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HISTOPATHOLOGICAL AND MOLECULAR SPECTRUM OF BREAST CARCINOMA WITH SPECIAL REFERENCE TO KI-67

Pathology

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ABSTRACT

Background: Breast carcinoma is the most common cancer in Indian women. Biomarkers like Ki67 aid in molecular subtyping and prognosis. **Objective:** To evaluate the histopathological and molecular characteristics of breast carcinoma and correlate Ki67 index with tumor grade and molecular subtypes. **Methods:** This cross-sectional study included 150 histopathologically confirmed cases of invasive breast carcinoma. IHC was performed for ER, PR, HER2, and Ki67. Tumors were graded and classified into molecular subtypes. Statistical analysis was done using SPSS v15.0, with ANOVA for correlations. **Results**: Mean age was 52.86 ± 12.17 years. IDC was the most common subtype (83.3%). Most tumors were Grade II (69%). ER, PR, and HER2 positivity were seen in 54%, 36%, and 47.3% respectively. Luminal B (HER2+) was the most common molecular type (24.7%), followed by Triple Negative (24%). Mean Ki67 increased significantly with grade (Grade I: 25%, Grade II: 37.71%, Grade III: 50.45%, p=0.020). ER- and PR-negative tumors had significantly higher Ki67 indices (p<0.001 and p=0.001 respectively). **Conclusion:** Ki67 index significantly correlates with tumor grade and hormone receptor status, supporting its role as a prognostic marker.

KEYWORDS

Breast carcinoma, Ki67, IHC, Molecular subtypes, Tumor grade

INTRODUCTION

Breast cancer is the leading cause of cancer in women around the world, accounting for one fourth of all female cancers. Breast cancer deaths in the South-East Asia region are expected to rise to 61.7% by 2040.[1] In 2024, invasive breast cancer cases in India are estimated at 310,720, with 16% occurring in women under 50.[2]

A panel of immunohistochemistry (IHC) biomarkers can classify breast cancer into four surrogate subtypes: hormone receptors (HR) (estrogen (ER) and progesterone (PR)), HER2 and Ki67. These classify luminal A-like (ER/PR positive, HER2 negative, low Ki67), luminal B-like (ER positive, PR low positive, high Ki67, HER2+ or HER2−), HER2 enriched (ER/PR negative, HER2+) and triple negative (ER/PR−, HER2−) tumors.[3,4]

Ki-67 is a nuclear protein that is present during the late G1, S, G2 and M phases of the cell cycle, reflecting the proportion of cell proliferation[5]. Ki-67 expression is also used for subdividing luminal-like breast cancers into luminal A and luminal B groups.[6]

MATERIALS AND METHODS

Design And Setting:

Cross-sectional study conducted at the Department of Pathology, GMC Kota, between Oct 2023 – Dec 2024.

Sample Size:

150 patients with histopathologically proven invasive breast carcinoma.

Inclusion Criteria:

- Female patients with untreated invasive breast cancer
- Adequate tissue sample

Exclusion Criteria:

- Prior chemotherapy or hormonal therapy
- Recurrent tumors

IHC Panel Used:

- ER: Rabbit monoclonal SP1
- PR: Rabbit monoclonal SP2
- HER2: Rabbit monoclonal SP3
- Ki67: Rabbit monoclonal SP6(All from Diagnostic Biosystems)

Ki67 Evaluation:

At least 1000 tumor cells counted; % nuclear positivity calculated.

Statistical Analysis:

SPSS v15.0; ANOVA and post hoc tests for group comparisons; p<0.05 as significant.

RESULTS

Table 1: Distribution Of Cases According To HPE Diagnosis

HPE Diagnosis	No.	%
IDC	125	83.3%
Invasive lobular carcinoma	5	3.3%
Mixed Ductal and Lobular Carcinoma	5	3.3%
Mucinous Carcinoma	6	4.0%
Papillary Carcinoma	4	2.7%
Metaplastic Carcinoma	3	2.0%
Other	2	1.3%

Histopathological examination (HPE) revealed that the most common diagnosis among breast cancer cases was Invasive Ductal Carcinoma (IDC), observed in 125 cases (83.3%). Invasive lobular carcinoma and Mixed Ductal and Lobular Carcinoma were each identified in 5 cases (3.3%). Mucinous carcinoma was found in 6 cases (4.0%), while Papillary carcinoma accounted for 4 cases (2.7%). Metaplastic carcinoma was present in 3 cases (2.0%), and other rare types (Medullary carcinoma, squamous cell carcinoma)were seen in 2 cases (1.3%). This distribution indicates a predominance of IDC among the breast cancer histological subtypes.

Table 2: Distribution Of Cases According To Grade

Grade	No.	%
Grade I	10	7%
Grade II	96	69%
Grade III	34	24%

The majority of breast cancer cases were classified as Grade II, accounting for 69% of the total cases. Grade III tumors were observed in 24% of patients, while Grade I tumors were the least common, comprising only 7% of cases.

Table 3 : Correlation Of ER With Ki67 Index

ER	Ki67 (%)		ANOVA	
	Mean	SD	F-value	p-value
Negative	49.43	23.35	4.71	<0.001
Positive	30.73	24.96		

The analysis of Ki67 proliferation index based on estrogen receptor (ER) status showed a significant difference between ER-negative and ER-positive breast cancer cases. The mean Ki67 expression was higher in ER-negative tumors (49.43 ± 23.35) compared to ER-positive tumors (30.73 ± 24.96), with an ANOVA F-value of 4.71 and a p-value of <0.001, indicating a statistically significant association between higher Ki67 levels and ER negativity.

Table 4: Correlation Of PR With Ki67 Index

PR	Ki67 (%)		ANOVA	
	Mean	SD	F-value	p-value
Negative	44.70	25.75	3.51	0.001
Positive	29.80	23.50		

The analysis of Ki67 proliferation index based on progesterone receptor (PR) status revealed a significant difference between PR-negative and PR-positive breast cancer cases. The mean Ki67 expression was higher in PR-negative tumors (44.70 ± 25.75) compared to PR-positive tumors (29.80 ± 23.50). The ANOVA analysis yielded an F-value of 3.51 and a p-value of 0.001, indicating a statistically significant association between higher Ki67 levels and PR negativity.

Table 5 : Correlation of HER2 With Ki67 Index

HER2	Ki67 (%)		ANOVA	
	Mean	SD	F-value	p-value
Negative	37.89	26.66	0.72	0.472
Positive	40.94	25.12		

The analysis of Ki67 proliferation index based on HER2 status showed no significant difference between HER2-negative and HER2-positive breast cancer cases. The mean Ki67 expression was 37.89 ± 26.66 in HER2-negative tumors and 40.94 ± 25.12 in HER2-positive tumors. ANOVA analysis yielded an F-value of 0.72 and a p-value of 0.472, indicating that the difference in Ki67 levels between the two groups was not statistically significant.

Micro-Photographs

Figure 1: Infiltrating Lobular Carcinoma of Breast showing malignant cells arranged in Indian File Pattern. (40X)

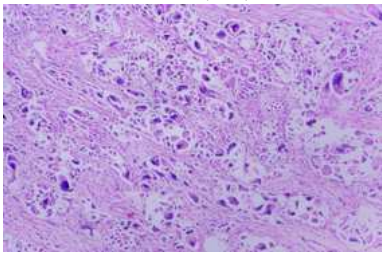

Figure 2 : Infiltrating Ductal Carcinoma (NOS) H & E (BR Grade III)

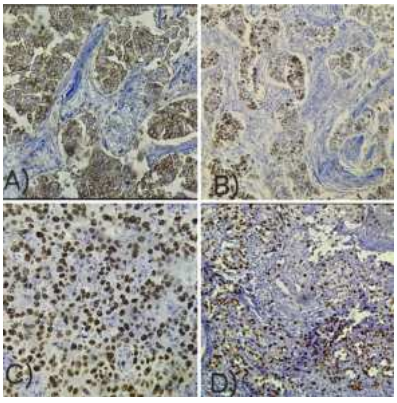

Figure 3: A)Breast carcinoma showing Her2 membrane positivity(3+) 200x, B)ER nuclear positivity, Alred score 7/8 (60%) 200x, C)Ki67 nuclear positivity (90%) 400x, D) Ki67 nuclear positivity (60%) 200x

DISCUSSION:

In our study, the mean Ki67 index was 25.00% in Grade I tumors, 37.71% in Grade II, and 50.45% in Grade III, with a significant p-value of 0.020. This increasing trend was also observed by Yerushalmi et al., who reported higher Ki67 expression in poorly differentiated tumors.[7] Urruticoechea et al. [2] also concluded that Ki67 correlates positively with tumor grade and is a strong indicator of proliferative activity.[8]

In terms of hormone receptor status, our study found Ki67 to be significantly higher in ER-negative (49.43%) and PR-negative (44.70%) tumors compared to ER-positive (30.73%) and PR-positive (29.80%) tumors. This trend aligns with findings by Inwald et al. , who demonstrated that hormone receptor–negative tumors tend to exhibit higher proliferative indices and more aggressive behavior.[9] Cheang et al. showed that high Ki67 expression effectively distinguished Luminal B from Luminal A tumors, supporting our molecular subtype findings where Luminal B HER2+ and triple-negative tumors exhibited higher Ki67 indices[10].

Our observation of elevated Ki67 in triple-negative breast cancers mirrors the findings of Medeiros et al., who reported a Ki67 index of 77.22% in triple-negative tumors, in contrast to 10.14% in luminal A cases.[11] Maeda et al. emphasized not only the prognostic value of Ki67 but also the variability introduced by manual scoring, suggesting the adoption of digital quantification for more consistent results.[12]

Table 5 highlights the correlation between HER2 status and Ki-67 proliferation index in breast cancer did not reach statistical significance, with HER2-positive tumors showing a slightly higher mean Ki-67 value (40.94 ± 25.12) compared to HER2-negative tumors (37.89 ± 26.66), but the difference was not statistically significant ($F = 0.72$, $p = 0.472$).Contrary to our study, Yerushalmi et al. (2010) found a significant association between HER2 positivity and high Ki-67 index, noting that HER2-positive tumors frequently exhibit high proliferation rates, which contributes to their aggressive nature and worse prognosis if left untreated.[7] The ambiguity in our results is attributed to unavailability of FISH(Fluoroscent In Situ Hybridisation) in our instituition for further detection of HER-2 positivity.Thus, no conclusion can be made regarding the correlation between HER- 2 positivity and Ki-67 value.

Sandilya et al. in an Indian cohort, demonstrated significant correlations between Ki67 and tumor size, grade, and hormone receptor status, thereby validating the applicability of our findings in a regional context.[13] Similarly, Pereira et al. showed that Ki67 is particularly useful in stratifying patients with intermediate-grade tumors where therapeutic ambiguity often arises.[14]

Taken together, our findings are in line with established global and national literature, confirming the role of Ki67 as an important prognostic biomarker. Its significant correlation with histologic grade and hormone receptor status supports its inclusion in routine diagnostic reporting, particularly in settings where access to molecular profiling is limited.

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