Original Article

ESTROGEN RECEPTOR, PROGESTERONE RECEPTOR & Her-2/ NEU POSITIVITY AND IT'S ASSOCIATION WITH TUMOR CHARACTERISTICS

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ABSTRACT:

BACKGROUND: Breast carcinoma is the most frequent malignancy of females which is worldwide in its distribution and is the second most common cause of cancer related deaths in females. In Pakistan it occurs more commonly in young ladies as compared to western women where it is more prevalant in older age group. The objectives of this study were to determine the frequency of ER, PR positivity& HER-2/neu receptor over-expression and its association with some of the characteristics of breast cancer like patient age, tumour size, histological grade and axillary lymph node involvement.

OBJECTIVE: To determine the receptor status of breast carcinoma and analyze them in correlation with tumor characteristics in women of District Faisalabad.

STUDY SETTING: The sample consisted of 123 surgical specimens of patients who had the diagnosis with invasive breast cancer received from January 2011 to January 2013.

METHODS: This study was conducted at Pathology Department of UMDC, Faisalabad & Meezan Lab Faisalabad from January 2011-13. Surgical specimens of patients with breast cancer who had undergone Modified Radical Mastectomy (MRM) sent to the histopathology labs were included in the study. The representative sections from resected specimens were referred for immunohisto-chemical (IHC) studies (HER-2/neu receptor and ER/PR) after detailed histopathological review. Patients who had refused for ER, PR & HER-2/neu receptor immuno-histochemistry were not included in this study. Data was analyzed by SPSS version 16.

RESULTS: The study included comprised of surgical specimens of 123 cases of breast carcinoma who had undergone modified radical mastectomy**.** The mean age of the patients included in this study was 46 years and majority of the patients were in 4^{th} decade of life. Estrogen receptor positivity was observed in 58.53% cases. Progesterone receptor was positive in 52.03% cases. The over expression of Her-2/neu protein was seen in 52.03%. Hormone receptor positivity and positivity, over-expression of HER-2/neu was not significant in relation to histopathological sub-type ($p>0.05$), number of axillary lymph nodes with metastatic IDC ($p>0.05$) and tumor size ($p>0.05$). It had a significant association with histological grade ($p<0.05$).

CONCLUSION: Hormone receptor status and Her-2/neu expression should be assesed in all patients of breast carcinoma because it carries high prognostic significance and is reported more prevalent in Pakistani women. Thus the positive cases of Her-2/neu may be offered Herceptin therapy and derive benefit from the specific chemotherapeutic agents. Similarly the ER, PR positive cases should get anti-estrogen therapy which can improve survival in these patients. This is more cost effective and will avoid unnecessary use of drugs**.**

KEYWORDS: Carcinoma breast, hormone receptor, HER-2/neu, modified radical mastectomy (MRM).

INTRODUCTION:

Breast cancer is a major worldwide health problem of women. It is most frequent cancer among western women and is the second most cause of cancer related deaths in these ladies.[01] It is the most frequently diagnosed cancer in Pakistani females.[02] The incidence and mortality rate due to breast cancer is variable in different regions. According to published data high incidence is reported in developed countries and low in developing regions of the world. Inspite of high incidence the mortality rate due to breast carcinoma is low in developed nations due to favorable survival and availability of health care facilities.[03]A large number of factors determine the prognosis of disease and treatment response.[04] Estrogen receptor(ER) and progesterone receptor (PR) expressions are the most important and useful factors which are currently available and determine the treatment response of tumor. These are intracellular steroid hormone receptors which have received substantial attention since 1986. [05] ER & PR have been detected in 50-85% of the breast cancer patients in measurable amount. The frequency and quantitative value of hormone receptors rise with age, and reach at highest levels in postmenopausal women. Availability of detection of Her-2/neu gene amplification plays important role in prognosis and in specifying the treatment.[06] It's over expression is associated with tumor aggressiveness and chemoresistance. This also predicts whether the tumor will respond to Trastuzumab. High grade tumors bearing mostly large size and associated axillary metastasis mostly found depict Her-2/neu positivity.[07]

Majority of the patients (>60%) in Pakistan present at an advanced stage of disease as compared to the western countries.[8, 9] It may be due to lack of awareness, inadequate health screening programmes or due to different biological behavior of tumor in this region. Present study was conducted to detect the percentage of positivity of ER, PR & Her-2/neu and its association with tumor characteristics.

Breast cancer development is attributed to variable risk factors but in majority cases the dominant factor is long term exposure to estrogen in genetically susceptible cases.[10] Various studies have suggested and confirmed this association i.e. estrogen exposure and breast cancer development.[11] Breast carcinoma also has genetic basis imparting two to three times greater risk in first degree relatives. Other predisposing factors are early menarche, nulliparity, late age at first birth and late menopause.[12] Existing old data depicts the exogenous intake of hormone imparts an overall increase to 2.5 fold.[13] Recent research work depicts the higher breast cancer risk among women using hormone replacement therapy. Estrogen hormone has been declared as a known human carcinogen since December 2002 by National Toxicology Programme. Hormone receptor positivity in tumor tissue predicts the better response of tumor to hormonal therapyandchemotherapy.[14]

Estrogen and progesterone receptor determination plays significant role in selection of patients who will receive benefit from endocrine therapy, and also provides information regarding prognosis.[15] Hormone receptor positive tumors derive greater benefit from endocrine therapy. The hormone receptor positive patients who do not have recurrence usually do not experience metastatic disease.[16]

The amplification & over expression of of HER-2/neu proto-oncogene is found in 25-30% of breast cancer and it indicated high grade/aggressive tumor bearing poor prognosis.[17] Several studies have been conducted for the analysis of HER-2/neu to predict the responsiveness of tumors to chemotherapy application of Transtuzumab in management of cases with metastatic breast disease.[18] Our study correlates the expression of these biomarkers with various tumor characteristics.

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MATERIALS & METHODS:

One twenty three patients of breast carcinoma were selected for study from January 2011 to January 2013 at Meezan lab and at Pathology Department of University Medical & Dental College, Faisalabad. Mastectomy specimens of patients having primary breast cancers were included in the study. Surgical specimens that exhibited poor preservation were excluded.

Relevant information of the patients was collected on a proforma. Mastectomy specimens were sliced and overnight fixation was done by keeping them immersed in 10% buffered neutral formalin. Tumor size was noted and representative sections of the tumour were submitted for histopathologic evaluation. Lymph nodes were isolated from axillary tissue and submitted for histopathologic examination. After completion of tissue processing the paraffin embedded blocks were prepared. Sections were cut at (4 micron) stained with haematoxylin and eosin. Slides sections were mounted with coverslips using DPX. Once the diagnosis of malignancy was established on histological examination, sections from the primary and metastatic tumour in lymph nodes were submitted for immunohistochemistry.

Immunohistochemical stains were performed according to manufacturer's specifications. For immunohistochemical determination of ER, PR and Her-2/neu, three sections of the same tumour of 3-5 um thick sections were cut and placed on separate slides with adhesive on their surface. De-paraffinization of the sections was carried out in xylene, followed by clearing with alcohol and rehydration in Tris Buffered Saline (TBS). Each section was then subjected to heat induced antigen retrieval technique by placing them in 0.1 M Tris-HCl at pH 10 in a domestic microwave (750 watts) for two cycles of 10 minutes each. The slides were cooled down at room temperature and washed twice with TBS for 2 minutes. In subsequent session slide for each immunohistochemical marker was separately treated with respective primary antibody for one hour followed by three changes of TBS for 3 minutes each. Biotinylated secondary antibody was placed on the slides for 20 minutes followed again by three changes of TBS of 3 minutes. Enzyme conjugate was applied for 2 minutes, after which slides were washed in TBS. In the end diaminobenzidine chromogen was applied for 10 minutes and slides were rinsed in distilled water followed by counterstain with haematoxylin and mounted with Canada balsam. The slides were screened manually. Quality control in each batch of immunostaing was maintained through running of positive and negative control for ER, PR & HER-2/neu.

Reporting criteria: An Allred score is semi quantitative system that takes into consideration the proportion of positive cells (scored on a scale of 0-5) and staining intensity (scored on a scale of 0-3). The proportion and intensity were then summed to produce total scores of 0 or 2 through 8. A score of 0 -2 was regarded as negative while 3 - 8 as positive (Table no 1)

After immunotaining each section was scanned at low magnification in order to assess the intensity throughout. The score equal or more than 3 was considered as positive. For consideration of HER-2/neu as positive the set criteria was to detect moderate or strong membrane staining in >10% cells.

Weak, incomplete membrane staining or cytoplasmic staining was considered negative.

RESULTS:

Age, tumor size, and presence or absence of nodal involvement, distant metastasis, sites of metastasis, skin involvement, chest wall involvement, recurrent disease and bilateral breast cancer were noted along with ER, PR & Her-2/neu status on specifically designed proforma. Mean $\pm$ standard deviation was recorded for continuous variables like age. The frequency and percentage were calculated for categorical variables like ER, PR and Her-2 /neu status. The association of ER, PR & Her-2/neu status with tumor characteristics was sought employing t test, chi square test and ANOVA wherever appropriate P value<0.05 was considered significant.

The size of tumor ranged from 1.0 to 11 cm with mean of 4.6 ± 2.17 cm. Majority of patients (101 of 123; 82.11%) included in our study had tumor size lesser than 5 cm. strong

association was found between tumor size and ER positivity.

Invasive ductal carcinomas (IDC)was the most frequently diagnosed cancer(92%) in our study population, the percentage of invasive lobular carcinoma was 3.25%, insitu ductal carcinoma %age was 1.62% and 0.87% was meta-plastic carcinoma. Among the cases of IDC 64/114(56.14%) were poorly differentiated tumors based on Bloom Richardson Grading system on light microscopy, 49/114 (42.98%) were moderately differentiated and 1/114(0.87%) was a case of well differentiated IDC (Table no 2).

Table no 1: Guidelines for interpretation of ER results by Allred Method.

Proportion	Intensity	Observation Score (PS)	Observation Score (IS)
0	None	0	None
1	1%	1	Weak
2	1-10%	2	Intermediate
3	10-33%	3	Strong
4	33-66%	4	
5	66-100%	5	

Sum of proportion score and intensity score

0-2	Negative
3-8	Positive

Table no 2: Distribution of patients based on light microscopic examination

Infiltrating Ductal Carcinoma NOS	116
Invasive lobular carcinoma	04
Metaplastic carcinoma	01
Ductal carcinoma in situ with well diff IDC	02

Two of our patients showed significant content of ductal carcinoma in situ. 68 of 123 (55.28%) of invasive carcinoma showed focal necrosis. 32.52% showed comedo necrosis. There was no association observed between necrosis and hormone receptor status. No noticeable relation was detected between necrosis and HER-2/neu receptor status ($P < 0.05$).

Desmoplasia is extensive stromal fibrosis and was observed in 64/123(52.28%) of invasive breast carcinoma. 52 & 21 cases with desmoplasia were ER and PR positive. Hormone receptor status was noted for these diagnosed cases of breast carcinoma. Estrogen receptor positivity was observed in 72/123(58.53%) cases. Progesterone receptor was positive in 64/123(52.03%) cases. Her-2/neu protein over expression was observed in 64/123(52.03%). Among 72 ER positive cases 52 were strongly positive while 20 cases. 64/123 cases were positive for progesterone receptor. Among the Progesterone receptor positive cases 43 were strongly positive and 21 cases revealed weak positivity. Over expression of Her-2/neu was found in 64/123 cases (table no 5). Among the Her-2/neu positive cases 35 were strongly positive with Allred score of 3+ and 29 cases were reported as equivocal. 45/123 cases were triple positive. While the remainder exhibit inverse relationship of hormonal positivity of tumors with Her-2/neu overexpression.

Ninety out of 123 cases (73.7%) were node negative. 58.6% cases presented with

axillary lymph node metastasis in 1-3 lymph nodes and 15% in 4- 9 lymph nodes. There was no significant correlation between metastatic potential and ER, PR or HER-2/neu status of tumor.

Table No 3: Distribution of cases according to their histological grades

Grade of the tumor	Number of cases
Well differentiated(Grade I)	04
Moderately differentiated(Grade II)	50
Poorly differentiated(Grade III)	69

Table no 4: Additional features of tumor on microscopic examination

Characteristics of tumor	No of patients
Comedo pattern of tumour necrosis	40/123
Tumour necrosis	68/123
Desmoplasia	64/123
Presence of nipple erosion alongwith skin ulceration	09/123
Skin retraction	02/123
Lymph nodes involvement	90/123

Table no 5: Results on Immunohistochemistry

ER positive 72		PR positive 64		Her-2/neu positive 64	
Strong +ve	Weak +ve	Strong +ve	Weak +ve	Strong +ve	Weak +ve
52	20	43	21	35	29

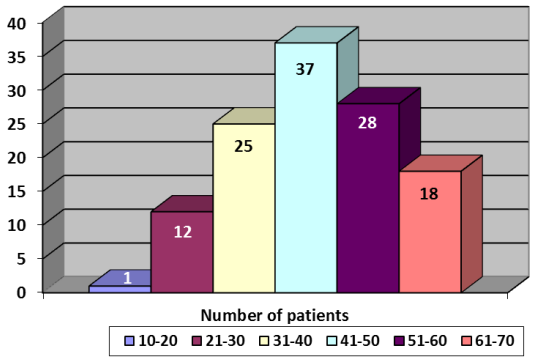

Figure No.1: Age distribution of patient having cancinour breast.

DISCUSSION:

In the management of breast cancer there is involvement of multiple disciplines i.e. pathologist, surgeon, oncologist etc. The application of therapeutic method and intensity being conditioned by type of tumor, its grade, presence or absence of metastasis, patient's age and their menopausal status. The specific immune markers help to predict treatment response, prognosis and manipulate the therapeutic plan.

The ER, PR receptor status of breast carcinoma can predict the response to adjuvant endocrine therapy. Hormone receptor positive breast cancers are more prevalent in western world as compared to Asian women where hormone receptor positivity is found to be low. [20, 21] This hormone receptor positivity is inversely related to Her 2/neu expression in western women.[21]

The management of breast cancer requires the evaluation of prognostic and predictive factors in order to divide the patients into two groups: those who are hormone receptor positive are more like to be benefited by adjuvant chemotherapy and other ones who are not likely to be benefited/treated by adjuvant therapy. ER/PR status and Her-2/neu gene over expression carries both prognostic and predictive values.[22, 23] In general hormone receptor positivity and HER-2/neu receptor negative breast cancer is

indicative of better prognosis & and vice versa.

This study was conducted to determine the frequency of ER, PR & Her-2/neu positivity and correlation of expression of ER, PR& Her-2/neu with some morphological parameters of tumors. The results obtained were found to be more or less similar to those reported in international literature. The mean age of the patients in the study conducted was 46 years and majority of the patients were in 4th decade of life. The youngest lady included in our study was 0nly 18 years old. (Table no 2).

As compared to commonly reported incidence regarding the age group of patients our results showed that 73.98% of the patients were young with age less than 60 years.[24' 25] Majority of them (46.8%) were between the ages of 41 and 50 years. This depicts occurrence of breast cancer in relatively younger age group as compared to West.[23] In our study, we did not find any significant association between the age of the patients and receptor positivity of tumor. We found that patients hormone receptor negative tumors in mostly young patients similar to results obtained by Farid Saleh and Saud Abdeen.[26]

Christopher *et al.* have documented a prevalence of 76-78% of ER, PR-positive breast cancers in the United States from 1992 to 1998 with a rise in the prevalence over the years.[27] However, only limited research work has been done in Asian communities on immunohistochemical status of breast cancer. A prevalence of 32.6% for ER-positive and 46.1% for PR-positive breast cancers has been documented in a study carried out in India.[28, 29] A Jordanian research article documented 50.8% ER-positive tumors and 57.5% of PR-positive tumors in their study sample.[30] The present study depicts ER positivity in 58.03% while PR positivity was 52%. The present study documents a prevalence of 54.3% for completely negative ER and 51.7% for completely negative PR. It indicates that the majority of the breast carcinomas in our study sample would not get benefit from endocrine therapy. However, detection of Her-2/neu overexpression is comparable with the rest of the world.

CONCLUSION:

The frequency of expression of hormonal receptors in breast cancer patients from city of Faisalabad is 58.53% comparable to documented research data. Although %age of HER-2/neu overexpression is higher (52.03%) in our population. The inverse relationship between hormone receptor expression and Her-2/neu expression was not significantly detected in our population (study population mostly comprising residents of Toba, Faisalabad, Jhang, Gojra, Sarghodha etc). ER, PR negativity and Her-2/neu positivity are associated with more advanced disease and poor outcome. There is significant association between histological grade of tumor and Her-2/neu positivity.

ER, PR &HER-2/neu receptor status should be checked in all patients (who have breast carcinoma) due to their prognostic and predictive significance so that they get benefit from specific treatment which can improve survival in these patients. It is more cost effective and avoids unnecessary use of drugs.

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5	Asso Prof Dr. Muhammad Khalid	Acquisition, analysis & interpretation of data
6	Dr. Mubasher Ahmad	Contribution in conception, drafting of article