Original Article ISSN (O):2395-2822; ISSN (P):2395-2814

Immunohistochemical Profile of Infiltrating Ductal Carcinoma Breast (Not Otherwise Specified) - A Study Of 100 Cases.

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Received: March 2017
Accepted: March 2017

ABSTRACT

Background: Breast carcinoma is the most common malignant tumor. Infiltrating ductal carcinoma-not otherwise specified is the most common histological pattern of breast cancer. Estrogen receptor (ER), progesterone receptor (PR) and human epidermal growth factor receptor (HER2/neu) are immunohistochemical prognostic and predictive markers. AIM: The aim of the study was to explore the correlation of these immunohistochemical markers to each other, age of the patient, histological grade, menopausal status, tumor size, lymph node metastasis and to find the frequency of occurrence of the four immunohistochemical sub-types of breast cancer. **Methods:** In our cross-sectional study, we included patients coming to the department of Pathology. Paraffin sections from 100 cases diagnosed with infiltrating ductal carcinoma (NOS) were analyzed by immunohistochemical means for ER, PR and HER2/neu expressions and collected data was analyzed statistically by chi-square method. **Results:** The mean age of the patients was 55.28 years (range= 24 to 80). Majority of tumors were of grade II. Majority of tumors were ER (63%) & PR positive (58%) and HER2/neu negative (93%) and of immunohistochemical subtype 2 i.e. ER/PR positive & HER2/neu negative. The expression of estrogen receptor & progesterone receptor correlated significantly with age, menopausal status, tumor size and tumor grade. HER2/neu expression correlated significantly with age, menopausal status & tumor size. HER2/neu didn't correlate with tumor grade. None of them showed correlation with axillary lymph node metastasis. ER and PR expression correlated with each other, but none was correlated with HER2/neu. **Conclusion:** Breast carcinoma in this North-West region of Indian population may be biologically different from that of rest of population as well as western population. Our results indicate the importance of ER, PR & HER2/neu in management of carcinoma breast.

Keywords: Immunohistochemistry, Estrogen receptor, Progesterone receptor, HER2/neu, Infiltrating ductal carcinoma breast-NOS.

INTRODUCTION

Breast carcinoma is the most common malignant tumor and the leading cause of deaths due to carcinoma in women.[1] In India, breast cancer is the second most common cancer (after cervical cancer). Breast cancer incidence increases rapidly after the age of 30.[2] Breast cancer in Indian women is seen in earlier age as compared to western counterpart. The peak age for breast cancer is around 40-45 years in India.[3] A large number of risk factors have been identified that modify a woman's likelihood of developing this cancer: age at presentation, family history, menopause age, breast feeding, etc.[2] The term IDC-NOS is used for type of breast carcinoma that cannot be subclassified into any specialized type.

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Various prognostic and predictive factors are used in the management of breast cancer. These include: ER, PR, HER2/neu, PTEN, BRCA1, circulating tumor cells, p53, plasminogen system and Ki67.[4,5] Estrogens contribute to breast cancer initiation and progression.[6] The presence of estrogen receptor & progesterone receptor is a powerful predictive factor for the likelihood of benefit from adjuvant hormonal therapy including aromatase inhibitors e.g. Anastrozole,etc. and selective estrogen receptor modulators i.e. tamoxifen etc. As a prognostic factor, ER and/or PR positivity is associated with reduced

mortality compared to ER & PR negative tumors.[7] It is observed that ER positive breast cancers which lack PR expression, are less responsive to hormonal treatment than those that are PR positive.[8-10]

HER2/neu amplification or overexpression is involved in oncogenic transformation and tumorigenesis in breast cancer. It may lead to: increased & uncontrolled cell proliferation, decreased apoptosis, increased cancer cell motility and angiogenesis and hence worse prognosis.[11,12] HER2/neu amplification is a very good predictor of response to trastuzumab, but not a very good predictor of response to chemotherapy.[1] HER2/neu amplification correlates inversely with estrogen and progesterone expression. It is observed that HER2/neu amplification leads to resistance to tamoxifen treatment.[1,13]

The molecular subtypes of breast cancers include: Luminal (two sub-groups: ER-positive, HER2/neu negative with low proliferation and ER-positive, HER2/neu negative with high proliferation), HER2/neu positive and basal-like. These molecular groups predict clinical outcome and response to therapy. Among these molecular subtypes, the basal-like subtype has worst prognosis.[2] The classification of breast cancer into subgroups on the basis of gene expression patterns in tumor tissue is regarded as the gold standard. But there is limitation to its usage in the clinical or research setting, due to the expensiveness and technical difficulty encountered while performing gene-expression profiling using paraffin-embedded material. Consequently, immunohistochemical markers are used to classify tumors into subtypes that are surrogates for those based on gene expression profiling.[14]

As compared to gene expression profiling, immunohistochemistry is widely available, no special training is required, large tumor area can be analysed with ease, lesser time required for interpretation and relatively inexpensive.[38]

Immunohistochemical classification:[14]

Subtype1= ER/PR positive, HER2/neu positive
Subtype2= ER/PR positive, HER2/neu negative
Subtype3= ER/PR negative, HER2/neu positive
Subtype4=ER/PR negative, HER2/neu negative

This classification provides both therapeutic and prognostic information.

The present study was done for immunohistochemical analysis for expression of estrogen receptor, progesterone receptor and Human epidermal growth factor receptor-2 (HER2/neu) followed by their statistical correlation with age at presentation, histological grade, menopausal status, tumor size, lymph node metastasis and to find the frequency of occurrence of the four immunohistochemical sub-types of breast cancer.

MATERIALS AND METHODS

This study was conducted on 100 patients coming to the department of Pathology at Govt. Medical College and Rajindra Hospital, Patiala. It analysed the expression of ER, PR and HER2/neu by immunohistocemistry in 100 already diagnosed cases of infiltrating ductal carcinoma breast (NOS). The detailed proforma was duly filled for each case and patient's consent was taken. The parameters included in proforma were- patient's age, menopausal status, lymph node involvement, tumor size, tumor grade etc. Slides were prepared from these paraffin blocks and standard operating procedure was followed for immunohistochemical staining for ER,PR and HER2/neu.[15] Immunohistochemical kit was provided by Biocare Medical Oncord, CA, USA. Paraffin sections were cut at 5 micrometer and melted at 65ºC in an oven for 2 hours. Tissues were rehydrated following xylene dip and immersed in Peroxidized buffer solution followed by wash with Tris buffer. Heat retrieval of antigen was done with citrate buffer in decloaking chamber for 40 minutes at 95 degrees centigrade and brought to room temperature after removing from decloaking chamber. Background sniper was applied and slides were kept in moist chamber. The primary antibody was added for one hour followed by wash with Tris buffer. Secondary antibody was applied for 30 minutes and washed with Tris buffer. DAB chromogen was added to the slides and incubate for 6 minutes followed by wash with distilled water. Counterstaining was done with Harris hematoxylin. Sections were dehydrated with alcohol followed by xylene dip and mounted with DPX & coverslip applied.

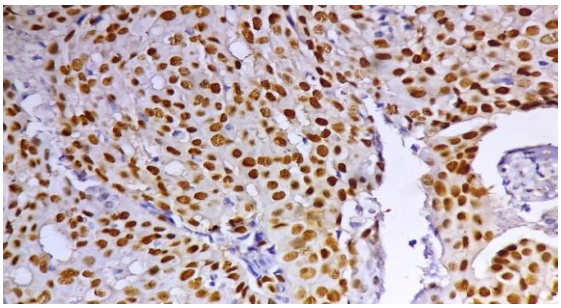

Figure 1: Photomicrograph showing strong ER positivity.

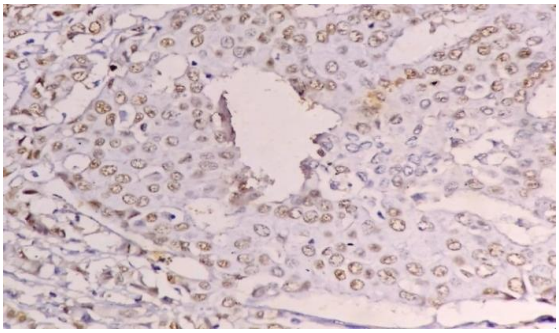

Figure 2: Photomicrograph showing moderate PR positivity.

ER and PR staining was interpreted with Allred Scoring system [As in Figures 1& 2] and HER2/neu was interpreted (3+, 2+, 1+, 0) with the help of ASCO/CAP 2013 guidelines [Figure 3].[16,17] ER, PR & HER2/neu, were statistically correlated with prognostic parameters like patient's age at presentation, menopausal status, lymph node status, size of tumor and tumor grade. Also frequency of IHC subtypes was calculated.

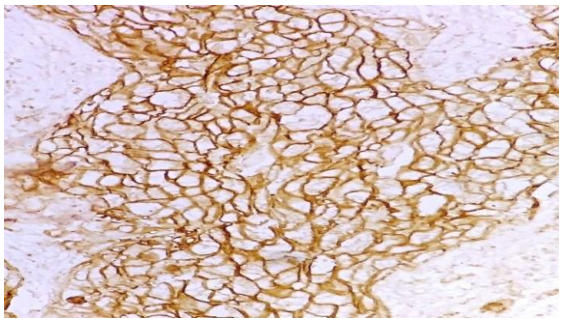

Figure 3: Photomicrograph showing HER2/neu positivity (3+).

RESULTS

This study was conducted on 100 paraffin tissue blocks of patients with IDC-NOS.The age of the patients varied from 24 to 80 years. Mean age was 55.28 years. The tumor size with maximum diameter ranges from 0.1 cm to 12 cm. The average tumor size was 4.3 cm. Majority of cases (43%) were in range of 2 to 5 cm. Grading of tumors was done according to Modified Bloom Richardson Grading system. [Table 1] reveals the clinicopathological parameters of all 100 cases.

Table 1: Clinicopathological parameters.

Variable	No./Percentage
1. Age	
<50 years >50 years	34 66
2. Menopausal Status	
Pre-menopausal Post-menopausal	18 82
3. Axillary lymph node	
Negative Positive	62 38
4. Tumor size(cm)	
<2 >2	29 71
5. Tumor grade	
I II III	26 43 31

Table 2: Immunohistochemical Profile.

	ER Status (No. of cases)	PR status (No. of cases)	HER2/neu status (No. of cases)
Positive	63	58	07
Negative	37	42	93
Total	100	100	100

[Table 2 & Figure 4] Reveal the Immunohistochemical parameters of all 100 cases.

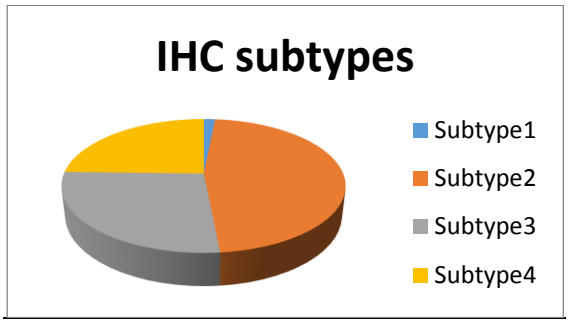

Figure 4: Immunohistochemical Subtypes.

Majority of ER positive (44.44%) and PR positive (43.10%) cases were of age >60 years. Majority of HER2/neu positive (71.43%) were of age <40 years in the present study. The expression of ER, PR and HER2/neu significantly correlated with age at presentation (p-value=0.000). 93.65% ER positive cases were post-menopausal. 91.38% PR positive cases were post-menopausal. 71.43% of HER2/neu positive were pre-menopausal. The expression of ER (p value=0.000), PR (p value= 0.004) and HER2/neu (p value=0.000) significantly correlated with menopausal status. 39.68% of ER positive cases had positive axillary lymph nodes for metastasis. 39.65% of PR positive cases had positive axillary lymph nodes. 28.57% of HER2/neu positive cases had positive axillary lymph nodes for metastasis. We did not observe any significant correlation between axillary lymph node status with ER (p value=0.651), PR (p value=0.689) and HER2/neu (p value=0.594) expression. 47.61% of ER positive tumors were of size between 2-5 cm. 46.55% of PR positive tumors were of size between 2-5 cm and 71.43% of HER2/neu tumors were of size <2 cm. There was seen significant correlation between tumor size and ER (p value=0.001), PR (p value=0.014) and HER2/neu expression (p value=0.028). Majority of ER positive (49.21%) and majority of PR positive (31.03%) tumors were of grade II, but majority of HER2/neu positive (57.14%) tumors were of grade III. The expression of ER (p value=0.003), PR (p value=0.031) significantly correlated with tumor grade, but HER2/neu expression (p value=0.298) didn't correlate significantly. ER and PR expression correlated with each other (p value=0.000), whereas expression of HER2/neu was inversely related to ER (p value=0.058) & PR expression (p value=0.102).

DISCUSSION

Breast cancer is the most common malignancy in women. It is highly curable, if diagnosed at early stage. Traditional morphological prognostic factors include: tumor size, tumor grade, axillary lymph node metastasis, etc. Now a days, more importance is given to biological molecular prognostic factors,

because a significant number of patients with early stage breast cancer harbor microscopic metastasis at the time of diagnosis. The biological prognostic markers in breast carcinoma include: ER, PR, HER2/neu, p53, Ki67, plasminogen activators & inhibitors, etc. Out of these, ER, PR and HER2/neu are most important prognostic and predictive markers.[18]

The present study was conducted on 100 patients coming to the department of Pathology at Govt. medical college and Rajindra hospital, Patiala, Punjab. In the present study, majority (63%) tumors were ER positive and 37% were ER negative. 58% tumors were PR Positive and 42% were PR negative. Only 07% were HER2/neu positive and 93% were HER2/neu negative. There was seen wide variation in the ER, PR & HER2/neu expression in breast carcinoma in different studies, possibly due to variations in different populations.[19-21] The results of HER2/neu positivity in our study (7%) were much lower as compared to other studies. The possible explanation for this is due to variations in different populations. Also HER2/neu assay results are influenced by multiple biological and technical factors. Many of the HER2/neu assays are not standardized. These different effects cannot be isolated.[22]

In the present study, ER and PR correlated with each other (p value=0.000), whereas expression of HER2/neu was inversely related to ER (p value=0.058) & PR expression (p value=0.102). Similar results were found in studies conducted by Siadati S et al,[23] Maha A et al,[24] Huang HJ et al,[25] and Al-Ahwal MS et al.[26]

In the present study, majority i.e. 61% cases were of subtype 2 i.e. ER/PR positive & HER2/neu negative, followed by subtype 4 i.e. ER/PR negative & HER2/neu negative (32%), then of subtype 3 i.e. ER/PR negative & HER2/neu positive (5%) and then of subtype 1 i.e. ER/PR positive & HER2/neu positive i.e. 2%. There was seen wide variation in the frequency of immunohistochemical subtypes in different studies.[19,21,14,27] The possible explanation for these differences is biological variability in different populations.

In the present study, there was seen significant correlation between age of the patient and ER (p value=0.000), PR (p value=0.000) and HER2/neu (p value=0.000) expression in present study. Studies by Desai SB et al,[28] Dodiya H et al,[29] Thang VH et al,[30] and Ganesan M et al,[31] showed similar results.

The majority of receptors positive cases were post-menopausal in our study. There was seen significant correlation between menopausal status of the patient and ER (p value=0.000), PR expression (p value=0.004) and HER2/neu expression (p value=0.000) in the present study. Studies by Wilking N et al,[32] Faheem M et al,[33] and Mahmood H et al showed similar results.[34]

In present study, we did not observe any significant correlation between axillary lymph node status with ER (p value=0.651), PR (p value=0.689) and HER2/neu (p value=0.594) expression in present study. Studies by Ambroise M et al,[19] Azizun Nisa et al,[35] showed similar results.

In present study, there was seen significant correlation between tumor size and ER (p value=0.001), PR (p value=0.014) and HER2/neu expression (p value=0.028) in the present study. Studies by Yadav R et al,[36] Prasad HLK et al,[37] and Bhagat VM et al showed similar results.[38]

In presnt study, there was seen significant correlation tumor grade with ER(p value=0.003) & PR(p value=0.031), study done by Thoreson S et al,[39] Onitilo AA et al,[14] and Dodiya H et al showed similar results.[29] No significant association was seen between tumor grade and HER2/neu expression (p value=0.298) in present study. Study conducted by Naeem M et al,[40] and Dodiya H et al showed similar results.[29]

Study limitations included lower HER2/neu positivity as compared to other studies.

CONCLUSION

The present study confirmed that the expression of estrogen receptor & progesterone receptor correlated significantly with age, menopausal status, tumor size and tumor grade. HER2/neu expression correlated significantly with age, menopausal status & tumor size. HER2/neu didn't correlate with tumor grade. None of them showed correlation with axillary lymph node metastasis. ER and PR expression correlated with each other, but none was correlated with HER2/neu.

These observations suggest that breast carcinoma in this North-West region of Indian population may be biologically different from that of rest of population as well as western population. These results could have clinical importance in management of carcinoma breast.

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How to cite this article: Kaur R, Kaur N, Singh H, Chand P, Kundal RK, Garg P. Immunohistochemical Profile of Infiltrating Ductal Carcinoma Breast (Not Otherwise Specified) - A Study Of 100 Cases. Ann. Int. Med. Den. Res. 2017; 3(3): PT17-PT21.

Source of Support: Nil, **Conflict of Interest:** None declared