Original Article

Evaluation of Ki-67 Proliferation Index in Breast Cancer Subtypes and Its Correlation with Various Histopathological and Other Prognostic Parameters

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Abstract

Background: The current classification of breast cancer is based on molecular markers. However, due to the unavailability of these markers in all healthcare institutes in developing countries like India, certain IHC markers such as ER, PR, HER2Neu, and Ki67 act as surrogate markers and play a crucial role in determining prognosis and targeted therapy for each patient. This study aims to evaluate Ki67 expression in breast carcinomas and its correlation with IHC status and other prognostic parameters of the tumor.

Materials and Methods: The study was conducted on 200 specimens of breast carcinoma cases received in the Pathology Department over a period of 18 months at RNTMC and MB Hospital, Udaipur. These specimens were processed and examined under a microscope to describe them in terms of histologic subtype, lymph node status, and histologic grade. IHC status of ER, PR, HER2Neu, and Ki67 was evaluated.

Results: The mean age of presentation was 48.3 years. The most common histologic subtype was Invasive Breast Carcinoma (NOS) (81%), and the IHC-based subtype was Luminal-A (54%). The most common histologic grade was Grade II (55%). High Ki67 expression was observed in many cases of Luminal-B, HER2neu-enriched, and triple-negative types of breast carcinoma. Low Ki67 expression was seen in the Luminal-A subtype. There is a significant correlation between tumor size, lymph node positivity, histologic grade, ER, PR, and HER2Neu status with Ki67 expression.

Conclusion: Ki67 is a significant biomarker of breast cancer because higher Ki67 correlates with higher tumor grade. Therefore, its evaluation, along with other IHC markers, can aid in the targeted treatment of the patient.

Keywords:

Breast Cancer, Ki67 antigen, HER2 Proto-Oncogene Protein

Introduction

Breast cancer is the most common malignancy in women worldwide, with increasing trends seen in India. It accounts for more than one in ten new cancer diagnoses each year globally [1]. In 2020, there were 2.3 million women diagnosed with breast cancer and 685,000 deaths globally, making it the world's most prevalent cancer [2].

The treatment of breast cancer depends on various histopathological and prognostic parameters. These include histological

subtypes of the tumor, lymph node status, histologic grade of the tumor, and immunohistochemical biomarkers like Estrogen Receptor (ER), Progesterone Receptor (PR), and Herceptin-2 neuro-oncoprotein (HER2 NEU) status. Apart from these biomarkers, another significant biomarker is Ki-67 (proliferation index). It is a non-histone nuclear protein described in 1983 and is expressed in all phases of the cell cycle except the G0 phase [3].

Cancer cells have a high proliferation rate. In breast cancer, immunohistochemical assessment of the proportion of cells with nuclear staining for Ki-67 has become the most widely used method for determining the proliferation rate of tumor cells.

Breast carcinomas are now classified based on molecular profiles. However, in limited-resource settings, certain IHC markers such as ER, PR, HER2 NEU, and Ki-67 play a crucial role in determining the prognosis and targeted therapy for each patient. Ki-67 expression varies across different grades and subtypes of the tumor.

The objectives of this study are to analyze Ki-67 expression in various breast cancer subtypes and correlate its expression with the age of the patient, tumor size, lymph node status, histologic subtype, histological grade (using the Modified Bloom-Richardson grading system), and IHC status of the tumor (ER, PR, HER2 NEU positivity).

Materials and Methods

This cross-sectional study was conducted in the Department of Pathology (Histopathology lab), R.N.T. Medical College, Udaipur, Rajasthan, over a period of 18 months following approval from the Institutional Review Board. A total of 200 patients clinically diagnosed with breast cancer, whose biopsies were received in the histopathology department, were included in the study.

The sample size was calculated using a two-sided test with $\alpha = 0.05$ and $\beta = 0.2$ (80% power). Assuming a moderate effect size ($r = 0.3$), the required sample size was determined to be $n = 196$ (for continuous outcomes). Considering the expected distribution of breast cancer subtypes, a rounded-off value of 200 was selected for robust statistical analysis. Informed consent was obtained from all study participants.

Detailed clinical history, physical examination, radiological investigations, and FNAC findings of these patients were collected. All Modified Radical Mastectomy specimens and patients of both genders, whose biopsies were received, were included in the study. Histopathological specimens from breast conservation surgeries such as lumpectomy, trucut biopsy, benign breast lesions, secondary metastatic breast cancer cases with prior malignancy history, and all post-chemotherapy or post-radiotherapy patients were excluded.

Biopsies were received in properly labeled containers with 10% formalin. They were fixed using neutral buffered formalin, examined grossly, and appropriate sections were taken following standard protocols. After fixation, dehydration, and clearing, sections were processed and embedded in paraffin wax blocks. Sections of 3-4 microns in thickness were cut perpendicular to the block surface. These sections were stained with routine Hematoxylin and Eosin (H&E) and mounted with coverslips.

Upon microscopic examination, histological subtyping and tumor grading were performed according to the Modified Scarff-Bloom-Richardson histologic grading system.

The most suitable tissue block was selected for immunohistochemistry (IHC) analysis of ER, PR, Her2neu, and Ki67. Tissue sections of 2-3 μm thickness were cut using a microtome with disposable blades. ER/PR expression indicated the amount of estrogen receptors (ER) and progesterone receptors (PR) present in the nuclei of tumor cells [4]. HER2-neu assay measured HER2-neu staining on the membrane of tumor cells [4]. Normal breast parenchyma present in the test block served as the internal control.

The Ki67 percentage was calculated by counting 1000 malignant cells from slides containing sections from tumor margins or hotspots showing mitotically active areas. Ki67 staining appeared as dark brown to black nuclear signals. This percentage represented the proliferation index, a marker of proliferation [5].

The Ki67 percentage was correlated with histopathological findings and IHC tumor status, as defined by the 2011 Saint Gallen Consensus Meeting. Tumors with a Ki67 index <14% were classified as low proliferation, based on comparison with PAM50 intrinsic multigene molecular test classification for luminal cancers [6].

Tumors were categorized into four broad groups based on IHC status and Ki67 proliferation index, as outlined in Table 1 [4].

Table 1: IHC-based subtypes of breast cancer

IHC based subtype	Clinico-pathologic definition
Luminal A (54%)	ER and/or PR positive, HER-2neu negative, Ki67 <14%
Luminal B (23%)	
Luminal B (HER-2neu Negative)	ER and/or PR positive, HER-2neu negative, Ki67>/=14%
Luminal B (HER-2neu Positive)	ER and/or PR positive, HER-2neu positive, any Ki67
Her-2neu Overexpression (12.5%)	HER-2neu overexpression, ER and PR absent
Triple Negative (10.5%)	ER and PR absent, HER-2neu negative

Results

In our study, a total of 200 breast carcinoma cases were selected, of which 199 were female patients and 1 was male. The mean age of presentation was 48.3 years (range 21 to 82 years). Of the 200 specimens received, tumor size was <2 cm in 37.5% of patients, 2-5 cm in 29%, and more than 5 cm in 33.5% of patients. Positive lymph nodes were found in 47% of specimens (1-3 nodes), 36% of specimens (4-9 nodes), and 17% of specimens (10 or more nodes).

The most common histologic type was invasive breast carcinoma (NST) (81%), followed by carcinoma with medullary features (9.5%). The least common was metaplastic carcinoma (1.5%). Ki-67 expression >14% was observed in 46 out of 162 cases of invasive breast carcinoma (NOS), 4 out of 9 cases of invasive lobular carcinoma, 3 out of 7 cases of mucinous carcinoma, and all cases of metaplastic carcinoma and carcinoma with medullary features. The most common histologic grade was Grade II (55%), followed by Grade III (39%), with Grade I (6%) being the least common.

ER positivity was seen in 77% of specimens, while 23% were negative. PR positivity was observed in 74% of specimens, and the remaining 26% were PR negative. HER2neu positivity was found in 29.5% of specimens, with 70.5% being HER2neu negative. Specimens with equivocal results on IHC were further tested using FISH.

The histologic subtypes were classified into four IHC-based subtypes: Luminal-A, Luminal-B, HER2neu overexpression, and triple-negative. Luminal-A was the most common subtype (54%), followed by Luminal-B (23%), HER2neu overexpression (12.5%), and triple-negative (10.5%), which was the least common.

A low Ki-67 proliferation index was observed in 64% of cases, while 36% had a high Ki-67 proliferation index. Low Ki-67 was commonly seen in Luminal-A type breast carcinoma, whereas high Ki-67 was associated with many cases of Luminal-B, HER2neu overexpression, and all triple-negative breast carcinoma cases. Tables 2 and 3 show the correlation between Ki-67 and various histopathological and prognostic parameters. A significant correlation was observed between Ki-67 and tumor size, lymph node positivity, histologic grade, ER, PR, and HER2neu status.

Table 2: Histopathological parameters evaluated in breast carcinoma patients

Variables	Ki67 <14% (n) (%)	Ki67>14% (n) (%)	p-value
Age (years)			0.941
<50 (48.5%)	52 (26%)	45 (22.5%)	
>50 (51.5%)	60 (30%)	53 (26.5%)	
Tumor size (cm)			0.001
<2 (37.5%)	46 (23%)	29 (14.5%)	
2-5 (29%)	23 (11.5%)	35 (17.5%)	
>5 (33.5%)	21 (10.5%)	46 (23%)	
Lymph node (number)			0.00001
1-3 (47%)	68 (34%)	26 (13%)	
4-9 (36%)	39 (19.5%)	33 (16.5%)	
>/=10 (17%)	5 (2.5%)	29 (14.5%)	
Histologic Grade			0.000013
I (6%)	11 (10.5%)	1 (0.5%)	
II (55%)	35 (17.5%)	75 (37.5%)	
III (39%)	18 (9%)	60 (30%)	
ER status			0.00001
Positive (77%)	125 (62.5%)	29 (14.5%)	
Negative (23%)	3 (1.5%)	43 (21.5%)	
PR status			0.00001
Positive (74%)	125 (62.5%)	23 (11.5%)	
Negative (26%)	8 (4%)	44 (22%)	
HER2 status			0.00001
Positive (29.5%)	8 (4%)	51 (25.5%)	
Negative (70.5%)	86 (43%)	55 (27.5%)	

Table 3: Association of Ki-67 with Histologic Subtypes

Histologic Subtype	Ki-67:- </=14% (% of total cases)	Ki-67:- >14%(% of total cases)
Invasive Breast Carcinoma (NST) (81%)	**116 (58%)**	**46 (23%)**
Carcinoma with Medullary features (9.5%)	**0**	**19 (9.5%)**
Invasive Lobular Carcinoma (4.5%)	**5 (2.5%)**	**4 (2%)**
Mucinous Carcinoma (3.5%)	**4 (2%)**	**3 (1.5%)**
Metaplastic Carcinoma (1.5%)	**0**	**3 (1.5%)**

Discussion

Four main breast cancer subtypes have been identified based on IHC markers – ER, PR, HER2NEU, and Ki-67. These include luminal type A, luminal type B, triple-negative type, and HER2neu overexpression. Various subtypes based on histology, such as invasive duct carcinoma, medullary, lobular, mucinous, metaplastic, and apocrine carcinoma, have also been described by the WHO.

Ki-67 is a monoclonal antibody and an immunohistochemical proliferation marker in many types of cancer. It has been widely studied among breast cancer patients. The name Ki-67 is derived from the city of Kiel in Germany, where this antibody was produced, and 67 refers to the original clone number.

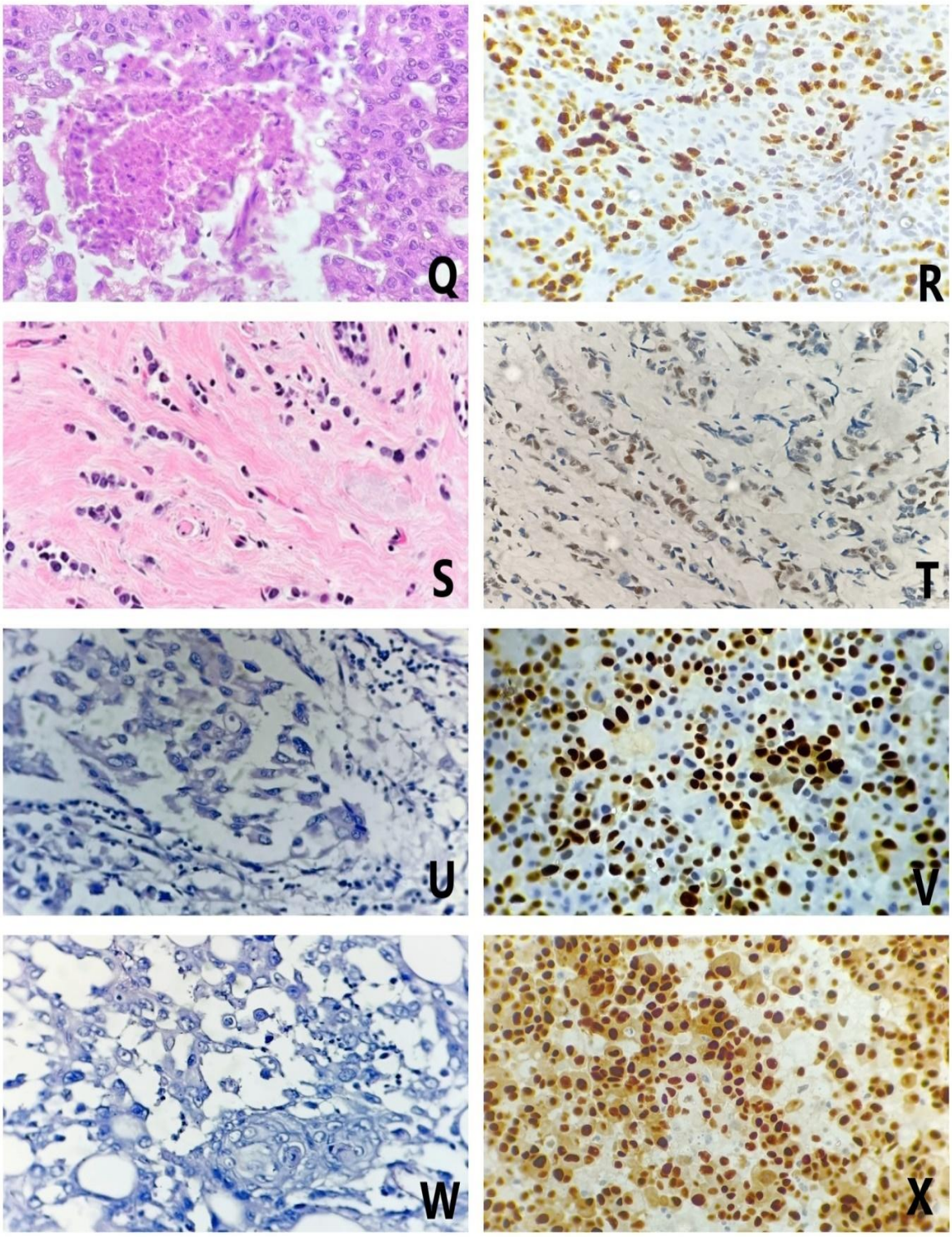

Figure 1: Photomicrograph of Hematoxylin and Eosin Images and Ki-67 immunostaining images at (400X magnification) Q- Invasive Breast Carcinoma NOS Grade III (H& E), R- Invasive Breast Carcinoma NOS Grade III (>14% Ki 67 expression) S- Invasive Lobular Carcinoma (H& E), T- Invasive Lobular Carcinoma (>14% Ki 67 expression), U- Carcinoma with Medullary Features (H& E), V-Carcinoma with medullary features (>14% Ki 67 expresssion), W- Metaplastic Carcinoma (H& E), X- Metaplastic Carcinoma (>14% Ki 67 expression)

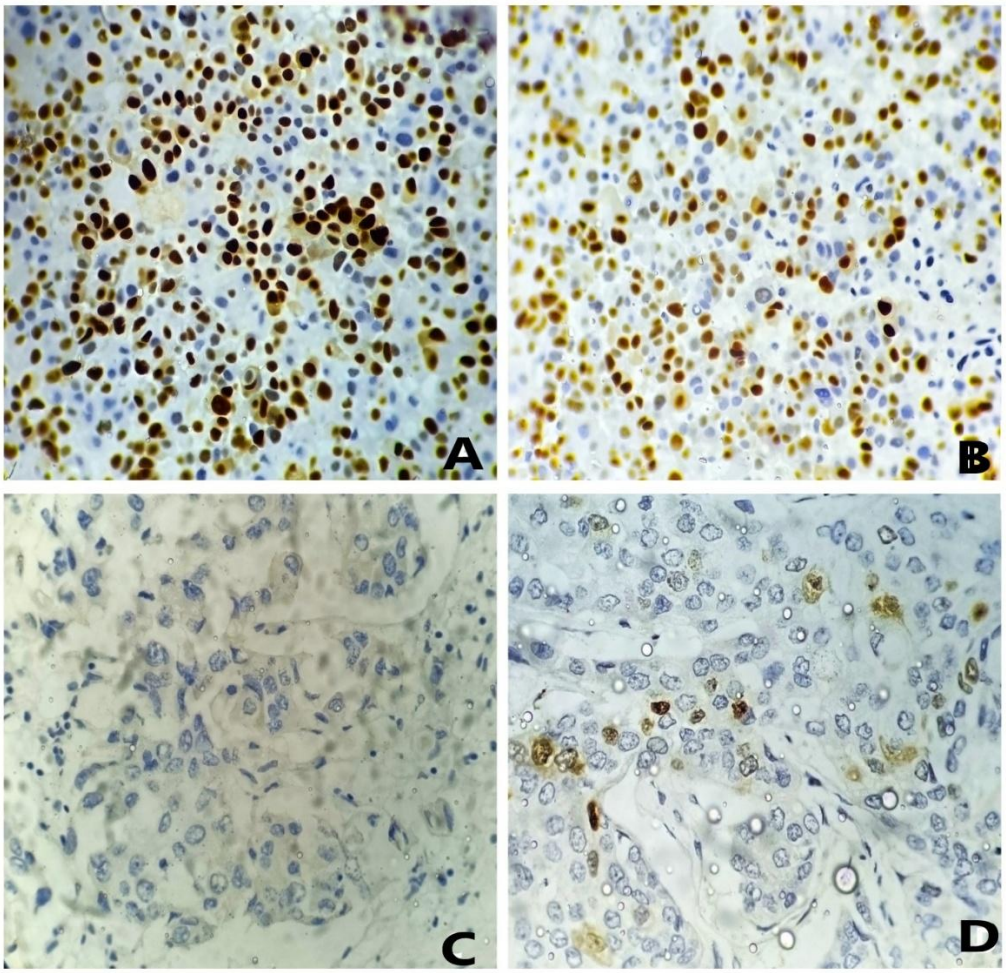

Figure 2: Photomicrograph (400X) IHC: Luminal-A subtype of breast carcinom(A-ER positive, B- PR positive, C-HER-2 negative, D-Ki-67 expression <14%)

In our study, evaluation of 200 breast carcinoma cases was conducted based on the following variables: age, tumor size, lymph node status, histologic subtype, histologic grade, and the status of ER, PR, HER2, and Ki-67. Observing the age of the patient at the time of diagnosis is crucial, as the incidence of breast cancer increases with age. The mean age of presentation in our study was 48.3 years. After comparing age with Ki-67, we could not establish any correlation. Similar results were found in a study conducted by Madani et al., where the mean age of presentation was 49.6 years, and no significant correlation was found between age and Ki-67 expression [7]. However, this finding did not align with other studies conducted by Nishimura et al., Li et al., and Spyratos et al., which found that higher Ki-67 expression significantly correlated with younger age groups [8,9,10].

A possible reason for the difference in results among studies could be the use of different cut-offs (such as 10% and 30%) for Ki-67 expression [8,9,10,11,12]. For accurate correlation, researchers need to agree on a standard and constant cut-off value.

The present study revealed that tumors with increasing size showed high Ki-67 expression, with the majority of tumors beyond 5 cm (23% of cases). Similar results were seen in studies conducted by Kontzoglou et al., Inwald et al., and Trihia et al. [13,14,15]. However, studies by Tahir et al. [16] and Shin et al. [17] found no significant correlation between increasing tumor size and Ki-67 expression.

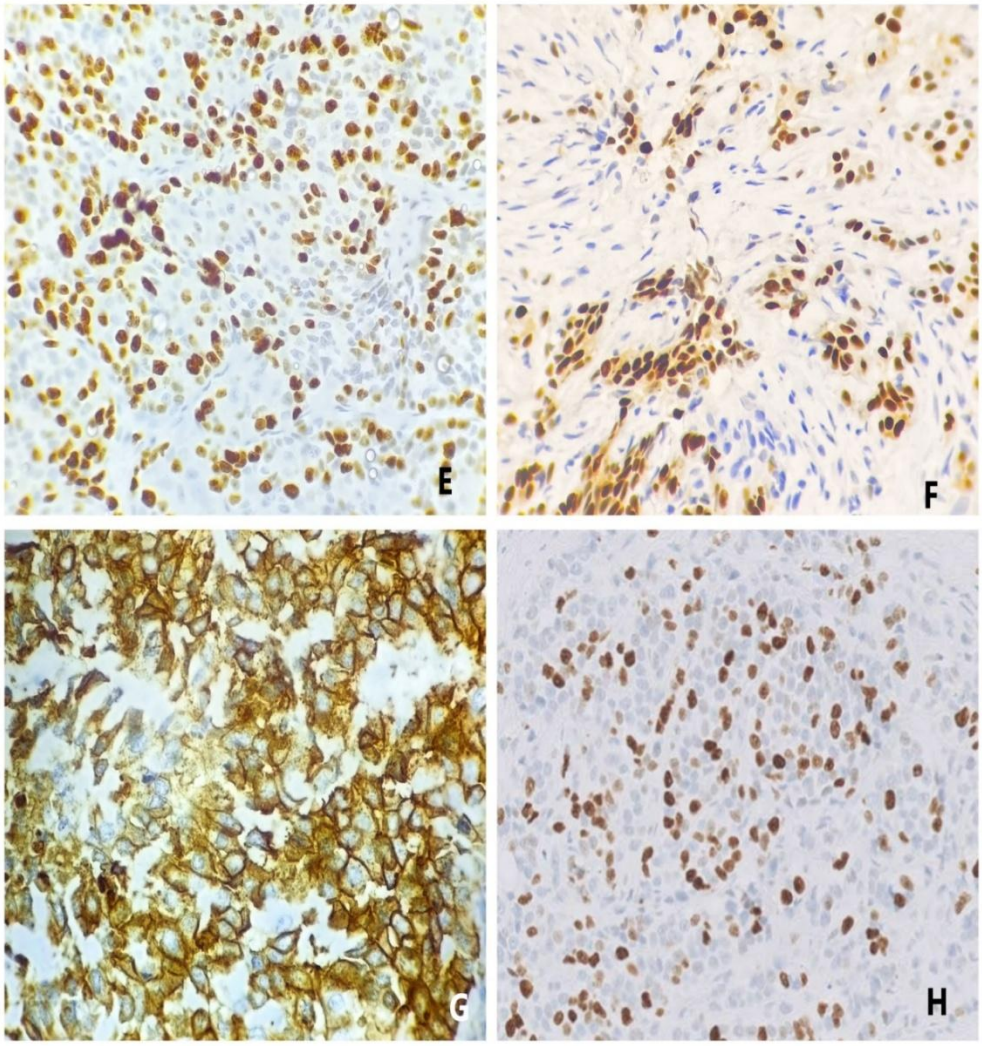

Figure 3: Photomicrograph 3 (400X) IHC: Luminal-B subtype of breast carcinoma (E- ER positive, F-PR positive, G-HER-2 positive, H- Ki 67 expression >14%)

The histology of the tumor plays an important role in prognostication. Breast cancer has different histological subtypes due to variations in cellular origin, genetic mutations, and hormonal influences. In our study, all cases of medullary carcinoma and metaplastic carcinoma showed high Ki-67 expression (>14%). Most cases of invasive breast carcinoma (NST) (58%), invasive lobular carcinoma, and mucinous carcinoma showed low Ki-67 expression (<14%). These findings were concordant with studies conducted by Inwald et al., Trihia et al., and Hashmi et al. [14,15,18]. However, studies by Mohammadizadeh et al. [19] and Gulati et al. [20] had conflicting results, showing that the majority of invasive breast carcinoma cases (71.4% and 68%, respectively) had high Ki-67 expression.

Axillary lymph nodes are common sites for breast cancer metastasis. Lymph node status can influence surgical decisions regarding whether to resect or preserve lymph nodes. Sentinel lymph node biopsy plays a crucial role in this process. Our study revealed that a higher number of positive resected lymph nodes correlated with higher Ki-67 expression. Similar results were obtained in studies by Madani et al., Nishimura et al., and Inwald et al. [7,8,14]. However, studies by Amini et al. [21] and Liu et al. [22] found no significant correlation between the number of positive lymph nodes and Ki-67 expression.

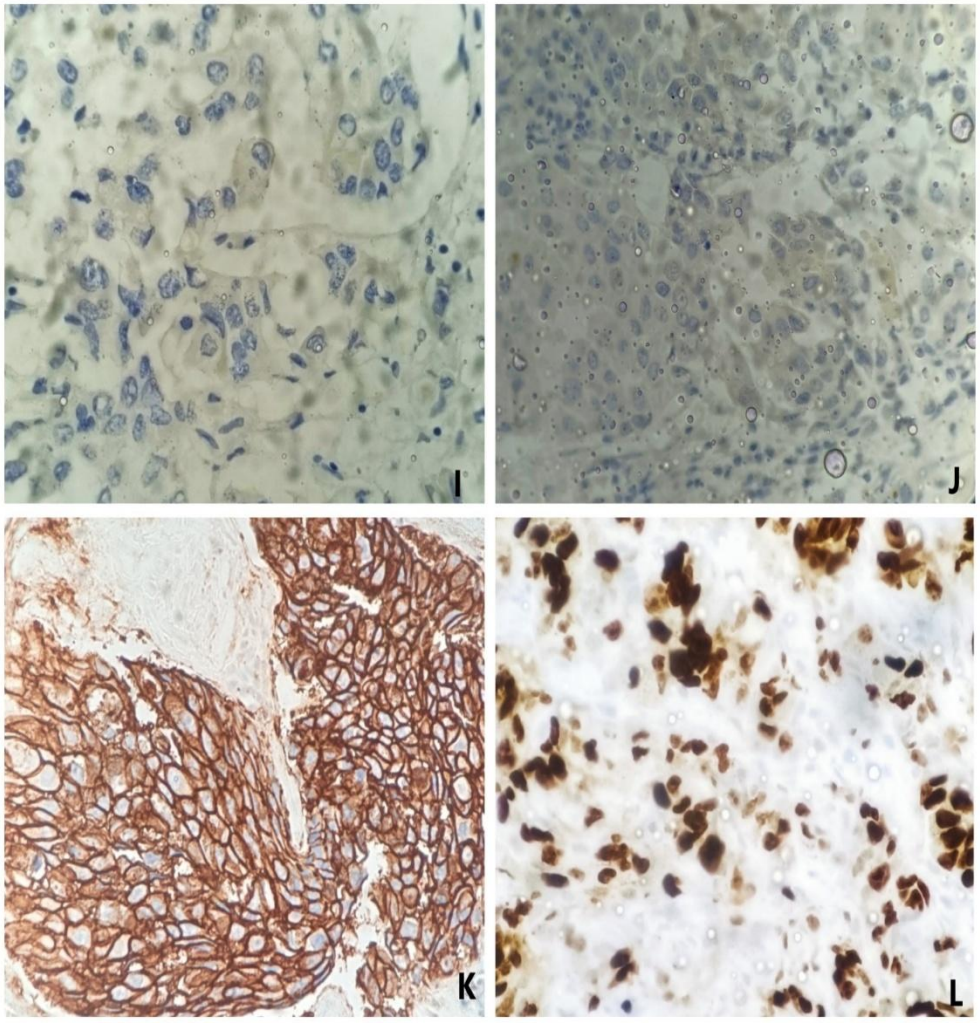

Figure 4: Photomicrograph (400X) IHC: HER-2 overexpression subtype of breast carcinoma (I-ER negative, J-PR negative, K-HER-2 positive, Ki 67 expression >14%)

Histological grade is a strong predictor of patient outcomes, reflecting tumor aggressiveness. Higher grades indicate more aggressive disease. Correlating histological grade with Ki-67 can provide significant insights into tumor behavior and aid in treatment planning. According to our study, the most common tumor grade, based on the Modified Bloom-Richardson grading system, was Grade II (55%), followed by Grade III (39%). Higher Ki-67 expression was observed in higher grades (Grade III). These findings were concordant with studies by Inwald et al., Trihia et al., and Gonzalez et al. [13,14,23], but discordant with the study conducted by Yavari et al. [24].

IHC-based classification of breast cancer provides a clearer understanding of hormone sensitivity, treatment preferences, and proliferation rates. In our study, high Ki-67 expression was observed in some cases of luminal-B, HER2neu-overexpression, and triple-negative breast carcinoma. Most triple-negative carcinomas were medullary, metaplastic, or high-grade invasive breast carcinoma (NOS). This correlates with several other studies by Nishimura et al., Kontzoglou et al., Inwald et al., and Colozza et al., which also found that higher Ki-67 indices significantly correlated with HER2 overexpression and triple-negative breast carcinoma [8,13,14,25].

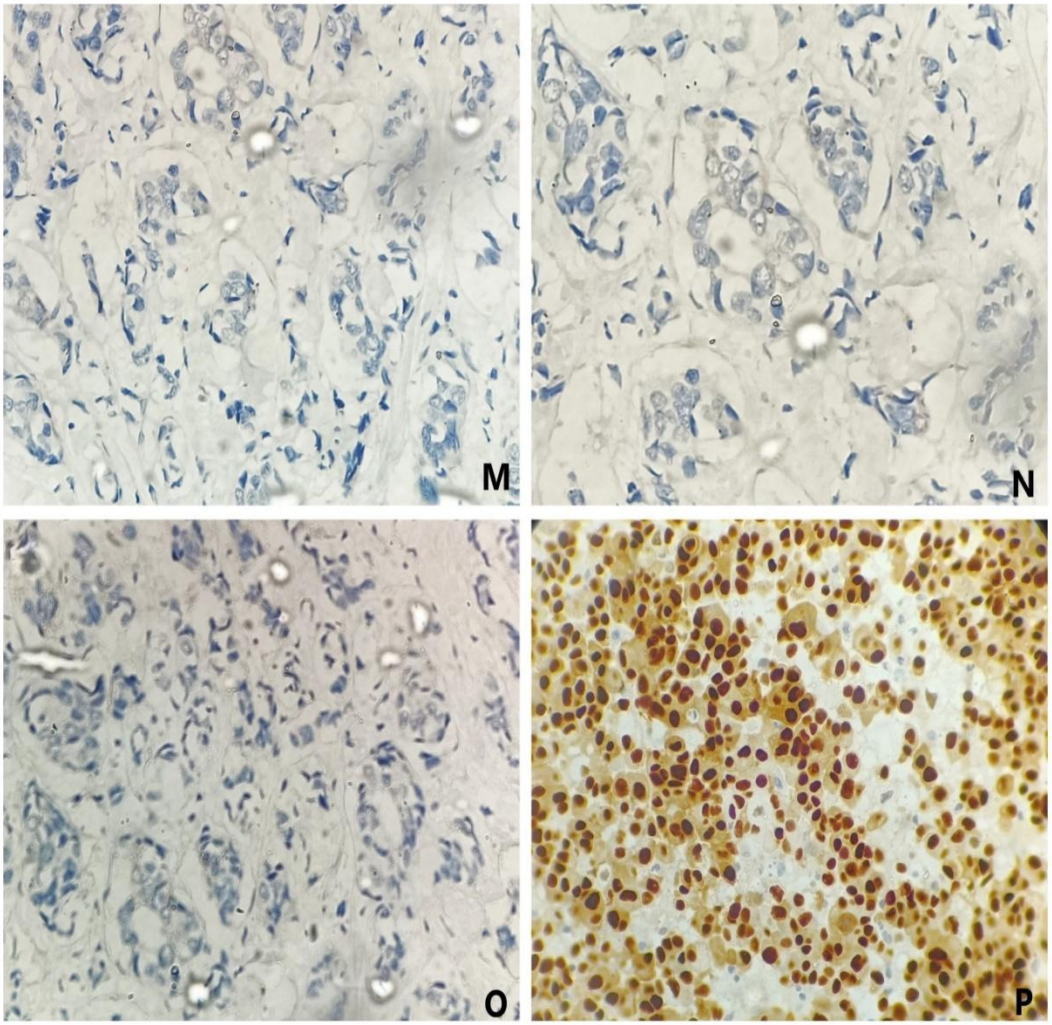

Figure 5: Photomicrograph (400X) IHC: Triple-negative subtype of breast carcinoma (M- ER negative, N-PR negative, O-HER-2 negative, P-Ki 67 expression >14%)

Limitations: Cut-off Value of Ki-67 – There is no universally accepted cut-off value for Ki-67, leading to discrepancies among previously conducted studies, which might have affected this study as well. Unavailability of Molecular Markers – The role of molecular markers in accurate subtyping and prognostication of breast cancer patients is crucial. However, our resource-limited setting, catering to the tribal belt of Southern Rajasthan, faced constraints due to the cost of molecular testing.

Conclusion

High Ki-67 expression is observed in some cases of luminal-B, HER2neu-overexpression, and triple-negative breast carcinoma. There is a significant correlation between tumor size, histologic grade, ER, PR, and HER2Neu status with Ki-67 expression. For better correlation between Ki-67 and various clinicopathological and prognostic parameters, a standardized and universally accepted Ki-67 cut-off is necessary.

Abbreviations

ER – Estrogen Receptor

PR – Progesterone Receptor

HER2NEU – Human Epidermal Growth Factor Receptor 2 / neuproto-oncogene

Ki-67 – Kiel-67

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Competing Interests – None declared.

Statement of informed consent: *Informed consent was obtained from all participants in the study. No identifying information about any patients is mentioned in the manuscript.*

Statement of human and animal rights: *The procedures followed in this study were in accordance with the ethical standards prescribed by the Institutional Review Board and National Ethical Committee, as well as the Helsinki Declaration of 1975, revised in 2000.*

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