ORIGINAL RESEARCH ARTICLE

Study of Estrogen Receptor, Progesterone Receptor and Human Epidermal Growth Factor Receptor Expression by Immunohistochemistry in Breast Carcinoma

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Abstract:

Introduction:

Over the last few decades there have been outstanding advances in breast cancer management leading to early detection and treatment of disease. Recent attention has been directed to immunohistochemistry (IHC) based classification of Estrogen Receptor (ER) / Progesterone Receptor (PR) and Human epidermal growth factor receptor/neu (HER2-neu) status which provides prognostic and therapeutic information and is inexpensive and readily available.

Aim:

The present study was undertaken with the view of correlating the histopathology of the tumor by way of tumor grade, various traditional prognostic markers and its immunohistochemistry profile with respect to Estrogen/Progesterone hormone receptors and Human epidermal growth factor receptor/neu status.

Material and Methods:

An observational study was conducted in the Department of Pathology, BKL Walawalkar Rural Medical College for two and half year from January 2015 to June 2017 including all the cases of breast carcinoma diagnosed on histopathology on Modified Radical Mastectomy (MRM) specimens and needle core biopsy. The cases with no prior oncological treatment and having complete clinical data were included and the cases with non-malignant conditions of breast were excluded. A total of 134 cases were studied. The surgical specimens were then evaluated immunohistochemically for ER, PR, HER2-neu markers.

Results:

Out of 134 cases studied, majority of the cases (92.5%) were of Invasive Breast carcinoma, No special type. Women of 31-50 years are more prone to the risk of the development of breast carcinoma. Grade III tumors were seen predominant with 56.67%. implying a poor prognosis. Percentage of ER positivity was 41.04%, PR positivity was 24.6%, Her2-Neu positivity was 26.9% and triple negative was 41.04%. Grade 3 tumor and triple negative cases indicate poor prognosis and poor outcome.

Conclusion:

From the present study it was concluded that with incorporation of immunohistochemistry based classification of both ER/PR and HER2-neu status into the histopathology report along with the traditional TNM staging and histological grading of breast carcinoma help in better therapeutic management and increases prognostic accuracy and is inexpensive and readily available.

Keywords:

Breast cancer, Immunohistochemistry, Estrogen receptor, Human epidermal growth factor receptor, Progesterone receptor, Triple negative.

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Introduction:

Breast Cancer is the commonest cancer in women worldwide. About one million new cases diagnosed every year and constituting over 20% of all malignancies among females. [1]

Worldwide, the incidence of cancer has been observed increasing. Based on the reports of

WHO Cancer Control and Prevention Programme for breast cancer, it is the most common cancer in women, accounting 16% of all female cancers. Breast cancer is known as cancer of developed world but majority of breast cancer deaths occur in developing countries. [2] In 2008, India recorded more deaths due to breast cancer than the USA. WHO forecasts that, by 2020, 70% of all breast-cancer worldwide will be in developing countries. [3]

Breast cancer is the most common cancer in women, in urban areas of developing countries due to increase in life expectancy, urbanization and western lifestyles. Most women with breast cancer are diagnosed in late stages in low and middle income countries due to lack of awareness on early detection and barriers to health services. In India, more than 100,000 new breast cancer patients are estimated to be diagnosed annually. [4]

Over the last few decades there have been outstanding advances in breast cancer management leading to early detection of disease and the development of more effective treatments resulting in significant decline in breast cancer deaths and improved outcome for women living with the disease. [5] Recent attention has been directed singularly at molecular classification of breast cancer.

While molecular and genetic testing is very elegant, prognostic and predictive, it is expensive and not yet widely available. [6]

In general, tumor size, nuclear grade, mitotic activity, lymphatic and vascular invasion and lymph node involvement are common clinical pathological features of breast cancer that can be detected by routine light microscopy. These parameters associated with the grading and staging of breast cancer are helpful in cancer treatment, clinical management and prognostic assessment. [7]

Immunohistochemical (IHC) assessment of hormonal markers such as ER and PR are important and useful predictive factor in breast carcinoma. In invasive breast carcinoma whose tumor cells lack Estrogen Receptor/Progesterone Receptor, they do not respond to hormonal therapy. Their status also has a prognostic value. Patients with ER/PR positivity have low risk of mortality in comparison to the patients with ER+/PR- or ER-/PR+ or both negative. [8,9]

HER2-neu is also known as Epidermal growth factor receptor 2. It has gained an importance as a significant prognostic marker. [10] Its amplification and over expression is associated with the poor prognosis in breast carcinoma patients with axillary lymph node metastases but there is no association with

negative lymph nodes. [11] HER2-neu can also be a predictive marker. [12]

Currently, neo-adjuvant chemotherapy has become the standard approach for locally advanced breast tumors as it helps to shrink the tumor in the early stage of carcinoma and make it convenient for breast conservative surgery.

Chemotherapy is also the mainstay in the treatment for almost all patients of metastatic breast carcinoma. [13] Several markers such as Estrogen receptor, Progesterone receptor and Human epidermal growth factor receptor 2-neu (HER2-neu) and their expressions have been used to study the breast carcinoma. Assessment of the status of these tumor markers has significant role in accessing the diagnosis, treatment and prognosis of breast carcinoma in patients. [8,9]

Thus this study was carried out with the aim of helping to correlate IHC and histopathological grade of breast carcinomas using the modified Bloom-Richardson system and hence, help in therapeutic management and prognosis of breast carcinomas.

Material and methods:

This Observational study was conducted in the Department of Pathology, BKL Walawalkar Rural Medical College, Chiplun from January 2015 to June 2017 which includes 134 cases of breast carcinoma diagnosed on histopathology including all the like needle core biopsies and MRM specimens. The patients should not have any prior oncological treatment and have complete clinical data. The cases with non-malignant conditions of breast were excluded.

Tissue processing was done by fixing the tissues in 10% buffered formalin overnight. The tissues were grossed and representative sections were taken and submitted for processing. Sections taken from paraffin embedded tissues were stained with hematoxylene and eosin and were examined. Grading was done according to modified Bloom Richardson grading system of WHO. [14] Most suitable tissue block were selected for ER, PR and HER2-neu markers and were sent to Tata Memorial Hospital, Mumbai. The ER/PR expression shows the amount of estrogen receptors (ER) and progesterone receptors (PR) present in tumor cells. HER2-neu assay measures the amount of HER2-neu staining present on the membrane of tumor cells. Allred's scoring was used for IHC. [15] Statistical Analysis was done, the data were tabulated and expressed as percentages.

Results:

Out of 134 cases diagnosed, majority of specimens received were Modified Radical Mastectomy (MRM) i.e 103 and rest were

needle core biopsies. The age of the patients ranged from 27 to 83 with mean age being 50.4 years (SD ±13.3years). Most of the cases belonged to the age group of 31-50 years with total of 74 cases, followed by 26 cases (19.40%) from 51-60 years, 25 (18.65%) from 61-70 years (Table 1). All were females except one which was male. Right breast was more prone comprising of 76 (56.7%) cases. Upper outer quadrant of breast was predominantly involved in 63 (47.01%) cases (Table 2).

The size of tumor in MRM specimen were measured between 2.0-5.0 cm in 67 cases (50%), followed by >5cms in 21 cases (27.6%) and ≤2cms in 15 cases (22.4%). The most common histologic type is Invasive Breast Carcinoma, No special type (IBC-NST) accounting to 124 cases followed by 3 case each of Invasive Lobular Carcinoma (ILC) and Ductal Carcinoma in situ (DCIS) and 2

case each of Mucinous and Medullary carcinoma. (Table 3). On extensive dissection of adjacent beast tissue and axillary tail, lymph nodes were retrieved in MRM, out which 48 cases showed positive metastatic deposits.

Modified Bloom Richardson scoring was done for all breast carcinoma cases (Table 4) and graded accordingly. Grade III was present in 62%, followed by 23% of Grade II and 15% of Grade I (Table 5). On histopathlogical examination, perineural invasion was noted in 38% of cases and lymphovascular invasion in 64.92% cases.

In our study 41.04% cases showed ER positivity, 24.6% showed PR positivity and 26.9% showed HER2-neu positivity. Only 2.2% cases showed triple positivity. However triple negative cases were 55 (41.04%) (Table 6). 21.6% belonged to luminal A group and 2.2% belonged to luminal B group (Table 7).

Table 1 - Age Distribution of carcinoma breast

Age	No. of cases
21-30	2 (1.5%)
31-40	37 (27.6%)
41-50	37 (27.6%)
51-60	26 (19.4%)
61-70	25 (18.6%)
71-80	6 (4.4%)
81-90	1 (0.7%)
Total	**134**

Table 2 – Quadrant Distribution

Quadrant	No of Cases
UOQ	63 (47.01%)
UIQ	37 (27.7%)
LOQ	19 (14.2%)
LIQ	10 (7.3%)
Central	5 (3.7%)
Total	**134**

Table 3 - Types of Carcinomas on histopathological diagnoses

Histological diagnosis	No.of cases
IBC-NST	124 (92.5%)
DCIS	3 (2.2%)
ILC	3 (2.2%)
Medullary C	2 (1.5%)
Mucinous C	2 (1.5%)
Total	**134**

Table 4 – RB Scoring and case distribution

Score	Tubule formation	Nuclear grading	Mitotic rate
1	4 (3%)	11 (8.2%)	24 (17.9%)
2	24 (17.9%)	32 (23.9%)	82 (61.2%)
3	106 (79.1%)	91 (67.9%)	28 (20.9%)
Total cases	**134**	**134**	**134**

Table 5 – Histologic grading in various types of carcinoma

Histological Grade	No of cases
I	20 (15%)
II	31 (23%)
III	83 (62%)
Total	**134**

Table 6 – IHC Status of cases under study

IHC Marker status	No of cases(out of 134)
ER positive	55 (41.04%)
PR positive	33 (24.6%)
HER2neu positive	36 (26.9%)
Triple positive	3 (2.2%)
Triple negative	55 (41.04%)

Table 7 – Luminal Classification of cases under study

Types	No of cases (out of 134)
Luminal A (ER+/PR+, HER2neu -)	29 (21.6%)
Luminal B (ER+/PR+, HER2neu +)	3 (2.2%)
HER2neu positive (ER-/PR-, HER2neu +)	23 (17.2%)
Triple negative (ER-/PR-, HER2neu -)	55 (41.04%)

Discussion:

The use of IHC in breast cancer has become an integral part of a complete and comprehensive histopathology report. In terms of prognosis and prediction of response to treatment, in addition to histological grade and tumor subtype, hormone markers, ER/ PR and HER2-neu have become the mainstay requirement for the oncologist. The present study was undertaken with the view of correlating the histopathology of the tumor by way of tumor grade, various traditional prognostic markers and its IHC profile with respect to ER, PR hormone receptors and Her2-neu. [16]

Estrogen receptors:

There are two forms of ER, ERα and ERβ which together mediate downstream events. ERα (classical ER), the major player in breast cancer is analyzed in clinical practice. ERβ on the other hand is a relatively newly recognized ER and its function particularly its role in breast cancer is poorly understood.

ER-positive cells in the normal breast does not indicate proliferative activity whereas ER-positive cells in atypical ductal hyperplasia and in invasive and in in-situ

carcinoma, show proliferative activity. The transformation from an ER-positive non proliferating cell phenotype into an ER-positive proliferating cell phenotype appears to be a critical switch and is one of the characteristic events of malignancy. The exact mechanism that is responsible for this switch is as yet not explained, although deregulation of transforming growth factor beta signaling is considered as the main culprit. Estrogen receptor (ER) positive tumors are heterogeneous and the efficacy of hormonal therapy depends on status of multiple other proteins along with other transcription factors such as FOXA1, GATA-3, growth factors and co-activator and co-repressorproteins. [17]

Progesteron receptors:

These are an estrogen-regulated protein found in the cells of breast tissue. Therefore its expression is believed to function as in ER pathway. [18] Assessment of both ER and PR is helpful in predicting response to hormonal therapy more accurately. There are few proposals which indicate that PR positive tumors are more likely to respond to tamoxifen. [19] The predictive value of PR positivity in the absence of ER is controversial. Breast tumors which are ER+ and ∕or PR+ show low mortality risk in comparison with ER- and ∕or PR- tumors. [20]

HER2-neu:

The HER2-neu gene is located on the long arm of chromosome 17 (17q12-21.32). It encodes p185 oncoprotein which is a receptor tyrosine kinase that can be associated with multiple signal transduction pathways. It has been found to be overexpressed in many types of human malignancies, notably breast, ovarian, gastric, pancreatic, prostatic, colorectal, cancers of the female genital tract and lung cancer. HER2-neu, also known as erbB-2 oncoprotein is overexpressed in 25 to 30 per cent of breast cancers. HER-2/neu overexpression in patients with breast cancer and positive lymph nodes is linked to poor prognosis with a reduced disease-free interval and shortened survival time, and similar linkage may exist in node-negative cases. HER2-neu gene expression level seems to be a significant predictor for response to some therapeutic agents.

Trastuzumab (Herceptin), a humanized monoclonal anti HER2-neu antibody, was approved by the US Food and Drug Administration as an adjuvant therapeutic agent for patients with metastatic breast cancer overexpressing HER-2/neu protein. As a result, evaluation of HER-2/neu status has become pivotal in determining patient's eligibility for trastuzumab treatment. [21]

A significant relationship between amplification of the HER2-neu (c-erbB-2) oncogene and adverse clinical outcome in patients with breast cancer was first noted by Slamon et al1 in 1987. Panjwani et al showed positive correlation of grade III with HER-2/neu amplification. [22]

The age range and mean age in present study was concordant with study done by Varughese AA et al [23] where as less similar to the study done by Engstorm MJ et al [24] in which mean age noted was much higher accounting to 72.5 years and range being 41-102 years.

The observations from the present study are concordant with other studies done by Ghosh S et al [25] and Ch'ng ES et al [26] which also shows female preponderance.

In the study conducted by Ambroise M et al [27] and Azizun – Nisa et al [28], the left breast was more commonly involved accounting for 59.2% and 57%respectively, but right breast was commonly involved in the present study.

The studies of Meena et al [29] and Costa M et al [30] show the most common quadrant involved was upper outer which was concordant with the present study (40%).

In study done by Ayadi L et al [31], and Ghosh S et al [25], observed that the most of the tumors ranges from 2-5 cm in size which constituted to 63.2% and 62.5% respectively which shows concordance with the present study.

Kumar A et al [32] and Varughese AA et al [23] observed that IBC -NOS type was the commonest histological type. In present study we encountered 122 cases of IBC-NST type which constituted 91%.

In our study maximum cases i.e, 65% of cases showed lymphovascular invasion which is similar to the study done by Ch'ng ES et al [26] in which they observed lymphovascular invasion is maximum with 77.8% of cases. Whereas in study done by Schoppmann SF et al [33] lymphovascular invasion was seen in less percent of cases i.e., 28.1%.

The tumors were graded using Modified Bloom Richardson grading system. Most commonly tumors of Grade III constituting 62% followed by Grade II group of 23% and Grade I were 15% and our results were concordant with studies done by Schoppmann SF et al [33] and Hui HU et al. [34] (Table 8)

In the present study, positive lymph node metastatic deposits were seen in 35.5% of cases. However the studies done by Schoppmann SF et al [33] and Chen S et al [35] observed maximum cases with metastatic lymph node.

Taking into account the immunohistochemical status, triple negative cases constitute the highest proportion of 55 cases (41.04%) in the present study which is similar to the studies done by Tiwari et al [36] and Nikhra et al. [16]

Table 8 - Histological grading and comparison with other studies

Authors	Schoppmann SF et al	Hui HU et al	Present study
Grade I	14.43%	31%	15%
Grade II	45.18%	44.1%	23%
Grade III	40.37%	24.9%	62%

Table 9 – Luminal Classification and comparison with other studies

Types	Tiwari et al (Out of 32)	Nikhra et al (out of 43)	Present study (out of 134)
Luminal A (ER+/PR+, HER2neu -)	7	14	29 (21.6%)
Luminal B (ER+/PR+, HER2neu +)	1	4	3 (2.2%)
HER2neu positive (ER-/PR-, HER2neu +)	6	10	23 (17.2%)
Triple negative (ER-/PR-, HER2neu -)	18	13	55 (41.04%)

Conclusion:

The present study was done to highlight the importance of histopathological examination in breast carcinoma not only in establishing diagnosis, but also histologically subtype, predict prognosis based on histological grade and IHC studies. From our study, it was concluded that Invasive Breast carcinoma, NST was the most common histopathological subtype of breast carcinoma. Women of 31-50 years are more prone to the risk of the development of breast carcinoma. Most cases of Grade 1 and ER/PR positivity expression implys a better prognosis. As the tumor grade increases, ER/PR expression decreases and HER2-neu expression increases and Grade 3 tumor and triple negative cases indicating for poor prognosis and poor outcome. Breast carcinoma with HER2-neu positivity or with triple negativity shows more aggressive nature.

Also a previous personal history of breast cancer, or a germline BRCA mutation all appear to be positively associated with Triple negative breast cancer (TNBC). This finding further supports the revised NCCN guidelines that recommend women 60 years of age or younger with TNBC to be referred for consideration of genetic counseling. In addition, there was a lack of association between TNBC and personal history of Atypical hyperplasia and Lobular carcinoma in situ. In order to develop more effective treatments, better surveillance and improved prevention strategies, it is critical to improve our understanding of the risk factors that are associated with the development of triple negative breast cancer.[37]

Conflict of interest: None to declare

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