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Role of epidermal growth factor receptor in breast cancer: An analysis of biomolecular receptor study and its clinicopathological correlation

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ABSTRACT

Introduction: Epidermal growth factor receptor (EGFR) is member of human epidermal receptor is frequently expressed in diverse forms of cancer. Many studies have studied the relation of EGFR positivity in breast cancer and its prognostic value, but yet no conclusions have yet been drawn. We attempt to study the receptor positivity in our patient and its correlation with various clinic-pathological prognostic predictors and outcomes. **Materials and Methods:** Data of 355 patients of breast cancer registered in our department between November 2014 and November 2016 and followed up until December 2016 was collected and reviewed for epidemiological and clinical features. **Results:** Results of total 355 patients analyzed, TNBC group, were most common (n = 152) (43%) followed by luminal A (25%). Median age at disease presentation was 45.3 years (24–73 years). The EGFR-positivity rate was 30.3%. EGFR-negative patients presented as early breast cancer significantly more than EGFR-positive patients (47.36% vs. 27.10% P = 0.046). Significantly, higher proportion of EGFR-positive patients presented with Grade 3 cancers (44.10% vs. 19.16% P = 0.049). Nodal involvement was significantly more in EGFR-positive patients (66.6% vs. 37.5% P = 0.0364). Pathological complete response (CR) was significantly associated with EGFR positivity (16.1% vs. 12.5% P = 0.0349). There were more recurrences in a surgically treated group with EGFR positivity than negative group, but this difference did not reach significance (18.1% vs. 5.2% P = 0.061). **Conclusion:** We found that our breast cancer was quite young with the median age almost two decades earlier than that of the west with very high number of patients presenting as an advanced stage and triple negative phenotypes. We found that EFGFR receptor positivity in almost one-third of the patients. This could be subgroup of patients which could be targeed by anti-EGFR therapy. This EGFR positivity also acted as surrogate for an aggressive disease which was shown by significantly larger proportion of advanced stage, high grade and node-positive disease present in receptor-positive patients. This subset showed a higher rate of pathological CR in patients subjected to neoadjuvant chemotherapy. There was trend of worse outcomes in surgically treated EGFR-positive patients which may be due to short follow-up period in our study. As we continue this study, EGFR positivity may emerge as a true prognostic marker of breast cancer.

Key words: Breast cancer, Epidermal growth factor receptor, Molecular, Receptor study

Introduction

Breast cancer can be considered to be a complex disease demonstrating heterogeneity at a clinical and histopathological level which has been attributed to distinct molecular signatures. Differences in gene protein expression pattern which have been observed in identical histopathological profile have led to a conceptual shift in breast cancer management from "everyone alike" to more personalized treatment.

Epidermal growth factor receptor (EGFR) is a member of the ErbB family of receptors, and its stimulation by endogenous ligands (epidermal growth factor or transforming growth factor-alpha) results in activation of intracellular tyrosine kinase, therefore, leads to the inhibition of apoptosis, activation of cell proliferation, and increases the metastatic potential.[1] Based on these properties, EGFR was investigated in many human malignant tumors, and it is now regarded as a potential target for cancer therapy. In breast cancer, various studies have shown EGFR can play a crucial role not only in the molecular diagnosis of breast cancer but also identify a subgroup of patients and become a marker for prognosis and survival.[2-4]

Very few studies have looked into the association between molecular subtyping and clinicopathological profile in Indian population. We attempt to study the receptor positivity in our patient and its correlation with various clinic-pathological prognostic predictors and outcomes. Understanding specific breast cancer subtypes and associated risk factors may better elucidate breast cancer treatment strategies for Indian population.

Materials and Methods

This study included a total of 355 patients of breast carcinomas diagnosed in our department from August 2013 to November 2016. All of the patients were diagnosed and treated in the Department of Surgical Oncology, King George Medical University, Lucknow, UP. For all patients, clinical and histopathological informations were noted in detail in specified pro forma.

Histopathology sample comprised either tissue obtained by tru-cut, incisional, excisional or post-surgical specimen form patients. After collection, the samples were sent for histopathological evaluation at the Department of Pathology K.G.M.U.

Histopathological categorization of breast carcinoma was done under College of American Pathologists (CAP) protocol which included the diagnostic information such as - specimen identification procedure, laterality, lymph node sampling, site and size of the tumor, histological type, tumor grading under the Nottingham modification of the Bloom-Richardson system, and evaluation of prognostic histopathological parameters. Immunohistochemical evaluation using streptavidin-biotin immunoperoxidase method was done.

Primary antibody used

1. ER - flex polyclonal rabbit - a Hu ER alpha, Clone EP1, RTU (DAKO AS/AS+)
2. Partial response (PR)- flex monoclonal Mo a Hu PR, Clone PgR636, RTU (DAKO AS/AS+)
3. HER2 - polyclonal rabbit a Hu c-erb2 oncoProtein, RTU (DAKO AS/AS+)
4. Ki67 - flex monoclonal Mo a Hu Ki67 Antigen, Clone MIB-1, RTU (DAKO AS/AS+)
5. EGFR - flex monoclonal Mo a Hu EGFR protein, Clone RTU (DAKO AS/AS+).

The slides were examined at ×40 magnification:

1. ER/PR status was elucidated by the following criteria. More than/=1% moderate to strong nuclear positivity was considered positive.
2. HER2NEU STATUS - it was calculated according to ASCO CAP protocol 2014.
   - Score 0 - it defined by no staining observed or membrane staining that is incomplete and is faint/barely perceptible and within ≤10% of the invasive tumor cells.
   - Score 1 + defined by incomplete membrane staining that is faint/barely perceptible and within >10% of the invasive tumor cells.
   - Score 2 + defined by circumferential membrane staining that is incomplete and/or weak/moderate and within >10% of the invasive tumor cells or complete and circumferential membrane staining that is intense and within ≤10% of the invasive tumor cells.
   - Score 3 + defined by circumferential membrane staining that is complete, intense in >10% of the invasive tumor cells.
3. Ki-67 index
   Immunostaining was quantitatively evaluated using light microscopy, in which the entire section was scanned at low-power magnification to determine areas with the highest numbers of positive nuclei (hotspot) within the invasive component. These were usually found at the periphery of tumors. Ki-67 labeling index (Ki-67LI) was expressed as the percentage of MIB1-positive cells among a total number of 1000 malignant cells at high-power magnification. High Ki67 was considered as >15% of such cells.
4. EGFR - a cytoplasmic expression of EGFR in tumor cells >5% was considered as positive.

Since this was not a clinical trial, the patient population was diverse, and the treatment received by patients was variable. Neoadjuvant chemotherapy (CT) and palliative CT given to the patients were mainly based on clinical staging and physician discretion. The type of therapy given can be broadly classified as anthracycline-based therapy, that is doxorubicin (adriamycin) plus cyclophosphamide, 5-fluorouracil, epirubicin, and cyclophosphamide, or taxane-based therapy.

Clinical outcomes

Response assessment of chemotherapy was done using the Recist criteria.

Complete response (CR): Disappearance of all target lesions. Any pathological lymph nodes (whether target or non-target) must have a reduction in short axis to <10 mm.

PR: At least a 30% decrease in the sum of diameters of target lesions, taking as reference the baseline sum diameters.

Progressive disease (PD): At least a 20% increase in the sum of diameters of target lesions, taking as reference the smallest sum on study (this includes the baseline sum if that is the smallest on the study). In addition to the relative increase of 20%, the sum must also demonstrate an absolute increase of at least 5 mm (Note: The appearance of one or more new lesions is also considered progression).

Stable disease (SD): Neither sufficient nor shrinkage to qualify for PR nor sufficient increase to qualify for PD, taking as reference the smallest sum diameters while on the study.

Variables were evaluated and analyzed statistically. Chi-square (χ^2) and Student *t*-tests were used to compare variables and tests were considered significant when $P < 0.05$.

Results

The present study was carried out with an aim to carry out an immune-histopathological (IHC) study of breast cancer cases and to correlate IHC findings with clinicopathological profile and outcome. For this purpose, a total of 355 patients falling in the sampling frame were enrolled in the study.

Age of patients ranged from 21 to 80 years. The age frequency curve indicated that the high proportion of the younger population is having the disease. Almost 2/3rd of the population was under 50 years of age. Mean age of patients was 45.38 ± 9.67 years.

Out of a total of 355 patients enrolled in the study, 346 were women and 9 were men. Among women, 188 (52.9%) were in premenopausal stage of their life whereas remaining 158 (45.1%) of women were in postmenopausal of their life. Except for 5 (1.8%) nullipara women, all the female patients were parous.

Maximum number of patients (n = 157; 45%) had locally advanced breast cancer, 134 (38%) were at early stage whereas 61 (17%) had metastatic disease.

On Luminal Subtyping by IHC patients could be classified into luminal A like which consituted 25%, luminal B like 15.6% Her 2 only 16.2% whereas triple negativeformed the largest subtype (43.2%).

Nearly 40.6% of the patients were hormone positive whereas 59.6% of the patients were hormone negative. Nodal positivity rate was 54% high Ki67 was seen in 64.4% of patients. Her2 Positivity was seen in 31.85.

The EGFR-positivity rate was 30.3%. EGFR-negative patients presented as early breast cancer significantly more than EGFR-positive patients (47.36% vs. 27.10% P = 0.046). Significantly, higher proportion of EGFR-positive patients presented with Grade 3 cancers (44.10% vs. 19.16% P = 0.049). Nodal involvement was significantly more in EGFR-positive patients (66.6% vs. 37.5% P = 0.0364). Pathological CR was significantly associated with EGFR positivity (16.1% vs. 12.5% P = 0.0349). There were more recurrences in a surgically treated group with EGFR positivity than a negative group, but this difference did not reach significance (18.1% vs. 5.2% P = 0.061).

Discussion

In our study, we found that the almost 2/3rd of the population was under 50 years of age. Mean age of patients was 45.38 ± 9.67 years. The age frequency curve indicated that the high proportion of the younger population is having the disease. When compared to the west, the mean age of occurrence of breast cancer in the US is 62 years.[5-7] The frequency curves indicate the differences in the occurrence of cancer wherein the west the disease is occurring in old age patients.[5-7] In Adedayo *et al*.[8] study, mean age of all subjects was 62.7 years (SD, 13.8; range, 27.9–95.8 years). In a study by Manjunath *et al*.,[9] the mean age of our patients was 53.0 years (standard deviation [SD] 11.38), with 76.4% (191 of 250) being <60 years of age. It appears to be that the breast cancer occurrence in India is almost a decade earlier than occurring in the west.

In our study, we found that 52.9% of the breast cancer patients were postmenopausal and 44.4% of the people were premenopausal patients whereas almost 3% of the patients were males. This represents a very high status of premenopausal patients having breast cancer.

Kakarala *et al*.[10] revealed in the analysis of the SEER data that the percentage of postmenopausal patients in the US is 75.5%. In the study during the subgroup analysis of Indian and Pakistani population of patient's percentage of postmenopausal women having breast cancer was 53.9% which is comparable to our study.

Another disturbing statistics in our study revealed that only 38.7% of the patients presented at early stage in our study whereas about locally advanced and metastatic patients contributed to 40.4% and 20.9%, respectively.

In the west, most of the patients present with early-stage breast cancer. According to SEER data,[6] 61% of patients would present at early stage, 31% of the patients at the locally advanced stage and only 6% of the patients would present at metastatic stage. Kakarala *et al*.[10] showed in Caucasian American women almost 88.5% presented with early breast cancer 7% in locally advanced stage and 4.5% in metastatic stage.

The higher proportion of breast cancer patients presenting late in our center is worrisome issue. The most important reason for this can be due to both lack of awareness of breast cancer and lack of screening programs for breast cancer. Due to robust screening programs in the west has lead marked reduction in late diagnosis of cancer as well as mortality rates.

On luminal subtyping of the patients, 36.8% of the patient were luminal A, 11.1% were luminal B 13.3% were her 2 only whereas triple negative subgroup formed the largest subgroup with almost 39% of the patients. This represents a very high proportion of triple negative patients.

Kurian *et al*.[11] did a SEER database analysis dividing the breast cancer into molecular subtypes. He showed that only 13% of the total population was triple negative. This proportion further dropped to 11.5% only white African women. The highest proportion of patients having triple negative disease was African black women which comprised 24% of the subgroup.

The studies done in Indian reveal the much higher proportion of triple-negative breast cancers compared to their western counterparts.[12,13] ranging from 25% to 33%. The triple negative subgroup represents the poor prognosis subgroup of the patients as it cannot be targeted any specific therapy against it thus limiting the therapeutic options.

Adriana *et al*. demonstrated the immunohistochemical expression of EGFR in 13.09% of the cases with invasive breast carcinoma. No expression was found in the normal mammary tissue, fibroadenoma, and atypical hyperplasia. EGFR is an important marker to stratify patients with breast cancer according to the molecular classification. The expression of EGFR correlated with the degree of differentiation, inversely with the lymph nodes immunohistochemical expression and significance of EGFR in breast cancer node status only in

basal-like carcinoma, and with distant metastases. In a subset of patients with breast cancer, EGFR could be considered an effective target for specific therapy.[14]

Tsutsui *et al*. indicated EGFR expression to have prognostic significance in recurrent breast cancer, whereas a multivariate analysis indicated ER status to be a more powerful prognostic factor than EGFR expression the patients with a positive EGFR expression tended to not respond to hormonal therapy and chemotherapy for breast cancer, whereas EGFR expression had an additional prognostic value.[15]

Sainsbury *et al*. showed a correlation between the presence of EGFR and high Bloom and Richardson grades that is independent of the size of tumor, lymph node state, elastosis, and round cell infiltrate.[16]

Tang *et al*. found that that EGFR overexpression has predictive value for better response to neoadjuvant chemotherapy in patients with TNBCs.[17]

We found that EFGFR positivity in almost one-third of the patients. This could be subgroup of patients which could be targeted by anti-EGFR therapy. This percentage was quite high as compared to other studies. This EGFR positivity also acted as a surrogate for aggressive disease which was shown by the significantly larger proportion of advanced stage, high grade, and node-positive disease present in receptor-positive patients. Studies relating to EGFR show similar outcomes. This subset showed a higher rate of pathological CR in patients subjected to neoadjuvant chemotherapy. There was trend of worse outcomes in surgically treated EGFR-positive patients which were not statistically significant. This might be due to the short follow-up period in our study.

As we continue this study, more trends might emerge. We could identify EGFR positivity as a true prognostic marker of breast cancer as well as a new target for therapy.

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