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Original Article

HER2/neu Expression Patterns in Breast Cancer - Epidemiological Trends from South-Eastern part of Rajasthan

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ABSTRACT

Background: Breast carcinoma is the most common malignancy among females and a leading cause of cancer-related mortality worldwide. The human epidermal growth factor receptor 2 (HER2/neu) is a key biomarker associated with tumor aggressiveness, therapeutic response, and prognosis.
Objective: To evaluate the correlation between HER2/neu expression and histopathological grade along with Ki-67 proliferation index in cases of invasive breast carcinoma.
Methods: A cross-sectional study was conducted on 191 cases diagnosed with breast carcinoma at tertiary care centre, during 2024-2025.
Results: Among the 191 cases, 17 (8.9%) were grade I, 144 (75.4%) grade II, and 30 (15.7%) grade III tumors, A statistically significant association was found between HER2 positivity (3+) and tumor grade (p = 0.0013), and significant association was also observed between HER2/neu status and Ki-67 proliferation index (p=0.0001), with HER2/neu-positive tumors demonstrating higher Ki-67 expression. Larger tumor size (>5 cm) was similarly associated with HER2/neu overexpression.
Conclusion: HER2/neu positivity (Score 3+) showed a significant association with higher tumor grade (p=0.0013) and Ki-67 index (p=0.0001).HER2/neu overexpression correlates with tumor aggressiveness and proliferation.

Keywords: *Breast carcinoma, HER2/neu, Ki67, histological grade.*

INTRODUCTION

Breast cancer is the most common malignancy among women worldwide and remains a leading cause of cancer-related mortality. According to global cancer statistics, approximately 2.3 million new cases were reported in 2020, accounting for a substantial proportion of the global cancer burden.[(1)] Recent updates from the Global Cancer Observatory indicate a continued rise in incidence, particularly in developing countries, including India.[(2,3)] In India, breast cancer has now surpassed all other malignancies in incidence among females, representing a major public health concern.[(4)] The Human Epidermal Growth Factor Receptor 2 (HER2/neu), encoded by the ERBB2 gene, is a transmembrane tyrosine kinase receptor that plays a critical role in cell proliferation, differentiation, and survival.[(5)] Overexpression or amplification of HER2/neu leads to aggressive tumor behavior, increased metastatic potential, and poor clinical outcomes.[(6-8)] HER2-positive breast cancers constitute approximately 10–20% of all cases and are associated with higher recurrence rates and reduced survival if untreated.[(9)]

Histopathological grading remains a cornerstone in assessing tumor aggressiveness and prognosis. The Nottingham modification of the Bloom–Richardson grading system is widely used to evaluate tumor differentiation based on tubule formation, nuclear pleomorphism, and mitotic activity. [(10,11)] Several studies have demonstrated a significant correlation

between HER2/neu expression and higher histological grades, suggesting its role in tumor progression.[12,13] Ki-67 is a well-established proliferation marker that reflects cellular growth activity and has been increasingly used in conjunction with HER2/neu to assess tumor aggressiveness.[14] Studies have shown that HER2-positive tumors tend to exhibit higher Ki-67 indices, indicating enhanced proliferative potential[15,16] Recent advances in molecular oncology emphasize the integration of histopathological parameters with molecular biomarkers for improved risk stratification and personalized treatment planning.[17] However, regional data from South-Eastern Rajasthan remain scarce. Therefore, the present study was undertaken to evaluate the association between HER2/neu expression, histopathological grading, and Ki-67 proliferation index in breast carcinoma patients from this region.[18]

MATERIALS AND METHODS

Study Design and Setting: This was a hospital-based cross-sectional observational study conducted in the Department of Pathology at Government Medical College and associated group of hospitals, Kota, Rajasthan, India. The study was carried out over a period of two years, from January 2024 to December 2025.

Study Population: A total of 191 consecutive cases of histopathologically confirmed breast carcinoma were included in the study. All patients underwent surgical resection (lumpectomy or mastectomy), and resected specimens were submitted for histopathological evaluation.

Selection Criteria: All cases diagnosed as invasive breast carcinoma on histopathological examination were included. Cases with inadequate tissue, poorly preserved samples, or incomplete clinicopathological data were excluded from the study.

Data Collection: Clinical and pathological data were retrieved from medical records and pathology reports. The variables collected included patient age, gender, tumor size, histological subtype, tumor grade, lymph node status, and biomarker status (HER2/neu and Ki-67).

Histopathological Examination: Tissue specimens were fixed in 10% neutral buffered formalin, processed routinely, and embedded in paraffin. Sections of 3–5 µm thickness were cut and stained with hematoxylin and eosin (H&E). Histological typing was performed according to the WHO Classification of Breast Tumors (2020). Tumor grading was carried out using the Nottingham modification of the Bloom–Richardson grading system, based on tubule formation, nuclear pleomorphism, and mitotic count. Tumors were categorized into Grade I (well differentiated), Grade II (moderately differentiated), and Grade III (poorly differentiated).

Immunohistochemistry (IHC): Immunohistochemical analysis for HER2/neu and Ki-67 was performed on representative tumor sections using standard protocols. Antigen retrieval and staining procedures were carried out as per manufacturer instructions.

HER2/neu Evaluation: HER2/neu expression was assessed based on membranous staining intensity and completeness in tumor cells, following the American Society of Clinical Oncology/College of American Pathologists (ASCO/CAP) 2018 guidelines. Scoring was performed as follows:

- **0 and 1+**: Negative
- **2+**: Equivocal (recommended for confirmation by in situ hybridization)
- **3+**: Positive

Only complete circumferential membranous staining was considered positive. Cytoplasmic staining was regarded as nonspecific and excluded from interpretation.

For statistical analysis, HER2/neu expression was dichotomized into:

- Negative (0 and 1+)
- Equivocal (**2+)** (recommended for confirmation by in situ hybridization)
- Positive (3+) (Equivocal cases were interpreted cautiously as per guideline recommendations.)

Ki-67 Proliferation Index: Ki-67 labeling index was determined by calculating the percentage of positively stained tumor nuclei. Based on expression levels, tumors were categorized as:

- Low (<5%)
- Intermediate (5–30%)
- High (>30%)

Statistical Analysis: Data were entered into Microsoft Excel and analyzed using appropriate statistical software. Categorical variables were expressed as frequencies and percentages. Associations between HER2/neu expression and clinicopathological parameters were analyzed using the Chi-square test. A p-value of <0.05 was considered statistically significant.

Ethical Considerations: The study was conducted in accordance with institutional ethical standards. As this was a retrospective observational study using anonymized patient data, informed consent was waived.

RESULTS

Table 1 - HER2/neu with Age Range (n=191)

HER2/neu	20-40	40-60	60-90
0	8	21	8
1+	5	8	6
2+	6	24	11
3+	12	59	23

Table 2 - HER2/neu Score with Age and Gender (n=191)

HER2/neu Score	Average Age	Gender Distribution
0	51.05	(F: 37)
1+	52.05	(F:19)
2+	53.1	(F: 40, M :1)
3+	52.81	(F:92, M:2)

Table 3 - Frequency Table: Tumor Size vs HER2/neu (n=191)

Tumor Size	0	1+	2+	3+
<2cm	12	4	8	16
>2cm and <5cm	18	9	18	41
>5cm	5	3	14	31

Table 1 shows the distribution of HER2/neu scores across different age ranges. The majority of patients with a score of 3+ fall in the 40-60 age range (59 patients), followed by the 60-90 age range (23 patients). As per Table 2, the average age of patients with HER2/neu scores ranges from 51.05 (score 0) to 53.1 (score 2+). The gender distribution shows a higher prevalence of females across all scores, with a total of 37 females in score 0, 19 in score 1+, 40 in score 2+, and 92 in score 3+. Table 3 illustrates the relationship between tumor size and HER2/neu scores. For tumors <2cm, the majority have a score of 3+ (16 patients), while for tumors >2cm and <5cm, the majority have a score of 3+ (41 patients). For tumors >5cm, the majority also have a score of 3+ (31 patients).

Histological Classification Counts (n=191)

Invasive/ Infiltrative ductal carcinoma and NST: 169
Invasive lobular carcinoma: 7
Medullary carcinoma: 4
Mucinous carcinoma: 3
Papillary carcinoma: 4
Invasive carcinoma with mixed ductal and lobular features: 4
Invasive carcinoma breast ST: 1

Table 4 - Frequency Table: HER2neu Score vs BR Grade (n=191)

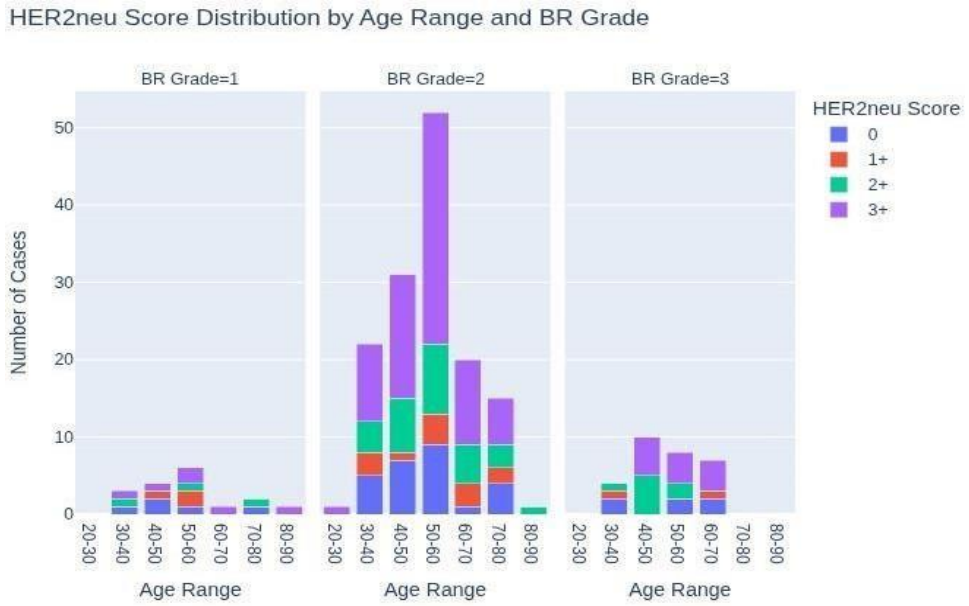
BR Grade	0	1+	2+	3+
I	5	3	3	6
II	26	14	30	74
III	6	2	8	14

Table 5 - Ki67 vs HER2/neu Status (n=191)

Ki67	HER2/neu Negative	HER2/neu Positive
Low (<5%)	28	34
Intermediate 5-30 %	4	17
High >30%	12	35

As shown in Table 4, the majority of patients with a HER2/neu score of 3+ have a BR Grade of II (74 patients), followed by Grade III (14 patients). For patients with a score of 0, the majority have a BR Grade of II (26 patients). Table 5 shows the relationship between Ki67 levels and HER2/neu status. For patients with low Ki67 (<5%), 28 are HER2/neu negative

and 34 are positive. For high Ki67 (>30%), 12 are HER2/neu negative and 35 are positive, indicating a possible correlation between high Ki67 and HER2/neu positivity.



Graph 1 - Showing HER2/neu Distribution by Age Range and BR Grade.

This graph is a stacked bar chart showing the distribution of HER2/neu scores (0, 1+, 2+, 3+) across three BR Grades (1, 2, 3) and various age ranges. BR Grade=2 has the highest number of cases, especially in the 40-50 and 50-60 age ranges, with the majority of cases having a HER2/neu score of 3+ (purple bars). BR Grade=1 and BR Grade=3 have fewer cases compared to Grade 2, with cases spread across age ranges 20-90, showing mixed HER2/neu scores.The age range 40-60 appears to have the most cases with high HER2/neu scores (3+), particularly in BR Grade 2. The graph suggests a correlation between BR Grade 2, higher HER2/neu scores (3+), and age ranges 40-60.

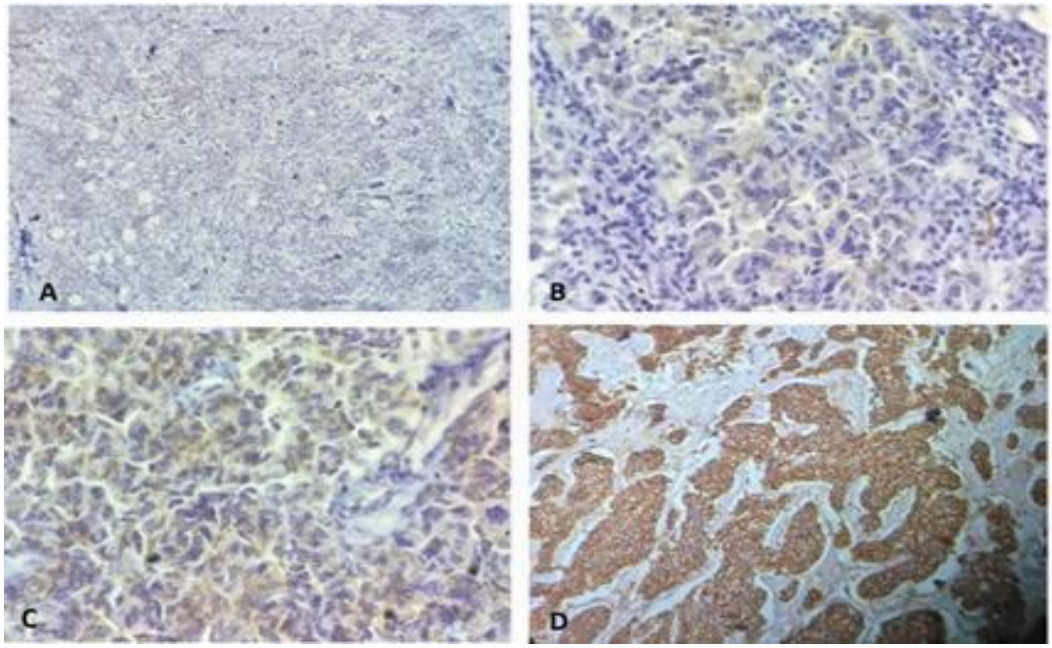


Fig:1 Representative examples of Human Epidermal Grouth Reseptor 2 (HER2), Immunohistochemistry (IHC) in breast cancer. A.HER2neu IHC Negative(0); B.HER2neu IHC Negative (1+); C.HER2neu IHC equivocal (2+); D.HER2neu IHC Positive (3+)

DISCUSSION

Breast carcinoma continues to be the most common malignancy among women globally and represents a major public health challenge, particularly in low- and middle-income countries such as India.[12,13,18] The present study evaluated the relationship between HER2/neu expression and key clinicopathological parameters, with special emphasis on histological grade and proliferative activity.

In this study, the majority of tumors were classified as Grade II (75.4%), followed by Grade III (15.7%) and Grade I (8.9%). This distribution is consistent with prior studies, which have reported a predominance of moderately differentiated tumors in breast carcinoma cohorts.[5,6,19] The high proportion of Grade II tumors may reflect intermediate tumor biology as well as delayed clinical presentation in resource-limited settings.

A statistically significant association was observed between HER2/neu expression and histological grade, with HER2 positivity increasing with higher tumor grade. This finding is in agreement with multiple studies demonstrating that HER2 overexpression is more frequently seen in poorly differentiated tumors and is associated with aggressive tumor behavior and adverse prognosis.[14,15] The biological basis for this correlation lies in the role of HER2 as a transmembrane tyrosine kinase receptor that activates downstream signaling pathways such as PI3K/AKT and MAPK, leading to enhanced cellular proliferation, survival, and resistance to apoptosis.[11,12,20]

An important observation in the present study was the strong association between HER2/neu expression and Ki-67 proliferation index. HER2-positive tumors demonstrated significantly higher Ki-67 expression compared to HER2-negative tumors, indicating increased proliferative activity. This finding is consistent with previous reports that have shown a positive correlation between HER2 overexpression and high Ki-67 index, both of which are markers of aggressive tumor biology.[13,16,17] The combined assessment of HER2 and Ki-67 may therefore provide improved prognostic stratification and assist in therapeutic decision-making.

Tumor size was also found to correlate positively with HER2 expression, with larger tumors (>5 cm) demonstrating higher rates of HER2 positivity. This observation aligns with earlier studies suggesting that HER2 overexpression is associated with increased tumor burden and more aggressive disease.[6,15] Larger tumor size at presentation may reflect delayed diagnosis, which remains a significant challenge in developing regions due to limited awareness and access to healthcare services.

With respect to histological subtype, invasive ductal carcinoma (no special type) was the most common variant in the present study, accounting for the majority of cases. This finding is consistent with global data and the WHO classification of breast tumors, which identifies invasive ductal carcinoma as the predominant subtype.[5] Other subtypes, including invasive lobular, mucinous, and medullary carcinoma, were relatively less frequent.

The age distribution in this study showed that HER2 positivity was most commonly observed in the 40–60-year age group, which corresponds to the peak incidence of breast cancer in the Indian population.[20,21] However, no statistically significant association between age and HER2 expression was identified, consistent with previous studies indicating that HER2 status is largely independent of patient age.[6,22] Female patients constituted the overwhelming majority of cases, reflecting the well-established epidemiology of breast carcinoma, with male breast cancer accounting for less than 1% of cases.[3,18]

From a clinical standpoint, accurate assessment of HER2/neu status is essential, as it serves both prognostic and predictive roles. HER2-positive tumors are associated with poor prognosis but demonstrate significant response to targeted therapies such as trastuzumab, which has markedly improved survival outcomes in this subgroup.[12] In addition, the strong association between HER2 expression and Ki-67 underscores the importance of combined biomarker evaluation in identifying high-risk patients and tailoring individualized treatment strategies.

Strengths and Limitations

The strengths of the present study include a relatively large sample size and a comprehensive evaluation of clinicopathological parameters in a regional population. However, certain limitations should be acknowledged. This was a single-center study, which may limit the generalizability of the findings. Furthermore, confirmatory testing using in situ hybridization (ISH) for equivocal (2+) HER2 cases was not performed in all instances, which may have affected the accuracy of HER2 classification.[16,17] Future multicentric studies with molecular confirmation and long-term follow-up are recommended.

Future Implications

Given the rising incidence of breast cancer in India, there is an urgent need to strengthen early detection programs and ensure standardized biomarker assessment. Integration of HER2/neu and Ki-67 evaluation into routine diagnostic protocols can improve prognostic accuracy and guide targeted therapy. Further research focusing on molecular subtyping and survival outcomes in the Indian population is warranted to optimize patient management strategies.

CONCLUSION

A total of 191 cases of invasive breast carcinoma were included in the present study. The majority of cases belonged to the age group of 41–60 years. Invasive ductal carcinoma (NST) was the most common histological subtype observed. HER2/neu expression was evaluated using immunohistochemistry (IHC) and scored according to ASCO/CAP guidelines, Score 0 and 1+ were considered negative, Score 2+ was considered equivocal, Score 3+ was considered positive.

A statistically significant increase in HER2/neu positivity (3+) was observed with increasing tumor grade. Grade I → Lower HER2 positivity, Grade II → Moderate increase, Grade III → Highest HER2 positivity. Equivocal (2+) cases were distributed across all grades but did not show a consistent trend. HER2-positive (3+) tumors showed a significantly higher Ki-67 proliferation index compared to negative and equivocal groups, indicating increased tumor aggressiveness. A statistically significant association was found between HER2 positivity (3+) and tumor grade ($p = 0.0013$). A highly significant correlation was observed between HER2 positivity and Ki-67 index ($p = 0.0001$). Equivocal (2+) cases were excluded from definitive positive correlation analysis where appropriate.

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