RESEARCH COMMUNICATION

Immunohistochemical Profile of Breast Cancer Patients at a Tertiary Care Hospital in South India

Moses Ambroise*, Mitra Ghosh, VS Mallikarjuna, Ann Kurian

Abstract

Aims: 1) To evaluate the estrogen receptor(ER), progesterone receptor (PR) and Her-2 /neu expression in invasive breast carcinomas by immunohistochemistry and 2) to compare the pattern of expression with clinico-pathological parameters like patient's age, tumor size, mitotic index, histological type and grade and lymph node metastasis. <u>Methods</u>: This is a retrospective study of 321 female invasive breast carcinomas diagnosed in the Department of Histopathology, Apollo Speciality Hospital, Chennai from January 2009 to June 2010. <u>Results</u>: The age of the patients ranged from 24 to 99 years, with a mean of 53.8, and the majority of the tumors were T2 (83.8% in range of 2-5 cms), predominantly histological grade 2 (57.3%), followed by grade 3 (33.3%). ER, PR and Her-2/neu expression was seen in 59, 51 and 27% of cases respectively. Triple-negative breast cancers constituted 25 % of our cases. We also found characteristic associations between hormonal receptor and Her-2/neu expression and various clinico-pathological parameters. <u>Conclusions</u>: The hormonal receptor expression appears to be lower in the Indian population compared to the West. A significant proportion of tumors in our study with Her2/neu overexpression also showed ER and PR positivity. Triple-negative breast tumors were most commonly grade 3, in women aged more than 50 years.

Keywords: Breast cancer - receptor status - Chennai, India

Introduction

Breast cancer is the most common cancer in women. Worldwide, more than one million women are affected by this disease and 4 lakh patients die of it every year (Gluz et al., 2009). In India, breast cancer was the second most common cancer in women prior to 1990 (Takiar and Srivastav, 2008). Even though the traditional high risk areas include Europe and North America (Saxena et al., 2005), the age-adjusted incidence rate of breast cancer in women in the United States fell sharply by 6.7% in 2003 (Ravdin et al., 2007). Contrary to this, though the cervix cancer rates are decreasing, breast cancer is on the increase and currently occupies the top rank among cancers in women in our country. While still relatively infrequent as compared to the Western world, breast cancer is already a major problem in India and a continued rapid rise is expected in the future, especially in view of the gradual change in the life style of Indian women (Takiar and Srivastav, 2008; Yeole, 2008; Moore et al., 2010).

The approach in managing breast cancers has undergone enormous changes over the last 20 years. The choice of conservative and reconstructive surgery today is more popular than mastectomy with increase in the use of systemic, hormonal and cytotoxic drugs following hormone receptor testing. Estrogen receptor (ER), progesterone receptor (PR) and Her-2/neu (Human Epidermal growth factor receptor Type 2) analysis have been accepted as established procedures in the routine management of patients with breast cancer. The combined expression of these three hormone receptors has thus become most informative in the molecular classification of breast tumors and their clinical assessment for treatment and further outcome. Studies have proved that racial and demographic features of patients can also influence the pattern of immunoexpression (Shet et al., 2009; Choi et al., 2003; Lund et al., 2009).

Recently a few studies from other parts of the country had appeared, featuring the role of hormone receptor tests in cancer breast (Kumar et al., 2007; Munjal et al., 2009; Shet et al., 2009; Vaidyanathan et al., 2010). To our knowledge, no such study has been reported from Chennai. This prompted us to undertake this study, which analyses the actual status of hormone receptor expression in breast cancer.

The objectives of the present study were: 1) to study the prevalence of immunohistochemical markers in sporadic breast cancer patients in Chennai region; and 2) to correlate the immunohistochemical expression with various clinicopathological parameters.

*Department of Histopathology, Apollo Speciality Hospital, Chennai, India *For correspondence: drmosesa@yahoo.com*

Materials and Methods

This study included a total of 321 female invasive breast carcinomas diagnosed in our department from January 2009 to June 2010.Only in-house cases and patients who had undergone Mastectomy or lumpectomy with or without axillary lymph node dissection were included in this study. Our patients were predominantly from South India, especially from Chennai region. ER, PR and Her-2/neu immunohistochemical analysis was done as part of the routine diagnostic workup for all these cases. For each case, the original H&E slides and estrogen and progesterone receptor and Her-2/neu immunostains were retrieved.

The histological type, tumor grade, mitotic count, lymph node involvement, estrogen and progesterone receptor and Her-2/neu status were assessed. The tumor size was recorded from the histopathological reports. For the sake of uniformity, all the immunostained slides were reviewed and evaluated using the following criteria in this study. The ER and PR results were screened manually and interpreted as positive when more than 10% of tumor cells showed positive nuclear staining, similar to previous studies. (Munjal et al., 2009; Vaidyanathan et al., 2010). The normal epithelial elements served as an internal control. The following method was used to score Her-2/neu overexpression: Cases showing no membrane immunostaining or in less than 10% invasive cancer cells were scored 0, cases with weak and incomplete membrane staining in more than 10% of invasive cancer cells were scored 1+, cases with complete membrane staining that was weak in intensity but with obvious circumferential distribution in at least 10% of cells were scored 2+ and finally cases with strong circumferential membrane staining in more than 10% invasive tumor cells were scored 3+. A score of 2+ and 3+ were considered positive (see Figure 1). In each case, negative benign glands were used
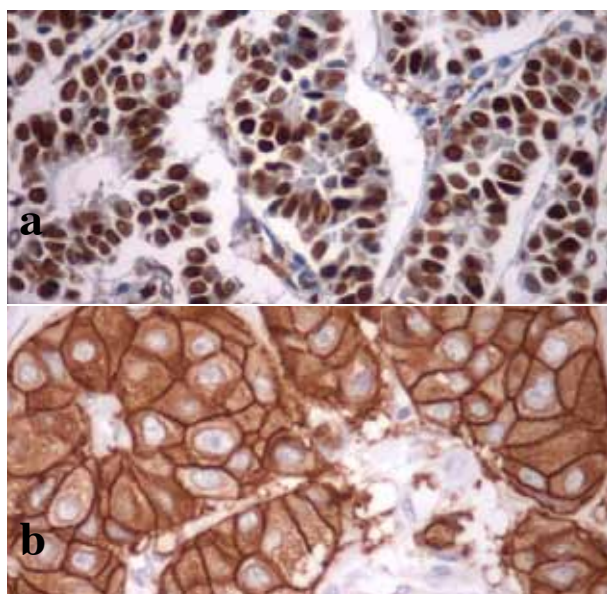

Figure 1. Immunohistochemistry. a) Invasive ductal carcinoma with ER-positive nuclear staining; b) invasive ductal carcinoma with strong circumferential membrane staining in more than 10% invasive tumor cells (Her-2/neu 3+,positive)

as internal controls for specificity of the immunoreaction and an invasive breast cancer case with known Her2/neu overexpression was used as an external control for each batch during the period of study for sensitivity of the immunoreaction. The following monoclonal antibodies were used for all the cases included in this study: 1) ER (Clone 6 F 11 from Serotec, Oxford, UK in a dilution of 1:100); 2) PR (Clone 1A6 from Serotec, Oxford, UK in a dilution of 1:100); 3) Her-2/neu (Clone CB11 from Biogenex, San Ramon, CA in a dilution of 1:100)

The following procedure was employed for all these cases. Representative blocks of formalin fixed paraffin embedded tissue of these cases were selected and 4 μm thick paraffin sections were floated on to slides previously coated with poly- L-lysine. Antigen retrieval was done by using Sodium Citrate Buffer (0.01 M, pH 6.0). Immunostain visualization was achieved with the standard streptavidin–biotin peroxidase technique. The slides were stained with 3, 3'-diaminobenzidine, counterstained with hematoxylin and mounted. During each batch, appropriate positive and negative controls were used. Fisher's exact test, Chi-square test and Mann-Whitney U test were employed for statistical analysis. The study was approved by the ethics committee of Apollo Hospitals, Chennai.

Results

Three hundred and twenty -one cases of invasive female breast cancers were included in our study. The age of the patients ranged from 24 to 99 years, with a mean age of 53.8 ±11.6 years. ER expression was seen in 59.19% and PR in 51.1% of cases and Her-2/neu positivity in 27.10%. Majority (59.2%) of the tumors were located in the left breast.

The clinical features and various combinations of ER and PR receptors are displayed in Table 1. ER expression correlated well with PR expression (P value < 0.0001). The percentage of ER but not PR positivity increased with age, though this difference was not statistically significant (P value-0.27). Her-2/neu expression was more frequent in younger individuals (P value 0.05).

Majority of the tumors measured in the T2 range of 2-5 cms with a percentage of 83.8%. T2 tumors showed an ER and PR positivity of 58.7% and 51.7% respectively. T1 tumors were more frequently ER, PR positive when compared to T3 tumors (P values -0.02 and 0.03). However, no significant variation was seen with the Her-2/neu expression.Her-2/neu positive tumors showed a higher mitotic index compared to the negative ones (P value-0.0005). The reverse trend was seen with ER and PR tumors (P value <0.0001 for both associations). Majority of the tumors in our study were histological grade 2 (57.3%), followed by grade 3 (33.3%). Regarding histological grade and Her-2/neu positivity, there was an increase from 6.67% in grade 1 to 29.9% in grade 3 tumors (P value -0.008). ER and PR expression were seen more frequently in grade 1 tumors compared to grade 3 tumors (P value<0.0001 for both associations). Lymph node status did not correlate with hormonal and Her-2/ neu expression. Infiltrating duct carcinoma (IDC) was the major histological type with a percentage of 96.3%. In the

Table 1. Clinicopathological Features and Receptor Status (N=321)

Her-2/neu status	Neg	234	72.9
	Positive	87	27.1
ER Expression	Neg	131	40.8
	Positive	190	59.19
PR Expression	Negative	157	48.9
	Positive	164	51.09
Tumor Grade	1 - 2	214	66.7
	3	107	33.33
Tumor size	<20 mm	27	8.41
	≥20 mm	294	91.6
Lymph Node status	Negative	125	41.8
	Positive	174	58.19
	Not available	22	
Age	20 – 50 Years	126	39.3
	51 – 65 Years	149	46.4
	> 65 Years	46	14.3
ER / PR	ER + PR +	151	47.0
	ER + PR -	39	12.2
	ER - PR +	13	4.05
	ER - PR -	118	36.8
Total		321	100

Data are No and % values

Table 2. Association of HER-2 status and ER and PR Expression

HER-2	Total	ER+	ER-	PR+	PR-
-ve	234 (72.9)	144(61.5)	90(38.5)	128(54.7)	106(45.3)
+ve	87 (27.1)	46(52.9)	41(47.1)	36(41.4)	51(58.6)
Total	321 (100)	190(59.2)	131(40.8)	164(51.1)	157(48.9)

IDC category, 57.9% of ER+ and 49.5% of PR + tumors were present while 72.2% of tumors were negative for Her-2/neu. The lobular carcinomas had expressed 100% of ER and PR positivity and only 14.3% of them were Her-2/neu+. Data for the association between HER-@ an dthe other two receptors are shown in Table 2.

The triple-negative breast cancers characterized by a lack of expression of ER, PR and Her2/neu receptors constituted 25 % of our cases. The triple-negative breast tumors were more commonly grade 3 (P value<0.0001). They were also larger in size compared to the rest, though this difference is not quite significant statistically (P value -0.06).There was no difference between Triple-negative tumors and non Triple-negative tumors with respect to age distribution.

Discussion

Our study comprised of 321 cases of invasive breast cancer with a mean age of 53.8 years. Mean age of Indian breast cancer patients is found to be lower when compared to the Western countries with an average difference of one decade (Stead et al., 2009; Sandhu et al., 2010).

In developed countries, majority of the patients have a negative lymph node status (Taucher et al., 2003; Huang et al., 2005; Stead et al., 2009). Indian and Asian studies have documented a greater percentage of breast carcinomas with lymph nodal metastasis compared to the Western figures (Saxena et al., 2005; Aryandono et al., 2006; Sandhu et al., 2010; Vaidyanathan et al., 2010).

Tumor size in our study was 2 cms and above in 91.5% of cases. This was comparable to other Asian and Indian studies (Aryandono et al., 2006; Azizun-Nisa et al., 2008; Vaidyanathan et al., 2010). In contrast, in a study from a western country (Taucher et al., 2003), the tumors are predominantly less than 2 cms. This could be due to the early detection programs prevalent in the western countries.

Our study showed that 59 % of cases had positive Estrogen receptor expression while 51 % expressed Progesterone. This is lower when compared to some Western studies which have reported 73 % ER positivity and 58 % PR positivity (Rhodes et al., 2000). A recent study from Mumbai also showed that hormone receptor expression in India is lower compared to the West (Shet et al., 2009). A study from Mumbai et al has also revealed lower ER (Murthy et al., 2007). Our study showed varying correlation between ER and PR expression. The percentage of tumors expressing PR but not ER was 4.05% in our study. A study from Mumbai has revealed it to be 6% (Shet et al., 2009). A Western study has also reported a very infrequent (3%) of this phenotype (Rhodes et al., 2000). In our study, the percentage of ER positivity was higher in the age group over 65 years. PR expression did not vary with age. Other studies have found that percentage of ER positivity increases with age whereas PR expression does not seem to have any significant variation (Saleh and Abdeen, 2007; Azizun-Nisa et al., 2008).

A significant inverse association was found between hormonal expression and tumor grade. The findings in other studies were also similar (Azizun-Nisa et al., 2008; Ayadi et al., 2008). A greater proportion of T1 tumors showed ER and PR positivity when compared to T3 tumors. This was observed in another study also (Saleh and Abdeen, 2007).

Her2/neu positivity was present in 27.10 % of our cases. In the Western studies the values ranged from 17 % to 27% (Taucher et al., 2003; Huang et al., 2005; Lal et al., 2005). A study from Malaysia (Kamil et al., 2010) showed that 31.5% of breast cancers were Her2-neu positive.

The frequency of Her2/neu positivity varies among Indian studies. In a study from Bangalore, South India Vaidyanathan et al., found a figure of 43.2% positivity by IHC and 25.5% by Genomic PCR (Vaidyanathan et al., 2010). Another study from the same region has documented 29% Her-2/neu positivity by IHC (James et al., 2008). A recent study from Indore, Central India has found 40.2 % of tumors to be Her2/neu positive (Munjal et al., 2009). Another study from Varanasi, North India revealed it to be 46.3 % (Kumar et al., 2007). The frequency of Her-2 positivity may change if we take into account only the positive cases detected by fluorescence in situ hybridization (FISH) analysis. However FISH was not performed in this study nor in the other recent studies from India.

The results in our study showed that Her-2/neu expression decreased with age. A previous Western study also showed similar results (Taucher et al., 2003), though other studies have showed no correlation between age and Her-2/neu expression (Kumar et al., 2007; Ayadi et al., 2008). In our study, Her2/neu positivity was expressed

in 6.7%, 28.8% and 29.9% of grade 1, 2 and 3 tumors respectively. Other studies also revealed that Her2/neu positivity is higher in grade 3 tumors and minimal in grade 1 tumors and lobular carcinomas (Taucher et al., 2003; Lal et al., 2005).

Our study could not demonstrate an inverse relationship between Her-2/neu and ER and PR expression. Though some studies could establish an inverse relationship, (Almasri and Hamad, 2005; Taucher et al., 2003) others did not find a statistically significant relationship. (Aryandono et al., 2006; Vaidyanathan et al., 2010). A significant proportion of tumors in our study with Her2/neu overexpression also showed ER (52.87%) and PR (41.38%) positivity (Table 4). Another extensive study also revealed that a substantial number of Her2-neu positive tumors expressed ER (49 %) or PR (24 %) (Lal et al.,2005). Her-2/neu positive tumors also showed a higher mitotic index compared to the negative ones, whereas the reverse was seen with ER and PR expression. Similar figures about ER and Her-2/neu expression were reported in another study (Saleh and Abdeen, 2007).

The triple-negative breast cancers characterized by a lack of expression of ER, PR and Her-2/neu receptors constituted 25 % of our cases. Studies from Western countries have showed that triple negative tumors have constituted 14 % to 29.5 % of breast carcinomas. (Lund et al., 2008 ; Vona-Davis et al., 2008 ; Tischkowitz et al., 2007; Lund et al., 2009 ; Stead et al., 2009). Studies have also shown that triple negative tumors vary markedly with ethnicity and have documented a higher incidence in African women compared to White women. In a study from Atlanta, USA (Lund et al., 2008) the prevalence of triple-negative breast cancers was found to be 29 % among African – American women and 13% among non-African-American women. A recent study of a cohort of women under age 55 revealed that triple negative tumors were the most common breast cancer subtype diagnosed among African–American women accounting for nearly 47%, compared to 22% among whites (Lund et al., 2009).

Majority of our triple-negative breast cancer cases (60.5%) occurred in women more than 50 years. This is in contradistinction to some Western studies (Tischkowitz et al., 2007) where triple-negative breast cancer predominantly affects younger women. However, in a study from Japan, a higher proportion of post menopausal women were present in the triple-negative breast cancer group (Iwase et al., 2010). The triple-negative breast tumors were more commonly grade 3 and larger in size compared to the rest in our study. Other studies have also documented similar features (Bauer et al., 2007; Vona-Davis et al., 2008).

To conclude, ER and PR positivity was seen in 59 % and 51 % of cases. The hormonal expression in our study suggests that hormonal expression in India is lower when compared to western countries.

Acknowledgement

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