Trends in Molecular Classification of Breast Carcinoma in a Tertiary Health Care Centre: A 5 year Retrospective Study

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ABSTRACT

Introduction: ER, PR and HER2 are the most important factors for predicting prognosis and response to treatment. In the present study we retrospectively measured the frequency of estrogen receptor (ER), progesterone receptor (PR), and Her 2 Neu positivity in breast cancer patients during a span of 5 years and classified them into luminal A (estrogen receptor/progesterone receptor [ER/PR]-positive and human epidermal growth factor receptor-2 [HER2]-negative), luminal B (ER/PR-positive and HER2-positive), HER2 (ER/PR-negative and HER2-positive), and triple negative (ER/PR- and HER2-negative) molecular subtypes. In this study we have also tried to correlate various molecular subtypes of breast cancer with age distribution, histological grade, lymph node status and distant metastasis.

Methodology: We conducted a 5-year retrospective study on 547 patients. Clinical data including the age, sex, lymphnode status along with histological type and, grade of the tumour were recorded and the cases were subjected for immunohistochemical evaluation of HER-2/neu, ER, PR receptor status.

Results: The results showed that HER2 neu was the most predominant immunomarker, while ER and PR was almost half of it. Among the molecular sub typing HER 2 type was the most prevalent constituting 53% of the cases, followed by almost triple negative and luminal A subtype with 20% cases each. Luminal B was least frequent with 9% of the cases. An inverse relationship exists between expression of her 2 neu and ER/PR.

Conclusion: Our study concluded that molecular subtyping of breast carcinoma is an important part of complete histopathology report, in terms of prognosis, recurrence and treatment. A shift in occurrence of breast malignancies towards younger age leads to increased Her-2 neu expression.

Keywords: Breast Carcinoma, Immunohistochemistry, Molecular subtypes.

INTRODUCTION

Carcinoma breast is the most common malignancy and the leading cause of death in females globally. The recent years have witnessed a rise in incidence as well as deaths, ranking it the most common in urban and second most common in the rural population of females in India.[¹] It is a heterogeneous disease with numerous molecular and clinical features due to its variable genetic make-up affecting the regulation of cell growth and differentiation.[²] Tumor markers are an important aspect of research in breast cancer, as they are linked to its treatment, prognosis, survival and histological subtypes.[³]

Human epidermal growth factor receptor 2 (HER2) gene, also known as ERB-B2 or HER2/neu is located on chromosome 17q11.[¹] Its amplification or over-expression of erb-b2 protein has been detected in 10 to 30 per cent of breast cancers[⁴] and affects the disease progression, metastatic potential and resistance to tamoxifen.[⁵] Its association has also been dictated with size, grade and stage of the tumour along with relapse and reduced overall survival, hence underlying its importance in detection.[²] The introduction of targeted therapy, anti-HER2 monoclonal antibody Trastuzumab (Herceptin) and HER1/HER2 dual receptor

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inhibitor, Lapatinib, has proved to be an effective therapy for patients undergoing gene amplification.\(^6,7\)

Estrogen is an important mitogen exerting its activity by binding to its receptor (ER) and its expression is seen in 50-80% of breast cancers. The response to hormonal and chemotherapy directly correlates with presence of hormone receptors (ER and PR) in the tumour tissue\(^8\). Hence, a more differentiated tumour has higher ER and PR positivity and a better prognosis. PR is a surrogate marker of a functional ER and is highly important in predicting the outcome of breast carcinoma. 60-70% invasive breast carcinomas express PR receptor with a higher positivity in older and postmenopausal age group.\(^9\)

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Majority of breast malignancies are luminal tumours. Luminal tumour cells look like cells of breast cancers that start in the inner (luminal) cells lining the mammary ducts. Among various molecular subtypes luminal A tumours have best prognosis, relatively higher survival and low recurrence rates\(^8,10\). on the contrary luminal B subtype are diagnosed at a younger age, carries poorer prognosis and linked with \(p53\) mutation.\(^11\) Triple negative or basal like tumours, constituting 15-20% of breast malignancies have cells with features similar to those of the outer (basal) cells surrounding the mammary ducts. These are aggressive tumours with poor prognosis and associated with BRCA 1 mutation.\(^12\)

Therefore, understanding of the hormonal receptors, hence the prognostic factors is very necessary for planning the disease investigation and treatment strategies. This study emphasizes on the expression of estrogen, progesterone and HER2 neu receptors among various histological variants of breast carcinoma along with molecular subtyping into luminal A (estrogen receptor/progesterone receptor [ER/PR]-positive and human epidermal growth factor receptor-2 [HER2]-negative), luminal B (ER/PR- and HER2-positive), HER2 (ER/PR-negative and HER2-positive), and triple negative (ER/PR- and HER2-negative) subtypes.

**MATERIALS AND METHODS**

A retrospective study with a total of 547 (either tissue or paraffin blocks) histologically proven breast cancer cases (including both in-hospital and referral cases) were included in the study, over a span of five years from 2010 to 2015. The study was conducted in the Department of Pathology, Jawaharlal Nehru Medical College, AMU, Aligarh.

**Clinical and Histological parameters:** Clinical data including the age, sex along with histological type, lymphnode status, grade and distant metastasis, wherever available was recorded in each case.

**Immunohistochemical evaluation:** All 547 cases were subjected for immunohistochemical study of HER-2/neu, ER, PR receptor. A 3 to 4 \(\mu\)m thick paraffin embedded tissue sections were placed on poly-L-Lysine coated slides. After deparaffinization, antigen retrieval and blocking of endogenous peroxidase, immunostaining was performed using primary antibody at 1: 100 dilution. Binding of the primary antibody was followed by the addition of diaminobenzidine (DAB) as a chromogen. Microscopic evaluation of the slide was done and different histological variants of breast carcinoma were categorized into 4 groups according to their Hormone Receptor positivity status. ER/PR+, Her2+ with Luminal B; ER/PR+, Her2− with Luminal A; ER/PR−, Her2+ and ER/PR−, Her2− with triple negative/basal-like tumors. Human epidermal growth factor receptor 2 scoring of IHC slides was done on light microscopy as per the recommended American Society of Clinical Oncology (ASCO) guidelines 2007. Scores 0 and 1 were considered as negative while scores 2 and 3 were considered as equivocal and positive respectively. Sections which showed strong membrane staining of normal epithelia of breast were rejected and subjected to a repeat IHC.

**RESULTS & DISCUSSION**

We conducted a 5 year retrospective study involving 547 cases of female patients with breast cancer and studied the expression of various immunohistochemical markers on each case (Fig.1, 2 & 3). Similar retrospective study was conducted by Jain et al and Ambroiseet al on 203 and 321 females respectively.\(^2,13\) Our study witnessed the maximum burden of disease ranging from 32-65 years of age with the mean age of presentation being 39 years, very similar to the study of Jain et al, Nikhra et al, Siadati et al and emphasizing the predominant presentation in younger age group.\(^12,14,15\)

However some studies like that of Ambroise et al document an older mean age.\(^15\) Figure I shows the total number of cases among various age groups.

![Fig 1: Total No. of cases in various age groups](image1.png)

![Fig 2: Shows the percentage distribution of cases according to the histopathological type of carcinoma.](image2.png)

Similar findings were recorded by Nikhra et al, Ghosh et al and Bhagat et al showing infiltrating ductal carcinoma(NOS).
as the most common histological type and lobular carcinoma as the second most common type.\cite{14,16,17}

The 510 cases of Infiltrating Ductal carcinoma were divided into grade I, grade II and grade III amounting to 8% (41), 64% (326) and 28% (143) cases respectively, on the basis of Scarff Richardson Bloom grading system including tubule formation, mitotic activity and pleomorphism. Our study is very much in concordance with Nikhra et al\cite{14}, and Jain et al\cite{2}, who obtained almost similar figures. Fig 3 shows percentage distribution of IDC cases.

![Fig 3: Percentage of IDC cases among various grades](image)

Lymph node being an important prognostic marker was also studied, and total number of cases were divided into absent/0 nodes, 1-3 nodes, 4-9 nodes and more than 9 nodes according to the total number of lymph nodes involved in each case. Fig 4 demonstrates the percentage of cases along with their lymph node status.

![Fig 4: Percentage of cases along with their lymph node status](image)

Of the total 547 breast carcinoma cases in our study, HER2 neu was the most predominantly expressed immunomarker seen in 62% (339) cases, while the prevalence of estrogen and progesterone was almost half as that of the former with only 28% (151) and 26% (140) cases respectively. Ductal and lobular carcinoma showed the maximum expression of her2neu in almost 60% cases, while metaplastic and medullary carcinoma had 33% of cases expressing above receptor. Almost all histological variants have similar prevalence in expression of ER, PR receptors ranging from 20-30 %. Mucinous carcinoma showed 40% expression of all the three receptors (Figure 5). Most of the studies noted relatively higher percentage of estrogen and progesterone positivity like Jain et al\cite{3} reported ER, PR positivity in 37% and 34% cases respectively while Nikhar et al\cite{4} noted ER positivity in 39% cases and PR positivity in 42% cases. Similarly Munjal et al\cite{18} demonstrated ER,PR positivity in 41% of their cases. This might be contributed to the inclusion of older or post-menopausal females in their study. While our study noted remarkably higher(62%) her2 neu positivity, Jain et al\cite{2}, Nikhra et al\cite{14}, Ambroise et al\cite{13} and Sharif et al\cite{19} demonstrated 35%, 32%, 27% and 31% her2neu positivity respectively. This can be explained by presence of predominantly younger patient population in our study. Among molecular subtyping (Fig 6) HER 2 type was most prevalent subtype constituting 53% of the cases, followed by almost equally occurring triple negative and luminal A subtype with 20% cases each. Luminal B was least frequent with only 45 (9%) of the cases. Metaplastic and Medullary carcinoma showed highest percentage of triple negative or basal like cancers. On the contrary HER 2 type was most common subtype in ductal and lobular carcinoma. Ambroise et al\cite{13} and Lund et al\cite{20} got 25% and 29% triple negative cases respectively. Similarly, various other studies have reported triple negative cases ranging from 13% to 47% amounting to regional and geographical variation and comparatively lesser prevalence in non-African American females.\cite{20} Our study also shows an inverse relationship between expression of her 2 neu and estrogen/progesterone, still a substantial amount (20%) cases were triple positive which is in concordance with Nisa et al\cite{9}, Sharif et al\cite{20} and Jain et al\cite{2}.

![Fig 5: Shows percentage positivity of ER, PR & HER2 neu amongst various histological variants of breast carcinoma](image)

![Fig 6: Shows percentage positivity of molecular subtyping in carcinoma breast among various histological variant.](image)
CONCLUSION
Our study concluded that Infiltrating Ductal carcinoma (NOS) is the most common histopathological sub-type of breast cancer. A positive correlation exists between ER and PR expression accompanied by an inverse correlation of these markers with HER-2/neu. At our centre there is predominance of her 2 neu expression in majority of breast carcinoma cases, probably because of early age at diagnosis and it indicates chances of better treatment outcome with herceptin. Thus, the use of IHC in breast cancer including hormone markers; ER/PR and HER-2/neu is an important part of a complete and comprehensive histopathology report, both in terms of prognosis and treatment.

What this study adds:
1. What is known about this subject?
   • Infiltrating Ductal carcinoma (NOS) is the most common histopathological sub-type of breast cancer.
   • A positive correlation exists between ER and PR expression accompanied by an inverse correlation of these markers with HER-2/neu.
   • The prevalence of Triple negative case range from 13% to 47% of cases amounting to regional and geographical variation.
   • ER/PR and HER-2/neu are an important part of a complete and comprehensive histopathology report, both in terms of prognosis and treatment.

2. What new information is offered in this study?
   • Our centre noted a predominance of her 2 neu expression in majority of 339 cases (62%) of breast carcinoma, which could be contributed to the inclusion of younger patient population in our study.
   • HER 2 type is the most prevalent subtype constituting 53% of the cases, followed by almost equally occurring triple negative and luminal A subtype with 20% cases each.

REFERENCES


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