RESEARCH ARTICLE

Correlation of Hormone Receptor and HER-2/neu Expression with Clinicopathologic Parameters in Primary Breast Tumors

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Abstract

Background: Breast cancer (BC) is a major health issue worldwide as well as in Pakistan. All women belonging to any race, ethnicity or lineage are in danger of developing breast cancer. Significant factors influencing the development of breast malignancies are the genetic background, environmental conditions, reproductive parameters, the consequences of female hormones both intrinsic and extrinsic, alteration of immune status, and biologic determinants. Materials and Methods: Overall 150 biopsy proven patients were included in the study. Samples were submitted for histopathology and determination of estrogen and progesterone receptor expression and HER-2/neu status. Associations with other characteristics like age, tumor stage, node involvement, histological grade were also studied. Results: Mean age at presentation was 46.7 years. The majority had invasive ductal carcinoma, 100 (84.7%), and were in stage pT3, 54 (45.7%). Important relationships (P<0.05) were found among ER, PR positivity, and Her 2 neu overexpression. However, no noteworthy link was identified amongst ER, PR, Her 2 neu and tumor grade, stage, age, lymph node involvement except for the menopausal status. Conclusions: In summary, breast cancer patients featured an advanced stage of disease, more lymph node involvement, and moderately high grade tumors and with more estrogen, progesterone receptor and HER-2 positive tumors.

Keywords: Breast cancer - hormone receptor - HER-2/neu - immuno-histochemistry

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Introduction

Globally, underdeveloped countries are going through communal and economic changes, the switch over to a more mechanized lifestyle has been leading towards diseases like cancers, associated with reproductive, nutritional, and hormonal risk factors. Incidence of different diseases has increased in many parts of the world, but there are huge disparities between affluent and underprivileged countries. Breast cancer (BC) is a major health issue of females worldwide as well as in Pakistan. Breast cancer with standardize incidence rate of 38.9 accounts for a one fourth of all cancer cases globally(Pimhanam et al.,2014). Incidence rates remain highest in more civilized nations, whereas mortality rate is higher in under privileged countries due to a shortage of facilities for initial diagnosis and medication. According to study by Ferlay et al. (2012) annual Age Standardized Incidence Rate (ASIR) for breast cancer in Pakistan stands at 50.3/100,000. The country based cancer registries are rarely considered for developing countries like Pakistan. Pakistan's population has the highest rate of breast cancer amongst all Asian countries (except Jews of Israel) (Alexander et al., 2002). Despite of advance approaches in early detection, surgical and adjuvant therapy of breast carcinoma, about 40,000 women expire each year (Ahmad, 2009). Breast cancer is well-characterized as a heterogeneous disease with genotypic and phenotypic diversity, composed of distinct subtypes, the criteria upon which the clinical management is based (Peppercorn et al., 2008). Human breast cells, growth multiplication and distinction between both normal and malignant, are controlled by steroid hormone and peptide growth factor receptors. The proteins found in and on breast cells that inform cells of growth upon receiving hormonal signs are called Hormone Receptors. The Estrogen Receptor (ER) is the classic and most acknowledged biomarker that is prevalent in breast tumor [6] (Welsh et al., 2012). The progesterone receptor (PR, well known as NR3C3 or nuclear receptor subfamily 3, group C, member 3), is a protein present within the cells. It is triggered by the steroid hormone progesterone. Epidermal growth factor receptors generally suspected in breast malignancy belong to the type I receptor tyrosine kinase family, which includes HER-1 (epidermal growth factor receptor), HER-2, HER-3, and HER-4. Amplification of the HER-2/neu gene, emerging in overexpression of the receptor, is found in 20%-25% of human breast tumors (Konecny et al., 2003). The malignant cells, positive for estrogen and progesterone receptor, may identify signals from their

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respective hormones that could urge their growth. Of every three breast cancer patients, approximately two test positive for hormone receptors (breastcancer.org ., 2015). A converse affiliation has been found among HER-2/neu amplification/over expression and the positive steroid hormone receptors in various clinical and experimental studies (Benz et al.,1993; Pietras et al.,1995).

Estrogen receptor status is considered one of the most widely accepted and decisive criteria for the implication for endocrine therapy. However, the ER positivity does not guarantee for response to endocrine therapy because most of them sooner or later develop metastases in spite of the treatment.

Progesterone Receptor (PR) status on its own is a predictive factor to benefit from concomitant endocrine therapy (Bardou et al., 2003). Progesterone receptor (PR) is a substitute marker of an active ER and valuable in assessing the performance of breast cancer (Mohammed et al., 2015). PR also help to categorize two subgroups of ER-positive tumors, ER+/PR+ tumors more liable to react to hormonal treatments, particularly tamoxifen, than ER+/PR- tumors (Fuqua et al., 2005).

HER-2/neu proto-oncogene amplification is mostly associated with cancer intensity and poor survival. HER-2/neu is usually analyzed to predict the reaction of breast cancer to chemotherapy and the use of transtuzumab (recombinant humanized antibodies) in the effective handling of patients with advanced breast cancer (Azam, 2009)

Triple Positive Tumors (TP) molecular classification comprising of the three basic molecular determinants. HER-2, ER and PR are routinely evaluated in clinical practice and play an important part in the personalized medication of breast cancer.

Triple Negative Tumors (TN) is an important category of breast tumors that are negative for ER, PR and HER2/neu, constituting approximately 15-20% of all individuals affected by breast cancer. TNBC patients are comparatively younger than the overall patients of breast carcinoma globally and with frequently higher-grade tumors, coupled with a greater possibility of reoccurrence and death, usually, within a period of 3 years of diagnosis (Bauer et al., 2007; Dent et al., 2007; Rakha et al., 2007). TNBC response to hormone therapy or HER2-directed agents is not much, so leaving cytotoxic chemotherapy as the one and only treatment of choice (Oakman et al., 2010; Davis et al., 2014).

The aims and objectives of the present study were to analyze the frequency of steroid hormone receptors and HER-2/neu overexpression in Pakistani subjects and to see their association with other clinical characteristics of disease especially age, menstrual status, involvement of nodes, histologic stage and grade.

Materials and Methods

Patients

Study Design: Cross-sectional comparative study.

Place of Study: Study was carried out at Ziauddin University, Karachi.

Patients were recruited from the breast cancer clinic of Jinnah Postgraduate Medical Centre (JPMC) and Baitul Sukoon cancer hospital, Karachi.

Statistical analysis

Statistical software named SPSS (Statistical software for social sciences)-version20 were utilized for data entry and computation as well. Data comprised of quantitative and qualitative information of patients. Quantitative variable i.e. age was presented as Mean ± SD, minimum, & maximum. Cross tabulation was used to depict the picture of classification of all qualitative variables including Tumor stage, Tumor grade, Status of ER, Status of PR, HER2 expression, menstrual history, Lymph Node involvement. To determine the association between them, Chi-square test was computed by considering P-value <0.05 statistically significant.

Bar chart was used to show the age category of patients. (Figure 1)

Ethical approval

The study was conducted after approval from the institutional Ethical Review Board of Ziauddin University.

Informed Consent

All individual who participated had given written consent prior to the study. Information with variables related to breast cancer were compiled on the pre-designed questionnaire.

Results

Our study included 150 cases of breast carcinoma. The record of ER, PR, and HER 2 /neu status of 32 patients could not be gathered at the time of study compilation, so these patients were not included. The study thus comprised 118 patients. Patients were eligible if they had invasive Breast Cancer, and no other malignant disease. Diagnosis was confirmed by histopathological evaluation of routine 4 micron thick hematoxylin and eosin stained sections. The TNM (tumor node metastasis) classification according to the revised American Joint Committee on Cancer (AJCC) was used to categorize the tumor stage at primary diagnosis. Histologic grading was done by Modified Bloom-Richardson System (MBR). ER, PR and HER2/neu were evaluated by immunohistochemical staining and expression was determined by applying the immune-reactive score, in keeping with American Society of Clinical Oncology and the College of

Table 1. Correlation of ER and PR Status with HER2 neu Expression

	HER2 expression		Total	P-value
	+ve	-ve		
	N (%)	N (%)		
Status of ER				
+ve	54 (71.1)	22 (52.4)	76(64.4)	
-ve	22 (28.9)	20(47.6)	42(35.6)	0.027
Total	76 (64.4)	42 (35.6)	118	
Status of PR				
+ve	54 (71.1)	20 (47.6)	74 (62.7)	
-ve	22 (28.9)	22 (52.4)	44 (37.3)	0.043
Total	76 (64.4)	42 (35.)	118	

Table 2. Correlation of Receptors with Tumor Grade and Stage

	PR Status		Total	P-value	ER Status		Total	p-value
	+ve N (%)	-ve N (%)			+ve N (%)	-ve N (%)		
Tumor Grade								
1	04(57.2)	03.(42.8)	07(5.9)		03(50)	03(50)	06.(5.1)	
11	45(65.3)	26(37.7)	71(60.1)		48(68.5)	22(31.5)	70(59,3)	
111	27(67.5)	13(32.5)	40(33.9)	0.804	26(61.9)	16(38.1)	42(35.9)	
Total	76(64.4)	42(35.6)	118		77(65.3)	41(34.7)	118	0.587
Tumor Stage								
T1	06 (60)	04 (40)	10(8.5)		07 (70)	03 (30)	10 (8.5)	
T2	26(53.1)	23(46.9)	49(41.5)		28(57.1)	21(42.9)	49(41.5)	
T3	41(75.9)	13(24.1)	54(45.8)		39(72.2)	15(27.8)	54(45.8)	
T4	03 (60)	02 (40)	05 (4.2)	0.18	03 (60)	02 (40)	05 (4.2)	
Total	76(64.4)	42(35.6)	118		77(65.3)	41(34.7)	118	0.575

	HER2 Status		Total	P-value
	+ve N (%)	-veN (%)		
Tumor Grade				
1	05 (71.4)	02 (28.6)	07 (5.9)	
11	47 (68.1)	22 (31.9)	69 (58.5)	
111	24 (57.1)	18 (42.9)	42 (35.6)	
Total	76 (64.4)	42 (35.6)	118	0.465
Tumor Stage				
T1	06(60)	04(04)	10(8.5)	
T2	29(59.2)	20(40.8)	49(41.5)	
T3	39(72.2)	15(27.8)	54(45.8)	
T4	03 (60)	02 (40)	05 (4.2)	0.441
Total	76(64.4)	42(35.6)	118	

Table 3. Association of ER, PR, & Her 2 Neu Expression with Different Parameters

Parameters	Status of ER (P value)		Status of PR (P value)		HER2 Status (P value)	
	Positive	Negative	Positive	Negative	Positive	Negative
Menstrual History	0.848		0.564		0.051	
Premenopausal	40 (64.5)	22 (35.5)	38 (61.3)	24 (38.7)	45 (72.6)	17 (27.4)
Postmenopausal	37 (66.1)	19 (33.9)	38 (67.9)	18 (32.1)	31 (55.4)	25 (44.6)
Skin involvement	0.551		0.249		0.655	
Present	43 (36.2)	25(36.8)	36 (70.6)	15 (29.4)	42 (62.7)	25 (37.3)
Absent	34 (68)	16 (32)	40 (59.7)	27 (40.3)	34 (66.7)	17 (33.3)
Nipple involvement	0.915		0.291		0.128	
Present	71 (66.4)	36 (33.6)	07 (58.3)	05 (41.7)	66 (62.3)	40 (37.7)
Absent	06 (54.5)	05 (45.5)	69 (65.1)	37 (34.9)	10 (83.3)	02 (16.7)
Lymphovascular invasion	0.915		0.291		0.259	
Present	41 (65.1)	22 (34.9)	38 (50)	38 (50)	37 (69.7)	25(40.3)
Absent	36 (65.5)	19 (34.5)	18 (42.9)	24 (57.1)	39(69.6)	17 (30.4)
Perineural Invasion	0.274		0.067		0.488	
Present	73 (67)	36 (33)	03 (33.3)	06 (66.7)	69 (63,3)	40 (36.7)
Absent	04 (44.4)	05 (55.6)	73 (67)	36 (33)	07 (77.8)	02 (22.2)

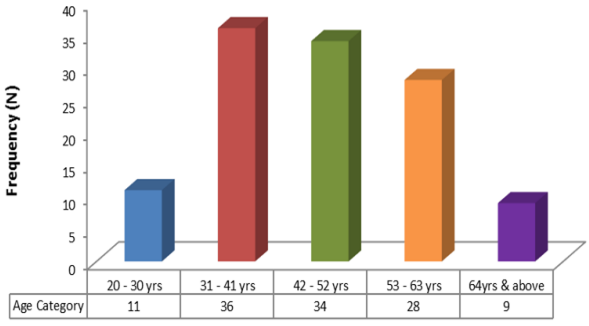

Figure 1. Age Categories of Patients

American Pathologists (ASCO/CAP) protocol. ER and PR affirmative interpretation involves at least 1% malignant cells presenting positive nuclear staining of any intensity. Receptor negativity is stated if <1% of malignant cells express staining of any intensity (Hammond et al., 2010). On the basis of ER and PR expression, patients are grouped as, ER+ve/PR+ve, ER+ve/PR-ve, ER-ve/PR+ve and ER-ve/PR-ve. Immunohistochemistry (IHC) for Her2, distinctive antibodies which detect the Her2/neu protein, presence of many copies caused the cells to change the color. The results of the IHC test can be: 0 (negative), 1+ (also negative), 2+ (borderline), or 3+ (positive - HER2 protein overexpression). (Wolff et al., 2013).The mean age was 46.69 years (range 22-76 years). The majority 62(52.5%) of the patients were premenopausal. Infiltrating ductal carcinoma (IDC) being the most prevailing histological variety of breast cancer in our study, constituted 100(84.7%) cases and invasive lobular carcinoma (ILC), 16(13.5%).

Association with Clinicopathological Factors

Among the various parameters, menstrual status

showed a statistically significant relation to Her-2/neu positivity (P=0.051). Significant association (P < 0.05) was found between ER, PR positivity, and Her 2 Neu over expression. Table 1. However no association was found between the steroid hormone receptors and HER2 status with the clinico-pathological factors like age, tumor grade and stage, skin and nipple involvement, lymphovascular and perineural invasion. Premenopausal females were 62(52.5%) and postmenopausal were 56 (47.5%).Table 2, 3. Lymph node involvement was seen in 78 cases (66.1%),while absent in 40 (33.9%).The commonest ER/PR/HER2 distributions was ER/PR+, HER2+, 51(50.2%) i.e Triple Positive, and ER/PR-,HER2-ve (Triple negative) were 18 (15.3%).

Discussion

Breast cancer occurs at young age in Pakistan as compared to the West (Mahmood, 2006). In our study mean age at diagnosis is 46.69 years with age range of 22-76 years. Approximately 48.6 years of mean age and range of (21 - 87 years) have been reported in other studies (Khokher et al., 2012; Badar et al., 2015). Study by Kakarala et al. (2010) on Asian Indian/Pakistani women showed higher frequency of invasive ductal carcinoma (69.1 *vs.* 65.6%) and lower invasive lobular carcinoma (4.2% *vs*. 8.2%) which is comparable to our study. According to the American Cancer Society, about two third cases of breast cancer are hormone receptor positive, to both estrogen and progesterone (Silberman , 2014). Our results also reveal that breast cancer patients have high hormone receptor positivity rates. We have 76(64.4%) ER and 74(62.7%) PR- positive cases. On the other hand our study shows 42(35.6%) HER-2/neu negative and 76(64.4%) positive cases, proportional to the results of 64% positivity and 36% negativity of HER2/neu (36%) as seen in the study by Gupta et al. (2015). Out of 118 cases, 51(50.2%) were Triple positive tumors and 18(15.3%) Triple negative tumors. The proportion of ER and PR positive patients was high in HER2/ neu positive patients and low in HER2/ neu negative patients. In contrast to study by Faheem et al. (2012), which showed inverse relationship of ER, PR with HER2/neu. Hormone receptor negativity turns out to be more significant correlate of survival than HER2/neu-positivity. United States National Cancer Institute's Surveillance Epidemiology and End Results (SEER) indicate immensely high frequency of breast cancer in premenopausal Asian Indian/Pakistani female's age less than 40 years, compared to Caucasian females. However in the study conducted, maximum number of premenopausal breast cancer patients are lying within the age category of > 40 years (significant P-value <0.05). The evaluation of menstrual characteristics affiliation with breast cancer in our women are highly subjected to recall bias. Prevalence of illiteracy in our society results in women not being aware of their ages at menarche and menopause (Gilani, 2010). The study by Moran et al. (2011) on Indian-Pakistani (IP) settled in US versus non-Hispanic white (NHW) female breast cancer, found more hormone receptor negative disease. One of the plausible answer for this may be younger age at the time of diagnosis, which is well documented to have link with ER negative tumors (Anders, 2008), but this is conflicting to our study which shows younger age at presentation but with more hormone receptor positive cases. Intake of estrogens from external sources is associated with increased frequency of ER positive breast cancers (Tewari, 2007).This is a debatable statement as Indian/Pakistani patients have reduced exposure to exogenous estrogen (i.e., contraceptive pills and hormone replacement therapy (HRT), but have more hormone positive tumors. Interestingly practice of self- breast examination and mammogram in females of subcontinent has been less frequently reported as compared with other Asian countries and whites (Sobani et al., 2012). While this may perhaps validate the appearance of disease at late stage in subcontinent patients. However there must be some biologic disparities with regards to young age at diagnosis with more advanced stage of disease, as majority of cases in our study are in stage pT3, which can't be simply accountable for early approach for screening or access to care alone. More extensive studies are required to cover the patients throughout the country and to judge their health-related quality of life after therapy and survival rate, in both receptor positive and negative patients. Certainly, these recommendations allow further research.

In conclusion, In Pakistan, breast cancer is a very common diagnosis, in spite of that we have to solely rely on the west for the statistical data to gather various factors that could be incriminated in the analysis of diseases like breast cancer. Large population based studies could draw attention to the probable risk factors and hormone receptor status within our population. Steroid hormone receptor evaluations in breast tumors represent the important method to deduce the biological nature of breast cancer for clinical management. Studies conducted by various institutions throughout the country should organize and bring together their data on breast cancer. This will help in designing programs for early detection and cancer control in the region.

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