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ROLE OF IMMUNO-HISTOCHEMISTRY IN SUBTYPING OF BREAST CARCINOMA AND ITS ASSOCIATION WITH HISTOLOGICAL GRADE AND LYMPH NODE STATUS

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Abstract

Background: Breast carcinoma is a biologically heterogeneous disease with variable prognosis and therapeutic response. Immunohistochemistry (IHC) plays a crucial role in molecular subtyping of breast cancer and provides important prognostic and predictive information beyond routine histopathology.

Objectives: To evaluate the role of immunohistochemistry in subtyping breast carcinoma and to study its association with histological grade and lymph node status.

Materials and Methods: This descriptive cross-sectional study included 50 histologically confirmed cases of breast carcinoma. Tumors were graded using the Nottingham modification of the Bloom–Richardson system. Immunohistochemical analysis for ER, PR, HER2/neu, and Ki-67 was performed, and cases were classified into molecular subtypes. Associations between IHC subtypes, histological grade, and lymph node status were analyzed.

Results: Invasive ductal carcinoma was the most common histological type. Luminal subtypes constituted the majority of cases. Luminal A tumors were predominantly associated with lower histological grades and lower lymph node involvement, whereas HER2-enriched and triple-negative subtypes showed significant association with higher histological grades, elevated Ki-67 index, and increased lymph node metastasis.

Conclusion: Immuno-histochemistry is an essential adjunct to histopathology in breast carcinoma, enabling accurate molecular subtyping and prognostic stratification. Its strong correlation with histological grade and lymph node status underscores its importance in guiding personalized management and therapeutic decisions.

Keywords: Immuno-histochemistry, Breast Carcinoma, Histological Grading, Lymph Node Status

Introduction

Breast carcinoma is the most common malignancy among women worldwide and remains a leading cause of cancer-related mortality, particularly in developing countries. According to global cancer statistics, breast cancer accounts for nearly one-quarter of all cancers diagnosed in women and represents a significant public health burden.[1] In India, the incidence of breast carcinoma has shown

a steady rise over the past few decades, with an increasing number of cases diagnosed at a younger age and at advanced stages of disease.[2] Breast carcinoma is a biologically heterogeneous disease, exhibiting wide variation in clinical presentation, histopathological features, response to therapy, and prognosis.

Traditionally, prognostication and therapeutic decision-making in breast carcinoma have relied on clinic-pathological parameters such as tumor size, histological type, histological grade, lymph node status, and clinical stage.[3] Among these, histological grading—most commonly assessed using the Nottingham modification of the Bloom–Richardson grading system—provides valuable prognostic information by evaluating tubule formation, nuclear pleomorphism, and mitotic activity.[4] Lymph node involvement is another critical prognostic factor, as axillary nodal metastasis is strongly associated with increased risk of recurrence and reduced overall survival.[5] However, these conventional parameters alone are insufficient to fully explain the biological behavior and therapeutic response of breast carcinomas.

Advances in molecular biology have led to a better understanding of breast cancer heterogeneity, resulting in the classification of breast carcinomas into distinct molecular subtypes. Gene expression profiling has identified intrinsic subtypes such as Luminal A, Luminal B, HER2-enriched, and Basal-like, each associated with different clinical outcomes and responses to therapy.[6] However, routine molecular testing using gene expression arrays is costly and not widely available, especially in resource-limited settings. As a result, immuno-histochemistry (IHC) has emerged as a practical and cost-effective surrogate for molecular subtyping of breast carcinoma in routine pathology practice.

Immuno-histochemical evaluation of estrogen receptor (ER), progesterone receptor (PR), human epidermal growth factor receptor 2 (HER2/neu), and the proliferation marker Ki-67 allows classification of breast carcinomas into clinically relevant subtypes that closely approximate molecular categories.[7] These IHC-based subtypes not only provide prognostic information but also guide therapeutic decisions, particularly the use of hormonal therapy and targeted anti-HER2 agents. ER- and PR-positive tumors generally show better prognosis and favorable response to endocrine therapy, whereas HER2-positive tumors benefit from anti-HER2 targeted therapy. Triple-negative breast carcinomas, which lack ER, PR, and HER2 expression, are associated with aggressive behavior and limited treatment options.[8]

In developing countries, where patients often present at advanced stages and access to advanced molecular testing is limited, immuno-histochemistry plays a pivotal role in the comprehensive evaluation of breast carcinoma. Correlating IHC-based subtypes with established prognostic parameters such as histological grade and lymph node status can enhance risk stratification and facilitate personalized management.

Materials and Methods

Study Design & Setting

A prospective observational study was conducted in the Department of Pathology of a tertiary care teaching hospital in North India over a period of one year from November 2024 to October 2025. The study included all histologically confirmed cases of invasive breast carcinoma received in the Department of Pathology during the study period. A total 50 cases of invasive breast carcinoma meeting the inclusion criteria were included in the study based on convenient sampling technique method

Inclusion Criteria

- All cases of invasive breast carcinoma diagnosed on:
  - Modified radical mastectomy specimens
  - Lumpectomy or wide local excision specimens
- Adequate tissue available for histopathological examination and immuno-histochemistry
- Cases with available axillary lymph node evaluation

Exclusion Criteria
- Cases of ductal carcinoma in situ (DCIS) without invasion
- Recurrent breast carcinoma cases
- Patients who received neoadjuvant chemotherapy or radiotherapy
- Inadequate tissue for immuno-histochemical evaluation

Specimen Processing
Surgical specimens were received in 10% neutral buffered formalin. Gross examination was performed according to standard protocols, noting tumor size, location, appearance, and lymph node status. Representative sections were taken from the tumor, margins, and axillary lymph nodes.

Histopathological Examination
Tissue samples were processed routinely, embedded in paraffin, and sectioned at 3–5 μm thickness. Sections were stained with Hematoxylin and Eosin (H&E) for histopathological evaluation.

Histological Grading
Histological grading of invasive breast carcinoma was performed using the Nottingham modification of the Bloom–Richardson grading system, based on:
- Tubule formation
- Nuclear pleomorphism
- Mitotic count

Tumors were categorized as Grade I, II, or III.

Lymph Node Evaluation
Axillary lymph nodes were examined for the presence or absence of metastatic deposits. Lymph node status was recorded as Node negative or Node positive. The number of lymph nodes examined and involved was documented.

Immuno-Histochemical Study
Immuno-histochemistry was performed on formalin-fixed, paraffin-embedded tissue sections using the standard avidin-biotin or polymer detection method. The following markers were evaluated:
- Estrogen receptor (ER)
- Progesterone receptor (PR)
- Human epidermal growth factor receptor 2 (HER2/neu)
- Ki-67 proliferation index

Interpretation of IHC Markers
- ER and PR were interpreted according to ASCO/CAP guidelines, with ≥1% nuclear positivity considered positive.
- HER2/neu was scored as 0, 1+, 2+, or 3+ based on membrane staining intensity. Scores of 3+ were considered positive, while 2+ cases were considered equivocal.
- Ki-67 index was expressed as the percentage of positively stained tumor cell nuclei.

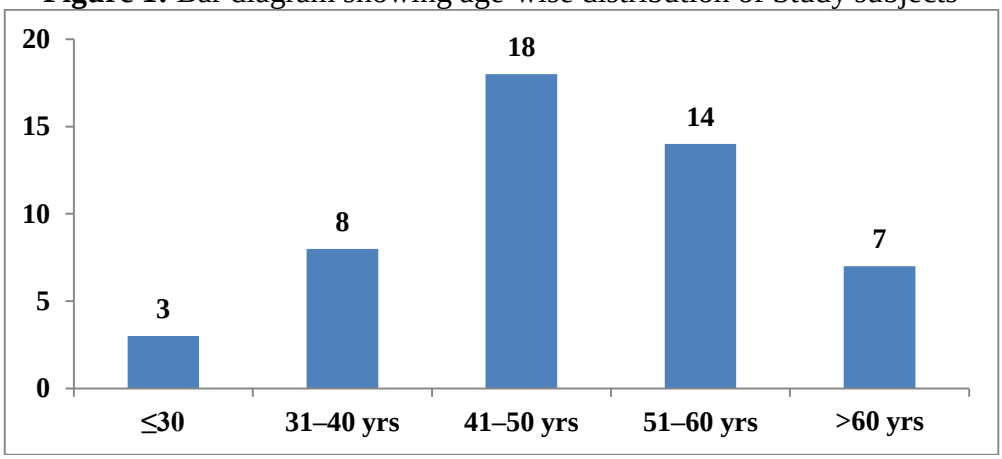
IHC-Based Molecular Subtyping
Based on IHC expression, breast carcinomas were classified into the following subtypes:
- Luminal A: ER and/or PR positive, HER2 negative, low Ki-67
- Luminal B: ER and/or PR positive with HER2 positivity or high Ki-67
- HER2-enriched: ER negative, PR negative, HER2 positive
- Triple-negative: ER negative, PR negative, HER2 negative

Statistical Analysis

Data were entered into Microsoft Excel and analyzed using SPSS software version 21 Associations between IHC subtypes, histological grade, and lymph node status were analyzed using Chi-square test and Fisher's exact test (where applicable). A p-value <0.05 was considered statistically significant.

Results

Figure 1: Bar diagram showing age-wise distribution of Study subjects



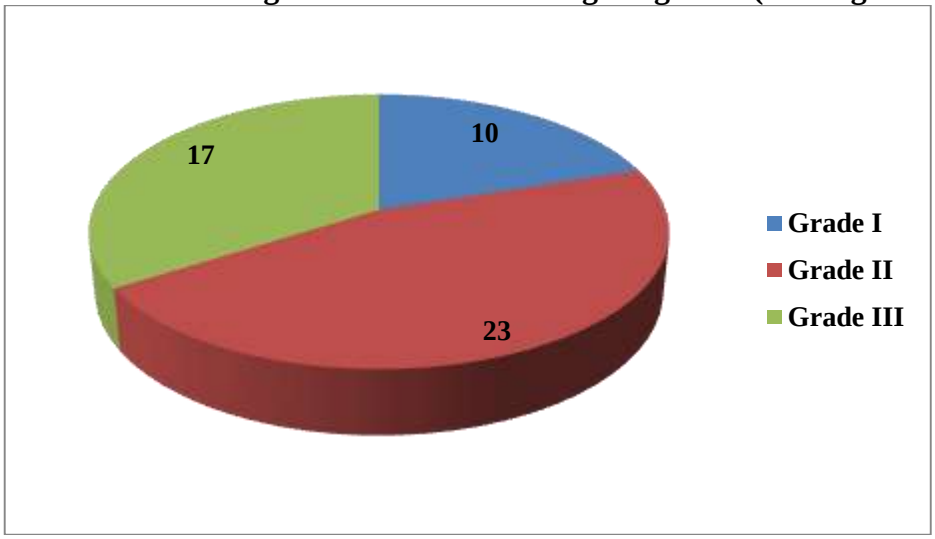
The age of patients ranged from 28 to 72 years, with a mean age of 48.6 ± 10.2 years. The majority of patients were in the 41–50 years age group (36%), followed by 51–60 years (28%).

Table 1: Histological Type of Breast Carcinoma

Histological type	Number of cases	Percentage (%)
Invasive ductal carcinoma (NOS)	43	86.0
Invasive lobular carcinoma	5	10.0
Others	2	4.0

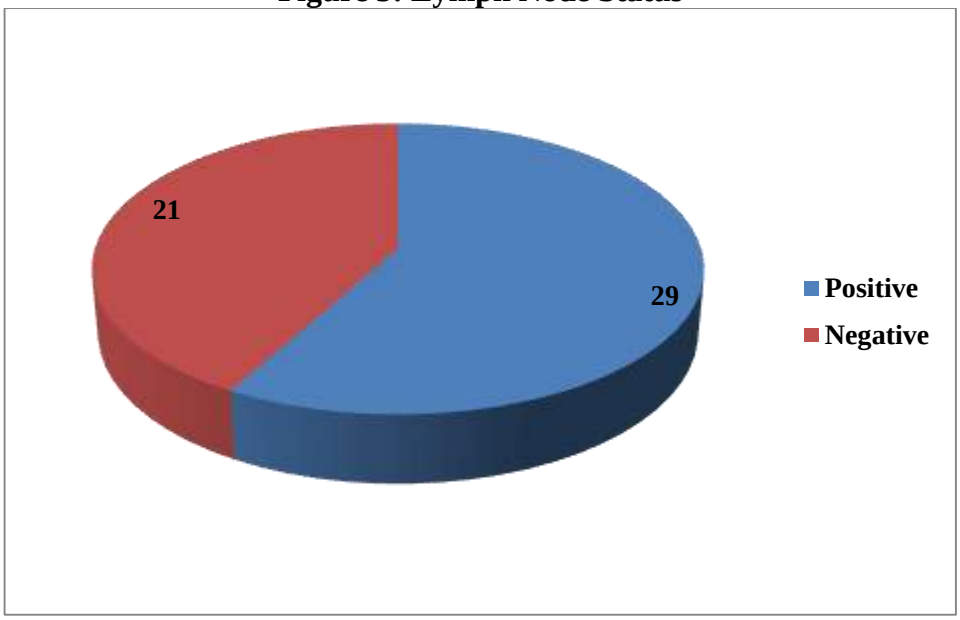
Invasive ductal carcinoma (IDC), not otherwise specified, was the most common histological type, accounting for **86%** of cases.

Figure 2: Pie chart showing distribution of histological grades (Nottingham Grade)



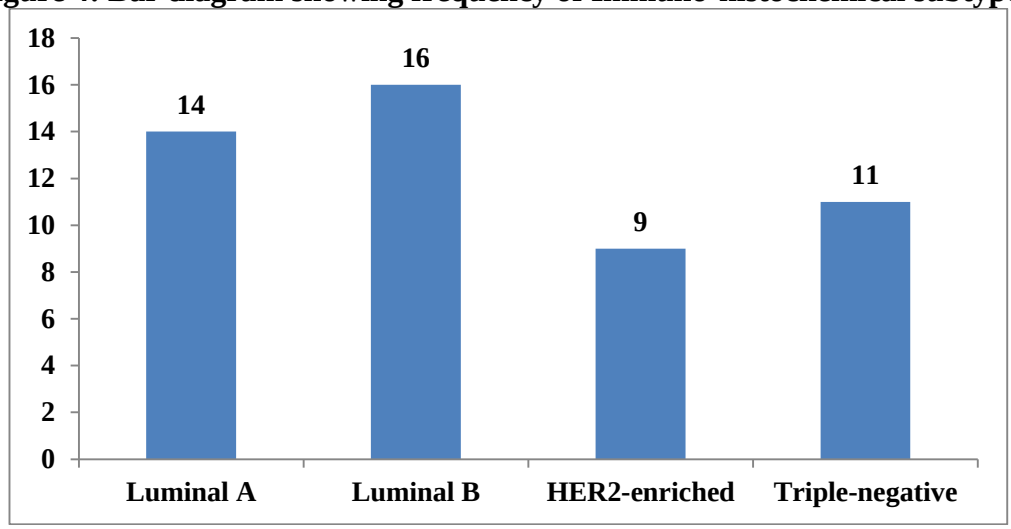
Most tumors were Grade II (46%), followed by Grade III (34%)

Figure 3: Lymph Node Status



Axillary lymph node metastasis was observed in 29 cases (58%), while 21 cases (42%) were node negative.

Figure 4: Bar diagram showing frequency of Immuno-histochemical subtypes.



Based on ER, PR, HER2, and Ki-67 expression, breast carcinomas were categorized into four IHC subtypes. Luminal B was the most common subtype (32%), followed by Luminal A (28%).

Table 2: Association between IHC Subtype and Histological Grade

IHC Subtype	Grade I	Grade II	Grade III	Total
Luminal A	7	6	1	14
Luminal B	2	9	5	16
HER2-enriched	1	3	5	9
Triple-negative	0	5	6	11

Luminal A tumors were predominantly Grade I and II, whereas HER2-enriched and triple-negative tumors showed a higher proportion of Grade III lesions. The association between IHC subtype and histological grade was statistically significant ($p < 0.05$).

Table 3: Association of IHC Subtype with Lymph Node Status

IHC Subtype	Node Positive	Node Negative	Total
Luminal A	6	8	14
Luminal B	9	7	16
HER2-enriched	7	2	9
Triple-negative	8	3	11

Lymph node positivity was more frequent in HER2-enriched (78%) and triple-negative (73%) subtypes compared to luminal subtypes. This association was found to be statistically significant ($p < 0.05$).

Discussion

In this study, invasive ductal carcinoma (IDC) constituted the predominant histological subtype, which is consistent with global and Indian literature reporting IDC as the most common breast malignancy. Histological grading using the Nottingham modification of the Bloom–Richardson system showed a higher proportion of Grade II and Grade III tumors, reflecting the tendency for patients in developing regions to present at a relatively advanced stage of disease. Studies done by Rakha EA et al in 2011 [3] and Elston CW et al in 1991 [4] have similarly documented a predominance of intermediate- to high-grade tumors at diagnosis, underscoring the need for robust prognostic stratification tools such as IHC.

Immuno-histochemical subtyping revealed luminal A and luminal B tumors as the most frequent molecular subtypes, followed by HER2-enriched and triple-negative breast carcinoma (TNBC). This distribution aligns with earlier Indian study done by Thakur et al in 2018 [9] and Asian study done by Ambroise M et al in 2011 [10] which report luminal subtypes as the most common, albeit with a relatively higher proportion of TNBC compared to Western populations. The predominance of luminal tumors suggests a significant proportion of patients may benefit from hormone-based therapies, emphasizing the therapeutic relevance of routine ER and PR testing.

A significant association was observed between histological grade and IHC subtype. Luminal A tumors were more frequently associated with lower histological grades, whereas HER2-enriched and triple-negative tumors showed a strong correlation with higher grades. These findings corroborate established evidence that hormone receptor–positive tumors tend to be better differentiated, while HER2-positive and triple-negative carcinomas are often poorly differentiated with aggressive histomorphological features consistent with findings of studies done by Goldhirsch et al in 2011 [11] and Rakha EA, Reis-Filho JS et al in 2010. [12] The high Ki-67 proliferation index observed predominantly in Grade III tumors further reinforces the link between increased proliferative activity and poor differentiation.

Lymph node metastasis was significantly more common in higher-grade tumors and in HER2-enriched and triple-negative subtypes. This observation is consistent with prior studies done by Prat et al in 2011 [13] and Carey et al in 2006 [14] demonstrating that aggressive molecular subtypes exhibit a higher propensity for nodal involvement and early dissemination. In contrast, luminal A tumors showed comparatively lower rates of lymph node positivity, supporting their relatively favorable biological behavior. The presence of nodal metastasis remains one of the most powerful prognostic

indicators in breast cancer, and its association with IHC subtypes highlights the importance of integrated pathological reporting.

The role of HER2/neu over expression identified in this study deserves particular attention. Tumors with HER2 3+ positivity showed higher histological grades and increased lymph node involvement, findings that align with the known aggressive nature of HER2-driven cancers. However, the identification of this subgroup is clinically crucial, as targeted anti-HER2 therapies have dramatically improved outcomes in this otherwise high-risk population as quoted by Slamon DJ et al in their study conducted in 2001. [15]

Triple-negative breast carcinoma constituted a notable subset in the present study and was characterized by high-grade morphology, elevated Ki-67 index, and frequent nodal metastasis. These findings are in agreement with existing literature describing TNBC as an aggressive entity with limited targeted treatment options and poorer prognosis.[16] The absence of ER, PR, and HER2 expression in these tumors underscores the diagnostic and prognostic value of IHC in identifying patients who may require more aggressive chemotherapy and closer follow-up.

Conclusion

The strong association of Immuno-histochemistry with histological grade and lymph node status highlights its importance in predicting tumor behavior and guiding individualized therapy. Routine integration of IHC markers is essential for optimal diagnosis, prognostication, and treatment planning in breast cancer patients.

Recommendations

- Immunohistochemistry should be performed routinely in all cases of breast carcinoma for accurate molecular subtyping.
- A standard IHC panel including ER, PR, HER2/neu, and Ki-67 should be incorporated into routine histopathology reporting.
- Treatment decisions should be guided by combined assessment of histological grade, lymph node status, and IHC profile.
- HER2-positive cases should undergo timely confirmation and be evaluated for targeted anti-HER2 therapy.

Limitations

- The study was conducted with a relatively small sample size, which may limit generalizability and also it was a single-center study, potentially introducing selection bias.
- Long-term follow-up and survival analysis were not included.
- Fluorescence in situ hybridization (FISH) confirmation for equivocal HER2 cases was not performed.

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