (%)	22 (52.4)	57 (57)	22 (47.8)	11 (68.7)	

negative tumors as compared to Her2/neu positive tumors. Parallel results were obtained in previous studies. The mechanism behind this negative correlation is ER and PR dependent down regulation of Her2/neu involve a complex molecular interaction as reported by Ciocca et al. [19] and some vitro studies [20, 21]. This may be one reason for Her2/neu over expressing women, resistant to tamoxifen. Both ER and Her2/neu positive tumors have poorer disease-free survival and overall survival when compared with ER positive and Her2/neu negative tumors. These findings demonstrate that Her2/neu positivity may be better predictor of response to tamoxifen therapy as compared to alone ER positive [22].

In our study, proportion of both ER and PR positive tumors were increasing with age (≥ 50 years) however Her2/neu positivity was decreased with advancing age (≥ 50 years). The proportional increase in ER and PR positivity with age was more marked for ER than for PR whereas some other studies have reported PR positivity with age was more marked than ER [3]. Further, Saleh and Abdeen [23] reported increased positivity of ER with age while PR positivity did not have any significant variation. A previous Western study also showed similar results, though other studies showed no correlation between age and Her2/neu expression [14, 24]. Tumor size in our study was 2 cm and 54.9 % of cases have T2 tumors. This was also similar to other Asian and Indian studies [9]. On the contrary to this, study from a western country, the tumors was predominantly less than 2 cm [25]. This may be due to early tumor detection programmes in the western countries.

In study no significant correlation was obtained between expression of hormonal receptors and histologic subtypes of breast cancer. Same results were also observed for Her2/neu expression. Further, positivity of hormonal receptor was found more in infiltrating ductal carcinoma (IDC) and infiltrating lobular carcinoma (ILC). Desai et al. [26] also reported the higher positivity of hormonal receptors in IDC and ILC. The grade of tumor in our study was significantly inversely correlated with the expression of PR (P value 0.0006) whereas in previous studies, both ER and PR

expression were significantly inversely correlated [9]. The lower grade tumors showed high ER and PR positivity as evident from the other studies [15, 27]. The relationship between grade of tumor and Her2/neu expression was found to be not significant and vast majority of Her2/neu negative tumors were grade III. In contrast, other studies were revealed higher expression of Her2/neu with higher grade tumor [22, 28–30]. The association between hormonal receptor and stage of tumors was found to be significant only for PR expression in the study. However, negative correlation observed for the expression of Her2/neu and PR expression with respect to stage. None of previous study revealed correlation results between receptors expression and stage of tumors.

The triple-negative breast cancers are characterized by a lack of expression of ER, PR and Her2/neu receptors. In our study, ER negative, PR negative and Her2/neu negative receptors constituted 13.2 % of our cases. In previous Indian study revealed 25 % cases of triple-negative breast cancer whereas Western countries have showed 14–30 % of triple-negative breast cancer [9, 31–35]. The negative expressions of triple receptors vary markedly with ethnicity and reported higher in African women as compared to White women [31]. The prevalence of triple-negative breast cancers was found to be 29 % according to study of Atlanta, USA and 13 % in non-African-American women [34]. Majority of our triple-negative breast cancer cases (78.4 %) occurred in women more than 50 years [9]. This is contradistinction to some Western Studies where younger women mainly suffering from triple-negative breast cancer except in a study of Japan revealed higher proportion of triple-negative in post menopausal women [34, 36].

Conclusion

In conclusion, the prevalence of hormonal receptors and Her2/neu in Western region of India, breast cancer patients are similar to the rest of the regions of India. There is negative correlation between expression of ER, PR receptors and Her/

neu expression. The lower grade tumor has higher hormone receptors expression. The hormone receptors expression in our study suggests that hormone receptors expression in India is lower as compared to Western countries.

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