## Original Article

# Correlation between Her2Neu Status with Molecular Classification, Cyclin D1 Status and Ki67 Expression in Intraductal Carcinoma of the Breast

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**Introduction :** With recent advancement of molecular biology, the prognosis and therapeutic strategy of breast tumours can be ascertained and predicted for a better prognosis.

**Aims and objectives :** The primary aim was to find out the correlation Her2Neu scoring and status with molecular classification of the breast. As the secondary outcome measure association will be looked for between Her2Neu status with Cyclin D1 and Ki67 expression in Invasive ductal carcinoma.

**Material and Methods :** The 57 cases with suspected breast carcinoma of intraductal carcinoma has been included in this study over a period of 18 months. Her2Neu expression and scoring done by Immunohistochemistry and Cyclin D1 expression was calculated semiquantitatively on the basis of positive nuclear staining fraction of tumour cells and their intensity. Ki67 exprssion has also been measured by IHC markers. The data analysed by SPSS version 25.0.

**Results :** The association between Her2Neu scoring ($p<0.001$) as well as Her2Neu status ($p<0,001$) have been found to be statistically significant. The association between Her2Neu scoring ($p=0.0359$) but not Her2Neu status ($p=0.2672$) with Cyclin D1 status has been found significant. The association between Her2Neu status and Ki67 status has been found to be significant ($p=0.004$).

**Conclusion :** The changes with increase in copy numbers and protein amplification of Her2, it has been shown the overexpression of this human epidermal growth factor receptor 2 is directly associated with early relapse, less survival time and poor prognosis.

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***Key words* : Breast carcinoma, Her2Neu status, Molecular classification, Cyclin D1 status, Ki67 index, Immunohistochemistry**

Breast cancer is most common in Indian women occurring in 1 in 4 women[1]. Her2Neu oncoproten (Humar Epidermal Growth Factor receptor 2) also known as Neu CD 340 p 185, is a protein encoded by ERBB2 gene located in long arm chromosome 17q12 with tyrosine kinase activity. It was the first oncogene in the samples of Invasive Ductal Carcinoma (IDC) and seen in 10-20% of breast carcinoma patients. It is a marker for sensitivity to Herceptin (Trastuzumab)[2]. The germline mutation (TP53 is commonly associated with breast cancers) seen in Li-Fraumeni syndrome. ER, PR, HER2 negative cancers (TN) or basal like cancers.

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***Editor's Comment :***

- The 57 cases with suspected breast carcinoma of intraductal carcinoma have been included in this study over a period of 18 months.
- The commonest type is Luminal A and the least common type is Her2positive type. The Luminal A type of tumours is commonly associated with Lobular carcinoma.
- The association between Her2Neu scoring ($p<0.001$) as well as Her2Neu status ($p<0.001$) have been found to be statistically significant.
- The association between Her2Neu scoring ($p=0.0359$) but not Her2Neu status ($p=0.2672$) with Cyclin D1 status has been found significant. The association between Her2Neu status and Ki67 status has been found to be significant ($p=0.004$).
- In this study, 40.4% patients had 3 Her2-Neu scoring, 90.9% patients had 3 Her2-Neu scoring, in LA Group 54.5% patients had 0 Her2-Neu scoring, in LB Group, 52.0% patients had 3 Her2-Neu scoring, in TN Group, 90.0% patients had 0 Her2-Neu scoring and the association of Her2-Neu scoring vs group was statistically significant ($p<0.0001$).

The response to chemotherapy is favourable with better prognosis in ER positive in HER2 negative breast cancers (24) Along with HER2, high Ki67 LI group of patients respond poorly to chemotherapy following

neoadjuvant endocrine therapy.[3]

The Molecular classification of breast carcinoma shows different hormonal receptors status and different immunohistochemistry (IHC) marker expression[4,5]. The description of different molecular subtypes on the basis of this has been discussed below (Table 1, Fig 1).

The (ER/PR/HER2) expressions are key for molecular subtype classification. The expression of Her2neu2 in DCIS (Ductal carcinoma in situ) is extremely important to predict the local recurrence whereas LA and TN types have comparatively low risk of recurrence[6].

## Materials and Methods

The primary aim of the presence study is to found the corelation between HER2Neu marker with other variables in invasive ductal carcinoma (IDC) of the breast ) the secondary outcome results are corelation with other variables. The consecutive 57 samples over a period of 18 months have been included for the study. Written informed consent from the patients or their legal guardians has been taken. Patients who are attending Tertiary Medical College and have been diagnosed with breast carcinoma clinically have submitted either mastectomy /lumpectomy specimen to the department included in the study and after screening confirmed Intraductal carcinoma cases are being selected for analysis. The cases with presence of fixation artefact, other types of benign / malignant breast tumor besides invasive duct carcinoma, patients who have received any kind of chemotherapy, radiotherapy, homeopathic or ayurvedic treatment and the cases where the written informed consent couldn't be obtained were excluded from the study.

Table 1 — *Molecular Subtypes of Breast Carcinoma*

| Molecular subtypes | Description |
|---|---|
| Luminal A | ER/PR positive, HER2 negative, low Ki67, low grade, slow growing seen in older patients and carries best prognosis. |
| Luminal B | ER/PR positive, either HER2 positive or negative with high Ki67, grow slightly faster has higher expression of gene proliferation carries worse prognosis than Luminal A subtype. |
| Triple Negative (TN) /basal like | ER/PR negative, HER 2 negative and more common in BRCA1 mutation. |
| HER2-enriched | ER/PR negative, HER 2 positive, grow faster, associated with nodal metastasis and have worse prognosis. |
| Basal like | Similar to Luminal A disease ER/PR positive, HER2 negative, low Ki67, seen in younger patients with visceral organ metastasis and has slightly poor prognosis than Luminal A disease. |

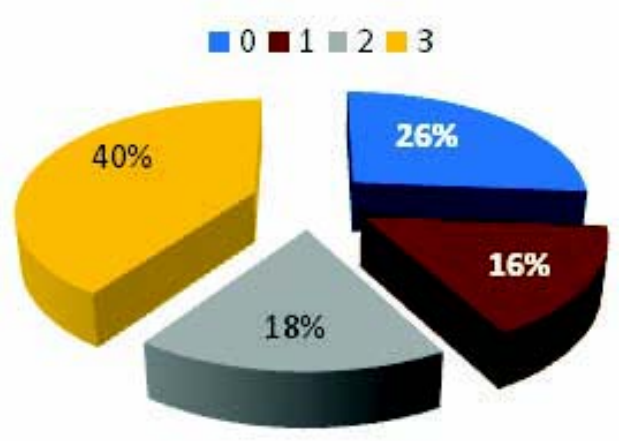


Fig 1

The correlation between Her2Neu scoring (0 to 3) and Her2Neu status (positive, negative and equivocal) with molecular classification, cyclin D1 status and Ki67are assessed. For statistical analysis data were entered and analysed by SPSS (Statistical Package for the Social Sciences) software (version 25.0; SPSS Inc, Chicago, IL, USA)[7].

## Results

In our study, 15(26.3%) patients had 0 Her2-Neu scoring, 9(15.8%) patients had 1 Her2-Neu scoring, 10(17.5%) patients had 2 Her2-Neu scoring and 23(40.4%) patient had 3 Her2-Neu scoring. In HER2 Group, 1(9.1%) patients had 2 Her2-Neu scoring and 10 (90.9%) patients had 3 Her2-Neu scoring. In LA Group, 6(54.5%) patients had 0 Her2-Neu scoring and 5(45.5%) patients had 1 Her2-Neu scoring. In LB Group, 3(12.0%) patients had 1 Her2-Neu scoring, 9(36.0%) patients had 2 Her2-Neu scoring and 13(52.0%) patients had 3 Her2-Neu scoring. In TN Group, 9 (90.0%) patients had 0 Her2-Neu scoring and 1(10.0%) patient had 1 Her2-Neu scoring. The association of Her2-Neu scoring vs group was statistically significant ($p<0.0001$) (Table 2, Fig 2).

In HER2 Enriched Group, 10(90.9%) patients had 1 Her2 Status and 1(9.1%) patients had 3 Her2 Status. In LA Group, 11(100.0%) patients had 2 Her2 Status. In LB Group, 13(52.0%) patients had 1 Her2 Status, 5 (20.0%) patients had 2 Her2 Status and 7 (28.0%) patients had 3 Her2 Status. In TN Group, 10 (100.0%) patients had Her2 Status. The association of Her2 Status vs group was statistically significant ($p<0.0001$) (Table 3, Fig 3). In Grade-1 Group, 2(33.3%) patients had 0 Her2-Neu scoring, 1(16.7%) patient had 2 Her2-

Table 2 — *Association between HER2-Neu scoring: Molecular Classification (Fig 5.20)*

| Molecular Classification | | | | | |
|---|---|---|---|---|---|
| **Her2-Neu scoring** | **HER2 n(%)** | **LA n(%)** | **LB n(%)** | **TN n(%)** | **TOTAL n(%)** |
| 0 | 0(0.0%) | 6(54.5%) | 0(0.0%) | 9(90.0%) | 15(26.3%) |
| 1 | 0(0.0%) | 5(45.5%) | 3(12.0%) | 1(10.0%) | 9(15.8%) |
| 2 | 1(9.1%) | 0(0.0%) | 9(36.0%) | 0(0.0%) | 10(17.5%) |
| 3 | 10(90.9%) | 0(0.0%) | 13(52.0%) | 0(0.0%) | 23(40.4%) |
| Total | 11(100.0%) | 11(100.0%) | 25(100.0%) | 10(100.0%) | 57(100.0%) |

$\chi^2$ = 61.7925; p-value:<0.0001

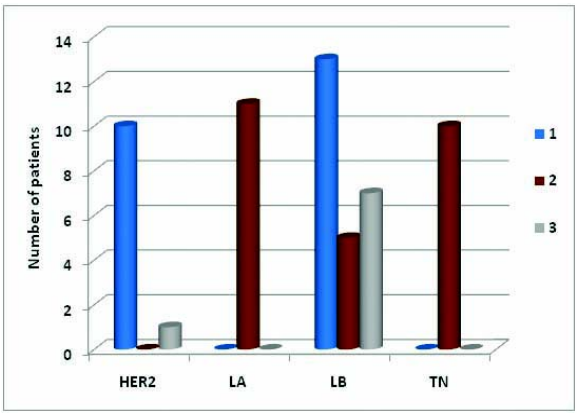


Fig 3

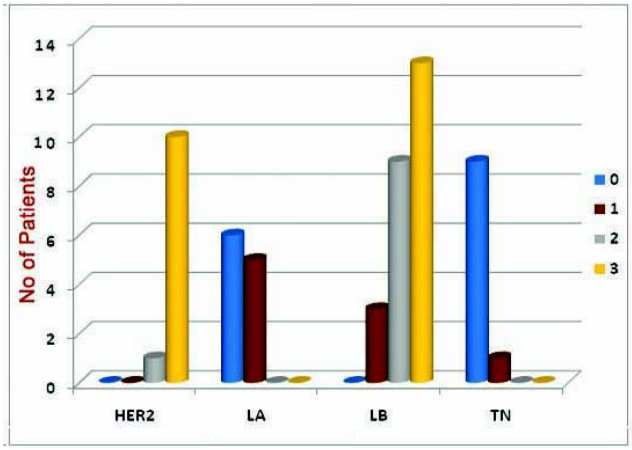


Fig 2

Table 3 — *Association between Her2Neu Status : Molecular Classification (Fig 3)*

| Molecular Classification | | Fig 3 | | | |
|---|---|---|---|---|---|
| **Her2 Status** | **HER2 Enriched** | **LA** | **LB** | **TN** | **TOTAL** |
| Positive : | | | | | |
| n (%) | 10(90.9%) | 0(0.0%) | 13(52.0%) | 0(0.0%) | 23(40.4%) |
| Negative : | | | | | |
| Row Col (%) | 0(0.0%) | 11(100.0%) | 5(20.0%) | 10(100.0%) | 26(45.6%) |
| Equivocal : | | | | | |
| Row Col (%) | 1(9.1%) | 0(0.0%) | 7(28.0%) | 0(0.0%) | 8(14.0%) |
| Total : | | | | | |
| Row Col (%) | 11(100.0%) | 11(100.0%) | 25(100.0%) | 10(100.0%) | 57(100.0%) |

$\chi^2$ = 45.1262; p-value:<0.0001

Neu scoring and 3(50.0%) patients had 3Her2-Neu scoring. In Grade-2 Group, 6(42.9%) patients had 0 Her2-Neu scoring, 3(21.4%) patients had 1 Her2-Neu scoring, 3(21.4%) patients had 2 Her2-Neu scoring and 2(14.3%) patients had 3Her2-Neu scoring. In Grade-3 Group, 1(25.0%) patient had 0 Her2-Neu scoring, 2(50.0%) patients had 2 Her2-Neu scoring and 1(25.0%) patient had 3 Her2-Neu scoring. In Grade-4 Group, 4(16.0%) patients had 0 Her2-Neu scoring, 2 (8.0%) patients had 1 Her2-Neu scoring, 3(12.0%) patients had 2 Her2-Neu scoring and 16 (64.0%) patients had 3Her2-Neu scoring. In Grade-5 Group, 2(25.0%) patients had 0 Her2-Neu scoring, 4(50.0%) patients had 1 Her2-Neu scoring, 1(12.5%) patient had 2 Her2-Neu scoring and 1(12.5%) patient had 3Her2-Neu scoring. The association of Her2-Neu scoring vs group was statistically significant (p=0.0359). (Table 4, Fig 4) In Grade-1 Group, 3(50.0%) patients had 1 Her2 Status, 2 (33.3%) patients had 2 Her2 Status and 1(16.7%) patient had 3 Her2 Status. In Grade-2 Group, 2(14.3%) patients had 1 Her2 Status, 9(64.3%) patients had 2 Her2 Status and 3(21.4%) patients had 3 Her2 Status. In Grade-3 Group, 1(25.0%) patient had 1 Her2 Status, 2 (50.0%) patients had 2 Her2 Status and 1(25.0%) patient had 3 Her2 Status. In Grade-4 Group, 15(60.0%) patients had 1 Her2 Status, 8(32.0%) patients had 2 Her2 Status and 2(8.0%) patients had 3 Her2 Status. In Grade-5 Group, 2 (25.0%) patients had 1 Her2 Status, 5(62.5%) patients had 2 Her2 Status and 1(12.5%) patient had 3 Her2 Status. The association of Her2 Status with cyclin D1 status has not been found to be statistically significant (p=0.2672). (Table 5, Fig 5). In the 3 x 2 contingency table, Ki67 expression (high and low) with Her2Neus status (positive, negative and equivocal) status have been checked. The chi square test has been done (Table 6), It has been found that the chi-square statistic is 11.0083 and the p-value is 0.004 (Table 6).

## Discussion

Breast carcinoma has different clinical presentation, different behaviours and progression and existing histological classifications fall way short to correlate

Table 4 — *Association between Her2-Neu scoring : Cyclin D1 Status (Fig 4)*

| Cyclin D1 Status | | | | | | |
|---|---|---|---|---|---|---|
| **Her2-Neu scoring** | **Grade-1 n(%)** | **Grade-2 n(%)** | **Grade-3 n(%)** | **Grade-4 n(%)** | **Grade-5 n(%)** | **Total n(%)** |
| 0 | 2(33.3%) | 6(42.9%) | 1(25.0%) | 4(16.0%) | 2(25.0%) | 15(26.3%) |
| 1 | 0(0.0%) | 3(21.4%) | 0(0.0%) | 2(8.0%) | 4(50.0%) | 9(15.8%) |
| 2 | 1(16.7%) | 3(21.4%) | 2(50.0%) | 3(12.0%) | 1(12.5%) | 10(17.5%) |
| 3 | 3(50.0%) | 2(14.3%) | 1(25.0%) | 16(64.0%) | 1(12.5%) | 23(40.4%) |
| Total | 6(100.0%) | 14(100.0%) | 4(100.0%) | 25(100.0%) | 8(100.0%) | 57(100.0%) |

$\chi^2$ = 22.1492; p-value:0.0359

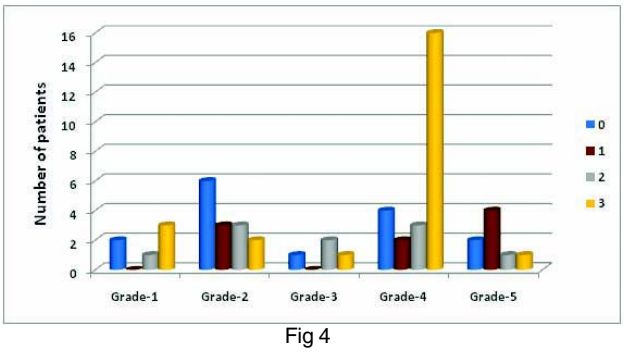


Fig 4

Table 5 — *Association between Her2 Status : Cyclin D1 Status (Fig 5)*

| Cyclin D1 Status | | | | | | |
|---|---|---|---|---|---|---|
| **Her2-Neu scoring** | **Grade-1 n(%)** | **Grade-2 n(%)** | **Grade-3 n(%)** | **Grade-4 n(%)** | **Grade-5 n(%)** | **Total n(%)** |
| 1 | 3(50.0%) | 2(14.3%) | 1(25.0%) | 15(60.0%) | 2(25.0%) | 23(40.4%) |
| 2 | 2(33.3%) | 9(64.3%) | 2(50.0%) | 8(32.0%) | 5(62.5%) | 26(45.6%) |
| 3 | 1(16.7%) | 3(21.4%) | 1(25.0%) | 2(8.0%) | 1(12.5%) | 8(14.0%) |
| Total | 6(100.0%) | 14(100.0%) | 4(100.0%) | 25(100.0%) | 8(100.0%) | 57(100.0%) |

$\chi^2$ = 9.9694; p-value:0.2672

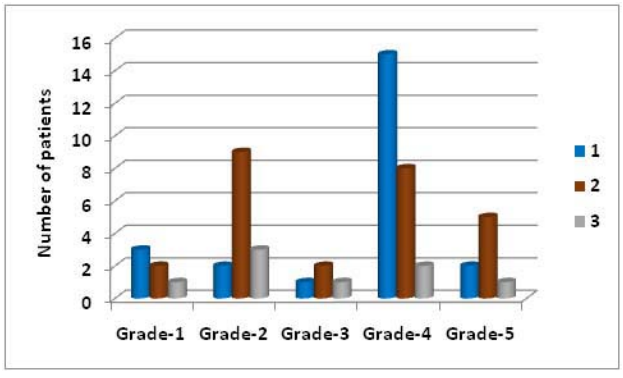


Fig 5

the trajectory of progression and making clinical decision.The molecular classification of breast cancer cell lines used in scientific studies are done by surrogate panel of IHC markers.

LA subtype has very good prognosis and shows less lymph node spread. It evolves slowly and with positive ER status shows favourable response to endocrine therapy[8,9]. LA type B has intermediate prognosis with potential for local regional recurrence (LRR)[10,11].

They are often being treated with breast conservative surgery and radiotherapy and the risk of LRR is around 5%. The endocrine therapy reduces LRR and mortality rate by more than 50%. Her2 overexpression carries higher risk of LRR (4-15%). Breast carcinoma cells are more vulnerable to injury when the Her2 pathway is activated[12].

Her2 over expression is found in nearly all cases of comedo type DCIS in 20-30% of IDC and in smaller percentage of invasive lobular carcinoma. Her2 gene amplification in breast cancer is associated with tumour invasiveness, progressive regional and distant metastasis and adverse prognosis[13].

The critical assessment with quantitative analysis has shown corelation between cyclin D1 and HER2 (Pearson's corelation = 0.90) and kappa corelation = 0.62). Cyclin D1 biomarker has also found to be inversely corelated to ER, PR HER2neu negative tumours. With growing public awareness, diagnostic facilities and availability biomarkers will guide the future management Her2 positive cancers arise from the pathway which shows amplification of HER2 in which is located in chromosome 17q[14].

In this study it has been found that both Her2Neu status and Her2Neu scoring are statistically significant ($p<0.001$) with molecular classification of IDC (Intraductal carcinoma of the breast) (Tables 2&3). The association of Her2-Neu scoring and cyclin D1 status (Table 4) has been found to be statistically significant ($p=0.0359$) however the same with Her2Neu status and cyclin D1 status (Table 5) are not found to be clinically

Table 6 — *Association between Her2Neu status and Ki67 expression*

| **Variable** | **Ki67 high** | **Ki67 low** | **Row Total** |
|---|---|---|---|
| Her2Neu Pos | 22 | 1 | 23 |
| Her2Neu Neg | 14 | 12 | 26 |
| Her2Neu Equiv | 6 | 2 | 8 |
| Column Total | 42 | 15 | 57 (Grand Total) |

$\chi^2$ = 11,0083; p-value:0.004

significant (p=0.2672). The association between Her2Neu status and Ki67 expression (Table 6) has emerged up as statistically significant (p=0.00407).

## Conclusion :

Immunohistochemistry may be used to subclassify the different breast tumours. The commonest type is Luminal A and least common type is Her2positive type. The Luminal A type of tumours are commonly associated with Lobular carcinoma. On the other hand Her2positive and TN (triple negative) groups show higher tumour grade and larger size when initially diagnosed usually in middle age group. The present study has some limitations like other varieties of invasive breast carcinoma like lobular carcinoma, metaplastic, medullary carcinoma have not been studied. A follow up longitudinal study with higher sample size and more biomarkers will be helpful to extrapolate the findings of present study. Despite these limitations, the present study is generates significant outcome which will be helpful for future research and will add scientific values with current knowledge.

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**Conflicts of interest :** None.

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