RESEARCH ARTICLE

HER-2/neu Status: A Neglected Marker of Prognostication and Management of Breast Cancer Patients in India

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Abstract

Background: Categorizing breast tumors based on the ER, PR and HER/Neu 2 receptor status is necessary in order to predict outcome and assist in management of breast cancer. Herfe we assessed this question in South Indian patients. Materials and Methods: A total of 619 formalin fixed paraffin embedded breast tumor tissues were collected from pathology archives after receipt of ethical clearance. With the help of primary and secondary conjugated antibodies, expression status of ER, PR and HER2/neu was determined. All the experimental data were assessed for correlations with histopathological features of tumors and clinical presentation of the subjects. Results: In the present study, the ages ranged from 20-87 years with a mean of 50.0±12.q years, and majority of the tumors (84%) were of infiltrating duct cell carcinoma type. Assessment of ER, PR and Her-2/neu expression showed that 46% were triple negative. Interestingly, an inverse relation between ER, PR and HER-2/neu was apparent in 41.2% (p<0.0001) of the tumors, of which 24.5% (p<0.0001) were ER and PR co-negative but HER-2 positive. Conclusions: ER and PR positive tumors are less common (i.e<30%) compared to HER-2/neu positive tumors (i.e>50%) in Indian breast cancer patients, underlining the need for effective diagnostic screening and specific therapeutic managements in order to improve the survival rate of patients in low resource countries such as India.

Keywords: Breast cancer - receptor status - ER - PR - HER-2 - developing countries - India

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Introduction

Breast cancer (BC) remains a persistent health burden accounting for increased number of deaths in both pre and post-menopausal women, worldwide. This disease shows heterogeneous presentation of histopathological features, molecular alterations and clinical symptoms (Lu et al., 2010). Currently 1.38 million women are estimated to be suffering with this disease, throughout the world with an approximate death rate of 400,000 women/ annum (Ferlay et al., 2010). Higher incidence rates are particularly observed in East African (19.3/100,000) and European (89.9/100,000) women (Kraft et al., 2010). In India, the annual breast cancer diagnosis rate has now reached to 23/100,000 women highlighting the alarming need for major social and medical concerns. Breast cancer management approaches have undergone enormous changes over the last two decades with targeted therapy based on hormone receptor status becoming the mainstay (Ambroise et al., 2011).

Estrogen and progesterone are the critical hormones involved in normal breast development and tumorigenesis which act upon after binding to estrogen receptor (ER) and progesterone receptor (PR) (Misrahi et al., 1987; Kumar et al., 2010; Shyamala et al., 2000). Breast carcinomas, that originate from intrinsically ER and PR positive luminal cells are designated as ER and PR positive carcinomas, respectively (Patil et al., 2011). Additionally, human epidermal growth factor receptor-2 (HER-2/neu) encoded by an oncogene, CerB2, is a transmembrane cell surface glycoprotein expressed at low levels in normal non-neoplastic epithelia, including breast duct epithelium. However, it's over expression is commonly evident in primary BC (Livi et al., 2011).

Screening for ER, PR and HER-2/neu status in breast tumors has become a standard method in determining the appropriate therapy for Breast cancer patient management throughout the world (Ambroise et al., 2011), triple receptor status detection in India is still not carried out routinely, even if ER and PR screening is done, HER-

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Table 1. Comparison of Age with Different Receptor Status of BC Patients

Receptor Status	<40 Years (%)	Z Value (Confidence Interval) P value	40-50 Years (%)	Z Value (Confidence Interval) P value	>50 Years (%)	Z Value (Confidence Interval) P value
ER +	42/194 (21.64)	46.88 (16.86%-28.10%) <0.0001	55/194 (28.35)	42.595 (22.12%-5.24%) <0.0001	97/194 (50.0)	28.75 (42.75%-57.25%) <0.0001
PR +	46/191) (24.08)	44.97 (18.20%-30.78%) <0.0001	56/191 (29.31)	41.65 (22.96 %- 36.31%) <0.0001	89/191 (46.59)	30.69 (9.35%-53.93%) <0.0001
HER-2/neu +	45/168 (26.78%)	40.57 (20.25%-4.14%) <0.0001	52/168 (30.95)	38.09 (24.05%-8.53%) <0.0001	71/168 (42.26)	31.36 (34.68-50.10%) <0.0001

*[-]: Negative, [+]: Positive

Table 2. Correlation Coefficients

	AGE	ER	PR
ER	0.0368	NA	0.9259
PR	-0.0435	0.9259	NA
HER-2/neu	-0.0098	-0.1233	-0.1032

*NA: Not Applicable , ER: Estrogen Receptor, PR: Progesterone Receptor, HER-2/neu Human Epidermal Growth Receptor -2

2 is not included as a marker in most centers, leaving a significant number of cases under-diagnosed and mistreated, thus accounting for increased mortality rates compared to the developed countries. Hence, this study aimed to evaluate the expression status of ER, PR and HER-2/neu by immunohistochemistry in breast tumors and establish their role in better prognostication and management of breast cancer patients from a cosmopolitan city in South India.

Materials and Methods

Ethical approval and consents

Approval for genetic studies in Breast cancer was taken from the Ethics Committee of Vasavi Medical and Research Centre, Vasavi Hospital, Hyderabad, India (VMRCE-3-2011)

Sampling

In this retrospective study, a total of 619 formalin fixed paraffin embedded tissue (FFPE) blocks of breast tumors removed during Modified Radical Mastectomy of BC cases were collected from pathology archives (2001-2007) of three different Hospitals located in a cosmopolitan city, Hyderabad, South India. All the BC patients were diagnosed by mammography and/or fine needle aspirate assay (FNAC) and later the surgical specimens were confirmed after histopathology by a competent pathologist.

Immunohistochemistry

Representative blocks of FFPE tissue (4 μm thick) sections were foated on to slides previously coated with gelatin and chromalum followed by antigen retrieval by Sodium Citrate Buffer (0.01 M, pH 6.0) method. The primary antibody treatment was done for each marker i.e ER (Biogenix, USA-AM272-2ME), PR (AM328-5ME) and HER-2/neu (AM134 -5ME). Immunostain visualization was achieved with standard HRP conjugate technique. Slides were further stained with 3, 3'-diaminobenzidine from Biogenix (USA), counterstained with hematoxylin, mounted and examined under light microscope at 40X magnification as described by our group earlier.

ER and PR expression was scored as per the method detailed by Munjal et al. (2009) and Vaidyanathan et al. (2010). Positive tumor tissues were those which showed nuclear staining in at least 10% of the cells examined, when compared to matched epithelial elements as an internal negative control. For HER-2/neu scoring, criteria proposed by Ambroise et al. (2011) was adopted by keeping known HER-2/neu positive as an external control.

Statistical analysis

SISA online statistical software was used to derive the correlation co-efficient values with the expression ratios of different receptor markers examined. The sub grouping of different receptor expression such as hormone receptors with HER-2/neu receptor were made by Z test using MedCalc software (12.2.1 version). P values of

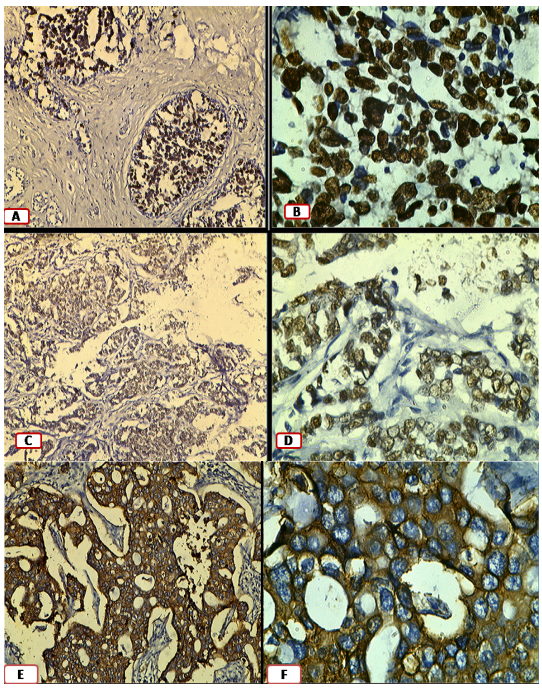

Figure 1. A) ER 10x, B) ER 40x, C) PR 10x, D) PR 10x, E) HER2/neu 10x, and F) HER2/neu 40x

Table 3. Triple Receptor Status with Age

Receptor Status	<40 Years (%)	Z Value (Confidence Interval) P value	40-50 Years (%)	Z Value (Confidence Interval) P value	>50 Years (%)	Z Value (Confidence Interval) P value
ER-PR-HER2 -	28/138 (20.28)	40.274 (13.91%-27.96%) <0.0001	62/138 (44.92)	26.99 (36.44%-53.6%) <0.0001	48/138 (34.78)	32.45 (26.87%-43.35%) <0.0001
ER-PR-HER2 +	24/81 (29.62)	26.99 (19.98%-40.79%) <0.0001	26/81 (32.09)	25.97 (22.14%-4.9%) <0.0001	31/81 (38.27)	23.42 (27.68%-49.74%) <0.0001
ER+PR+HER2-	11/55 (20)	25.52 (10.43%-32.97%) <0.0001	15/55 (27.27)	23.04 (16.13%-40.95%) <0.0001	29/55 (52.72)	14.38 (38.79%-66.33%) <0.0001
ER+PR+HER2+	9/26 (34.61)	14.12 (17.21%-55.66%) <0.0001	6/26 (23.07)	16.82 (8.96% -43.63%) <0.0001	11/26 (42.30)	12.33 (23.34%-63.07%) <0.0001

*[-]: Negative, [+]: Positive

<0.05 were considered to be statistically significant.

Results

A total of 619 tumors had a record of IHC analysis, 14 were male and 605 were female breast cancer sample by origin. The overall age of the patients ranged from 20-87 years with a mean age of 50.0±12.06 years. The mean age of males was 52.4±13.02 years and that of females was 49.9±12.04 years. Among the cases, 42.2% were >40 years, 38.95% were between the age group 40-50 years and 18.95% were <39 years, age was not recorded in 4.68% of patients (Table 1). The histopathological sub typing revealed that majority of the tumors were of infiltrating duct cell carcinomas (IDCC) (84%, p<0.0001) type followed by >9% invasive duct cell carcinoma (INDCC) and 3% of duct cell carcinomas (DCC).

Expression ratios of ER, PR and HER-2/neu

A total of 619 tumors were examined for ER and PR status, 32.56% were positive for ER (p<0.0001) and 32.23% for PR (p<0.0001). Co-positivity to ER and PR was seen in 26.77% of the samples with a correlation coefficient of r=0.9259 (p<0.0001) (Table 2, Figure 1). HER-2/neu positivity was assessed in 330 cases and was found to be 3+ definitely positive in 35.71% (p<0.0001) (Figure 2) of the tumors while 15.93% were 2+ i.e equivocal, these required further confirmation by Flourescent in situ hybridisation technique which was not carried out in any centre during the period of study. Hence the samples which showed equivocal positivity of HER-2/ neu i.e 2+were not included for analysis. The total number of samples with all three markers assessed were 300, and triple negativity was evident in 46 % of the tumors. Interestingly, an inverse relation between ER, PR and HER-2/neu was apparent in 41.20% (p<0.0001) of the tumors, of which 24.54% (p<0.0001) were ER and PR co-negative but HER-2 positive (Table 3, Figure 3).

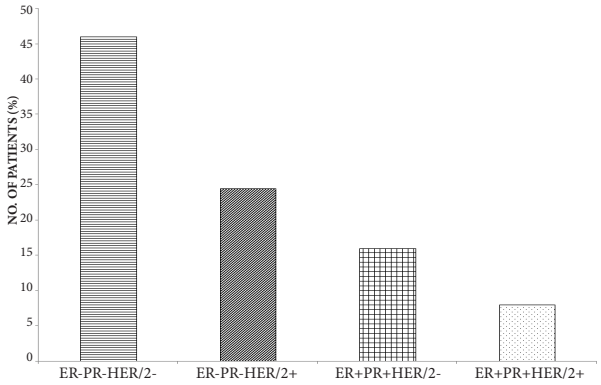

Figure 2. Combined Receptor Status of Breast Cancer Patients

Discussion

The expression ratio of ER, PR and HER-2/neu receptors in breast tumors is population specific, but it is still unclear whether the variation is due to physiologic, exogenous or ethnic factors. In the present study, 32% of the breast tumors from women of South India are found to be co-positive for the ER and PR expression. This value is lower than reports from Northern India, which state that 40% of the breast tumors are positive for ER and PR status (Desai et al., 2000; Dutta et al., 2008). The cumulated ratio of ER and PR expression in breast tumors of Indian women is found to be much lower than that of Western population, where >50% are co-positive for ER and PR (Barnes et al., 1996). Studies from Canada and Australia also reported that 73% and 59% of the BC were ER positive while 58.10% and 61% were PR positive respectively (Rhodes et al., 2000; Francis et al., 2007).

It is noteworthy that 51.64% of BC cases in the present investigation were found to be positive for HER-2/neu status. This value is in agreement with the previous findings (Dutta et al., 2008), where HER-2/ neu positivity was seen in 57.2% of BC cases. But, in follow-up studies, HER-2/neu positivity was seen only in 29% of Invasive BC cases from Bangalore city, South India (Marsiglinate et al., 1993). Vaidyanathan et al from the same region reported it to be 43.2% positivity in BC cases (Vaidyanathan et al., 2010). This finding was further supported by a group from Indore city Central India which showed 40.2% positivity and Varanasi city from Northern India exhibited 46.3% positivity (Kumar et al., 2007; Munjal et al., 2009). Reports from Asian

countries like Malaysia, Pakistan, Saudi Arabia indicated 31.5%, 45.8%, 28.3% Her2-neu positive breast cancers respectively (Al-Ahwal et al., 2006; Naeem et al., 2008; Kamil et al., 2010). Studies from USA have reported that 17-27% of the BC patients are positive for HER-2/neu expression (Taucher et al., 2003; Huang et al., 2005; Lal et al., 2005), while 15-20% of BC cases were reported to be HER-2/neu positive in UK (Lovekin et al., 1991). These studies without any doubt suggest that HER-2/neu positive status is seen in a higher percentage of patients in our population when compared to other parts of the world.

In the present study, an interesting inverse relation between the HER-2/neu positivity to that of the ER and PR negativity was found. To the best of our knowledge, the present investigation is the first report from India highlighting the fact that >40% of the BC tumors are positive for ER, PR or HER/Neu2, of which 24.54% are ER, PR co-negative but HER/Neu2 positive. This trend of ER, PR co-negative with HER/Neu2 positivity appears to be common to South Asians, as it is evidenced by the studies from Pakistan, SriLanka (Naeem M et al., 2008; Al-Ahwal MS, 2006), Jordan and Saudi Arabia (Almasri et al., 2005; Ratnatunga et al., 2007). Studies from Chicago and Serbia has also pointed out this inverse relation between ER/PR and HER-2/neu status of patients with breast cancer (Ariga et al., 2005; Ivkovic-Kapicl et al., 2007).

In India, IHC based detection of HER-2/neu was introduced into the clinical practice since 2000, but was initially available only in quaternary level health centers. However it gained some momentum by 2007 and is now accessible in most of the secondary labs, throughout the country. But still HER-2/neu testing is very subjective, due to the high cost of [Herceptin] therapy which is the appropriate therapeutic unaffordable by most patients. Higher mortality rates of BC cases are usually common in developing countries like India than the developed countries, despite their comparatively lower prevalence rate. Diagnostic and detection methods followed for BC management in developed countries need to be replicated for BC patients of developing countries (Masood et al., 2010). Since, the last decade HER2/neu is considered as an important therapeutic target in standard breast cancer treatment across the globe. Trastuzumab (herceptin) in combination or sequence with cytotoxic chemotherapy greatly improves the prognosis of HER-2/neu positive BC patients (Higgins et al., 2011).

According to available literature, tamoxifen, an anti-estrogenic compound targets the ER positive tumors, by altering the conformation of ER upon its binding and recruits co-repressor proteins to block the transcriptional activations of ER responsive genes and subsequent arrest of tumor growth. The critical threshold of co-activator or co-repressor proteins in cancer cells determines their sensitivity to specific therapeutic agents. Estrogen, when bound to ER is known to down regulate the transcription of HER-2/neu, via the Estrogen Response Element [ERE] present on this gene (Massarweh et al., 2007) but tamoxifen induces the transcription of HER-2/Neu. Studies have indicated that MEKK1, a downstream mediator of HER-2/neu signaling, activates ER and stimulates the agonist activity of tamoxifen. Thus, HER-2/neu positivity and increased downstream signaling could potentially convert tamoxifen from a breast cancer cell inhibitor into a stimulating agent. Clinical data clearly indicate that ER and HER2 copositive breast cancer cells respond to trastuzumab but are resistant to hormonal therapies such as tamoxifen alone. Trastuzumab restores the sensitivity of ER and HER2 copositive cells to tamoxifen.

It is also seen that ER-ve, HER2+ve breast cancer which is expected to be more aggressive than ER and HER2 copositive breast cancer, as it would respond well to trastuzumab plus hormonal therapy (Liu et al., 1995; Oh et al., 2001). However, the inappropriate treatment of HER-2/neu positive cases with drugs that are specifically responsive to ER and PR positive BC which is due to lack of knowledge about HER-2/neu status is a laxity in treatment which leads to bad prognosis. This emphasizes that the evaluation of HER-2/neu status must be made mandatory along with ER and PR, for planning appropriate therapy for BC patients.

In conclusion, a higher percent of HER-2/neu positive breast tumors are found in Indian women. The evidence of crosstalk between the HER-2 and ER signaling pathways in breast cancer and the availability of biomarker directed therapy calls for attention towards the mandatory detection of HER-2 status in all classes of populations to provide an appropriate treatment that gives good efficient result and help in decreasing mortality of BC patients in resource limited countries. Proper planning awareness among women of both high and low socioeconomic groups is the demand of the present state.

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