Section: Pathology

Original Research Article

IMMUNOHISTOCHEMICAL AND MOLECULAR PROFILE OF BREAST CARCINOMA AND ITS CORRELATION WITH CLINICOPATHOLOGICAL PARAMETERS IN A TERTIARY CARE HOSPITAL

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Received : 13/06/2026
Received in revised form : 06/08/2026
Accepted : 20/08/2026

Keywords:
Breast cancer, hormone receptor, triple negative, molecular subtype, proliferation index, histological grade.

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DOI: 10.47009/jamp.2026.8.4.220

Source of Support: Nil,
Conflict of Interest: None declared

Int J Acad Med Pharm
2026; 8 (4); 1317-1321

ABSTRACT

Background: Breast cancer remains a highly heterogeneous malignancy requiring detailed pathological and biomarker evaluation for optimal management. This study analyzed the microscopic features and immunohistochemical (IHC) profile including Estrogen Receptor (ER), Progesterone Receptor (PR), Human Epidermal Growth Factor Receptor 2 (HER2), and proliferative index (Ki-67)—in total of 150 patients diagnosed with invasive breast carcinoma (IBC) and evaluate the prevalence of triple negative breast cancer (TNBC). **Materials and Methods:** A retrospective analysis of 150 cases was performed. Demographic data including age and gender, histological features including subtype, Nottingham histologic grade and immunohistochemical profile including ER, PR, HER2 status and Ki-67 proliferation index were analysed. **Results:** Patients with invasive breast carcinoma predominantly comprised females (97.3%) with a mean age of 55.6 years. Grade 2 tumours were the most common (54.0%), followed by Grade 3 (38.7%) and Grade 1 (7.3%). Immunohistochemical biomarker profiling revealed ER positivity in 61.3%, PR positivity in 44.6%, and HER2 positivity in 35.3% of cases. Triple-negative breast cancer (TNBC) accounted for 23.3% of cases. ER/PR positivity was significantly associated with low grade tumors (p value -<0.0001, fischer exact test). Elevated Ki-67 was strongly associated with higher histologic grades. Triple Negative breast cancer cases showed high proliferation rate (mean 58.7%), and were mostly high-grade tumors. **Conclusion:** Majority of breast cancers are of luminal subtype. Triple negative breast cancer constitutes significant proportion of cases and shows aggressive features. Our findings underscore the critical role of combining routine histomorphological grading with Immunohistochemical analysis and molecular subtyping, particularly in view of significant burden of higher grade and triple negative cases within the studied population.

INTRODUCTION

Invasive breast carcinoma (IBC) is the most frequent cancer in women and accounts for maximum cancer related mortality in women, estimating around 2.4 million new cases (11.8%) in 2024 worldwide.[1] In India, female breast cancer accounts for 13.5% of all cancer cases and is leading cause of cancer related mortality in 2020.[2] Breast cancer is a genetically and morphologically heterogenous disease with multifactorial etiopathogenesis.[3] Histopathological grading and biomarker identification is pivotal for effective patient management. The Nottingham Histologic Score (Elston-Ellis modification of the Scarff-Bloom-Richardson system) serves as the gold standard for morphological grading, evaluating tubule formation, nuclear pleomorphism, and mitotic activity.[4]

Newer modalities such as immunohistochemistry, microassay techniques, and cytogenetics have facilitated accurate diagnosis, better prognostication, research and application of newer treatments.[3] Based on immunohistochemical expression of hormone receptors including Estrogen Receptor (ER), Progesterone Receptor (PR), and Human Epidermal Growth Factor Receptor 2 (HER2), breast cancer has been classified into Luminal A subtype, Luminal B subtype, Her2 positive and Triple

ISSN (O): 2687-5365; ISSN (P): 2753-6556

negative subtype.[5] This profiling is based on consideration that two distinct types of epithelial cells are found in human mammary glands: Basal cells and luminal epithelial cells.[3,5]

Triple negative cancers are characterized by lack of expression of ER, PR, and Her2 neu.[3,6] These cancers occur in approximately 10–25% of all breast carcinomas.[3,5] Triple negative breast cancers (TNBCs) are clinically known to be more aggressive and less responsive to standard treatment and are associated with poor overall patient prognosis.[7-10]

The clinical data on TNBC in Asian population are limited. The present study was designed to evaluate the immunohistochemical (IHC) expression ER, PR and HER2, alongside the Ki-67 proliferation index and to study the clinicopathological patterns, distribution of histologic grades and biomarker profiles in breast cancer, providing insight into the biological trends of breast cancer.

MATERIALS AND METHODS

This cross-sectional study was conducted in the Department of Pathology, Geetanjali Medical College and Hospital, Udaipur, Rajasthan, following institutional ethical clearance. A total of 150 cases of breast cancer diagnosed histopathologically over a period of 20 months were included in the study. Clinical and demographic details were recorded for all patients. Histopathologic evaluation was done following formalin fixation, standard processing and haematoxylin and eosin (H&E) staining. Histologic typing was performed according to the World Health Organization (WHO) classification. Nottingham Grading System. Immunohistochemical markers including ER, PR, Her2 and Ki-67 were performed using standard protocols on paraffin sections. ER and PR were evaluated using the Allred score and Her2 was evaluated using Her2 scoring system as per ASCO/CAP guidelines.[11] Ki-67 was expressed as percentage proliferation index in tumor cells. The tumors were classified into Luminal A type (ER and/or PR positive, Her2 negative, Ki-67<14%), Luminal B type (ER and/or PR positive, Her2 positive or Her2 negative, Ki-67 >14%), Her2 positive subtype (ER and PR negative, Her2 positive), and Triple negative (ER, PR and Her2 negative).[12,13]

The clinicopathological and morphological features were correlated with immunohistochemical profile of the patients.

RESULTS

Out of 150 patients, 146 were female (97.3 %) and 4 were male (2.7 %). The age ranged from 29 to 88 years, with a mean age of 55.6 years. Maximum number of cases were seen in patients more than 40 years of age. [Table1] The most commonly encountered morphology was Invasive breast carcinoma, no special type (ductal), accounting for 141 cases (94.0 %). Other variants observed included Lobular Carcinoma (4.0 %), Mucinous Carcinoma (0.7 %), and variants showing medullary or mixed features (1.3%).

Based on the sum of the structural and nuclear criteria as per Nottingham score (Modified Scarf Bloom Richardson grading system), the tumors were classified as Grade I, II and III. 11 cases were Grade I (7.3%), 81 cases were Grade II (54.0%) and 58 cases were Grade III (38.7%)

[Table 1] shows trend of tumour grade distribution across different age brackets. Overall, most cases were seen in the age bracket of 50 to 60, while maximum high-grade cases were seen in the age bracket of 40 to 50. On calculating the p value using Fisher exact test it was observed that there is significant association between a patients age and tumour grade (p value = 0.0294). Younger patients, less than 40 years at presentation showed significantly higher proportion of Grade 3 tumours, while older patients (ages 60 and above) presented with lower grade tumors more frequently.

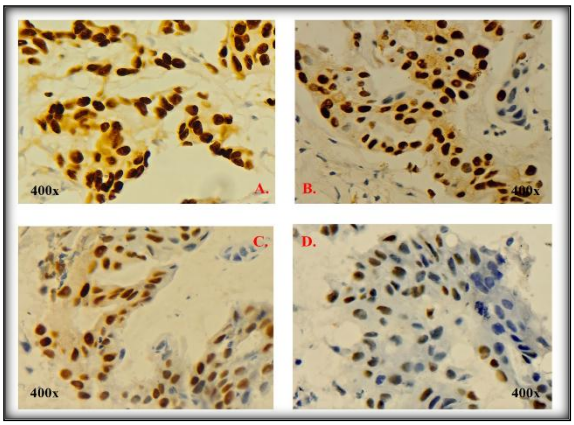

Figure 1: ER expression showing Intense (A) and Moderate (C) nuclear staining. PR expression showing Intense (B) and Moderate (D) nuclear staining.

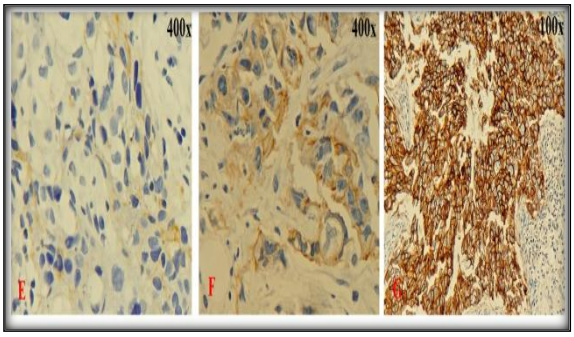

Figure 2: Her2 IHC showing barely perceptible incomplete membrane staining (E), weak to moderate membrane staining (F) and intense complete membrane staining (G).

The expression patterns of Hormone receptors and Her 2 were compared to tumor grade. [Table 2] ER/PR positivity was significantly associated with low grade tumors (p value -<0.0001, fischer exact test). Her 2 expression showed no statistical

ISSN (O): 2687-5365; ISSN (P): 2753-6556

significance when compared with tumour grade in our study.

Table 1: Age and Grade distribution in breast cancer (N = 150)

Age Group	Grade 1	Grade 2	Grade 3	N (%)
20–29	0	1	0	1 (0.67%)
30–39	1	3	7	11 (7.33%)
40–49	2	16	18	36 (24.0%)
50–59	3	31	15	49 (32.67%)
60–69	2	17	11	30 (20.0%)
70–79	7	9	4	20 (13.33%)
80–90	0	2	1	3 (2.0%)
Total	15	79	56	150 (100%)

Immunohistochemistry [Figure 1 & 2] showed ER was positive in more than half of cases (92 cases, 61.34%), PR was expressed in 67 cases (44.6%) and Her2 overexpression was seen in 53 cases (35.3%). 35 cases (23.3%) were negative for ER, PR as well as Her2 IHC, qualifying as Triple-Negative Breast Cancer.

Table 2: ER, PR and Her2 expression across various tumour grades

IHC marker	Status	Grade 1	Grade 2	Grade 3	Total cases
ER	Negative	2	17	39	58
	Positive	13	62	17	92
PR	Negative	3	33	47	83
	Positive	12	46	9	67
HER2	Negative	11	36	27	74
	Equivocal	0	16	7	23
	Positive	4	27	22	53

All the cases for which Ki-67 (proliferation index) was performed (95 cases) were further classified into various molecular subtypes. Proliferation indices ranged from 10% to 90%. Higher mean Ki-67 indices correlated significantly with high-grade morphology (pvalue<0.0001). Low grade tumors showed low proliferation index (mean 16.8), intermediate grade tumors showed higher proliferation rates (mean 27.7), whereas high grade tumors showed the greatest proliferation index (mean 52.5). The highest proliferation index (70% or higher) were found exclusively in Grade 3 tumors.

Molecular subtypes were correlated with mean age at presentation, tumor grade and proliferation index. [Table 3] All cases of Luminal A type breast cancer were low grade. Among Luminal B subtype, lower proportion of cases (22.7%) showed high grade morphology. Her2 enriched subtype showed high grade features in more than half of cases (61.1%). Triple negative tumors were high grade in majority of cases (78.9%). The correlation between molecular subtype and histological grade of the tumors was statistically significant (p value <0.001). No significant correlation was found between molecular subtype of breast cancer and age at presentation. However, proliferation index was significantly associated with molecular subtype of breast cancer. Luminal A subtype maintained a strictly low proliferation index (mean 13.9%), while Triple Negative cases showed high proliferation rate (mean 58.7%), indicating the aggressive potential of triple negative subtype. Luminal B cases when split into HER2-negative and HER2-positive variants express high replication rates regardless of hormone status.

Table 3: Molecular subtypes of breast cancer – frequency distribution and associations with clinicopathological characteristics

Molecular subtype	No.of cases	Mean Ki 67	Mean Age	High Grade tumour %
Luminal A	14	13.9%	60±12.74	0%(0/14)
Luminal B (HER2-Negative)	31	36.8%	56±10.78	22.5%(7/31)
Luminal B (HER2-Positive)	13	31.9%	55±12.65	23.07%(3/13)
HER2 Enriched	18	31.9%	52±8.04	61.11%(11/18)
Triple Negative	19	58.7%	52±12.88	78.94%(15/19)
Grand Total	95	P value -<0.001	P Value -0.177	P Value-<0.001

DISCUSSION

Breast cancer is heterogenous malignant neoplasm with varied morphological subtypes and clinical behaviors.[14] In this study, most of the patients were more than 40 years of age (92%) with only a few cases in younger age group. Maximum number the cases (n=141, 94.0%) showed invasive breast carcinoma, no special type (ductal) morphology. Few cases of special subtypes were noted including Lobular Carcinoma (4.0 %), Mucinous Carcinoma (0.7%), and variants showing medullary or mixed features (1.3%). Maximum number of cases were intermediate grade (54.0%), followed by high grade tumors (38.7%) and low grade (7.3%). The clinicopathologic features in this study are similar to

ISSN (O): 2687-5365; ISSN (P): 2753-6556

previous studies. Majority of breast cancers upon morphologic subtyping, fall in the category of invasive breast carcinoma, no special type (ductal). A smaller proportion of breast cancers include special types that include lobular carcinoma, mucinous carcinoma, medullary carcinoma, metaplastic carcinoma, apocrine carcinoma etc.[12,14]

Nottingham score (modified Scarf-Bloom-Richardson system) remains the standard system of tumor grading in breast cancer.[4] The strong correlation observed between high grade of the tumor owing to advanced Nottingham scores, and high proliferation index confirms that morphological assessment continues to serve as an excellent surrogate for underlying tumour kinetic activity.

Immunohistochemical studies showed ER positivity in majority of breast carcinomas (61.4%). Her2 was positive in 35.3% of breast cancers and 23.3% of cases were negative for all three markers ER, PR and Her2. Hormone receptors including ER and PR are important predictive and prognostic factors for breast cancer. They are expressed in around 70-80% of breast cancers.[3,11,12,13] Her2 gene overexpression is one of important events in early breast tumorigenesis. Her2 expression is seen in 15-25% of breast cancers on IHC, and Her2 status is important in deciding proper management.[3,12,13] Triple negative breast cancer constitutes around 10-24% of breast cancers. They are negative for ER and PR and do not show Her2 overexpression. Triple negative breast cancer is seen more commonly in African-American women. These are aggressive tumors with poorly differentiated morphology and higher proliferation index.[3,5] Triple negative breast cancer has been associated with younger age at presentation and more aggressive clinical course.[3,13]

The current study was undertaken to look at the clinical profile, histomorphological patterns and proliferation index in triple negative breast cancers, and to compare these parameters in breast cancers of other molecular subtypes.

The overall rate of TNBC in our study (23.3%) is comparable to results obtained by previous studies.[15] Majority of breast cancers are Hormone receptor or Her2 positive. However, 10-25% of breast cancers are negative for ER, PR and Her2.[3,5,13] Previous studies have described triple negative cancers ranging as low as 11-12%,[8,10,16] to as high as 30-32%.[6,17] In our study, the mean age at diagnosis was younger in TNBC patients (51.5 years) as compared to non-TNBC group (56.5 years). Similar results were seen in previous studies.[6,8] More patients (78.2%) in TNBC group presented with grade III tumors as compared to non triple negative patients. TNBC has been associated with high tumor grade in previous studies.[5,8,10,15] Triple negative breast cancer presents in a younger age group and shows aggressive features such as high-grade morphology. There was significant correlation observed between molecular subtype of tumors and grade in this study, with triple negative tumors showing high grade morphology (78.9%) in major proportion of cases, whereas luminal subtype tumors were mostly low grade (82.8%). Triple negative breast cancer is associated with histologically aggressive features including higher nuclear grade, mitotic activity, high nucleocytoplasmic ratio and necrosis.[9,10,13,18] Luminal subtype of breast cancer is associated with lower tumor grade and better prognosis.[13,16]

Triple negative breast cancers show considerable morphological and molecular heterogeneity. Majority of these tumors are high grade, poorly differentiated tumors with increased rate of proliferation.[3,14] They may show morphological features of invasive ductal carcinoma, or other variants such as metaplastic, apocrine or medullary features.[9,14,19] Triple negative breast cancer has been divided into basal like and non-basal like according to expression of basal cell IHC markers.[3,8,16]

Breast duct epithelium comprises luminal cells, that show expression of ER and BR, and basal cells, which show expression of CK 5/6 and CK 17.[5,14] Many authors still use terms “basal-like cancer” and triple negative cancer interchangeably; however, they are not same. Majority of basal like cancer are triple negative, but around 25% of them may show expression of HER-2 or hormone receptors.[5] Conversely, major proportion of TNBC have basal like immunophenotype.[3,5] TNBC has been associated with aggressive clinical course, high tumor grade and increased mitotic activity.[3,5,10]

Our study showed that among Triple negative breast carcinoma, majority of tumors were high grade (78.9%). Her2 positive tumors also comprised a large proportion of high grade (61.1%) On the other hand luminal type breast cancer comprised mostly of low-grade tumors. Similar association was found by GM Reddy et al,[19] and Ramchandwani et al.[13] Triple negative breast cancer shows poorly differentiated morphology with higher tumor grade and high proliferation rates in majority of cases.[3,13]

Ki-67 index showed significant correlation with high grade morphology. Low grade tumors showed low proliferative index, while high grade tumors showed Ki-67 index values on the higher side. Proliferation index is an independent factor with prognostic and predictive importance. Previous studies have shown that high Ki-67 index expression is seen more commonly in younger age group, higher tumor grade and Her2 positivity.[13] TNBC is aasociated with high Ki-67 index.[9,13] Ki-67 index not only provides measure of cell proliferation, but it is also an indicator of tumor aggressiveness, response to treatment and clinical outcome.[3,13]

Current study displays the spectrum of clinicopathological parameters and immunohistochemical profile in breast cancer. While hormone receptor positive disease remains the single largest group (61.4%), there is a notably high prevalence of Triple Negative Breast Cancer (23.3%). TNBC is widely recognized for its aggressive clinical course, lack of targeted endocrine interventions, and reliance on systemic cytotoxic

ISSN (O): 2687-5365; ISSN (P): 2753-6556

chemotherapy. Therefore, it is important to identify these tumors for early intervention and appppropriate patient management.

CONCLUSION

Our analysis highlights the diverse landscape of invasive breast cancer through histomorphology and routine immunohistochemistry. The high proportion of Grade 2 and 3 tumors, alongside a substantial TNBC subgroup, calls for more robust localized screening measures to detect disease at earlier pathological stages. TNBC presents at a younger age, with higher grade and shows high proliferation rates, reflecting aggressive nature of these tumors. Compiling this clinicopathological information for patients ensures proper clinical management, enabling oncology teams to optimise treatment into targeted endocrine, anti-HER2, or aggressive cytotoxic therapy.

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ISSN (O): 2687-5365; ISSN (P): 2753-6556