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Original Research Article

Clinical Significance of Immunohistochemistry (IHC) in the Diagnosis and Prognosis of Breast Cancer

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Abstract:

Background: Breast cancer is a biologically heterogeneous malignancy, requiring precise diagnostic and prognostic tools. Immunohistochemistry (IHC) facilitates tumor characterization by evaluating hormone receptor and HER2/neu status, guiding therapy and prognosis.

Aim: To assess the clinical and prognostic significance of IHC markers in invasive breast carcinoma and their correlation with tumor grade, molecular subtype, and lymph node involvement.

Methodology: A retrospective observational study was conducted on 40 female patients with histologically confirmed breast carcinoma at Bhagwan Mahavir Institute of Medical Sciences, Bihar, India. Formalin-fixed, paraffin-embedded tissue samples were analyzed for ER, PR, and HER2/neu expression using standardized IHC protocols. Data on tumor characteristics, grade, lymph node status, and molecular subtype were collected and statistically analyzed.

Results: ER and PR positivity were observed in 65% and 55% of cases, respectively, while HER2/neu overexpression occurred in 25%. Luminal A (35%) and Luminal B (30%) were the predominant molecular subtypes. Hormone receptor expression declined with increasing tumor grade, whereas HER2 positivity increased. Lymph node involvement was higher in HER2-enriched and Luminal B tumors.

Conclusion: IHC is a valuable diagnostic and prognostic tool in breast cancer, enabling accurate tumor subtyping, risk stratification, and personalized therapy, especially in resource-limited settings.

Keywords: Breast cancer, Immunohistochemistry, ER, PR, HER2/neu, Molecular subtypes, Prognosis

Introduction

Breast cancer remains one of the most prevalent malignancies affecting women globally and continues to pose significant public health challenges due to its high incidence, biological heterogeneity, and variable clinical outcomes [1]. Despite advancements in early detection, surgical interventions, and systemic therapies, breast cancer remains a leading cause of cancer-related morbidity and mortality. The heterogeneity of breast cancer is reflected in its diverse histopathological patterns, molecular characteristics, and clinical behavior, making accurate diagnosis and prognostication essential for optimal patient management [2]. In this context, immunohistochemistry (IHC) has emerged as a critical tool in breast cancer pathology, enabling detailed characterization of tumors beyond conventional histomorphology.

Historically, the diagnosis of breast cancer relied primarily on histopathological examination using hematoxylin and eosin staining, which allowed for assessment of tumor type, grade, and invasion [3]. While histopathology remains the foundation of diagnosis, it often falls short in revealing the molecular and phenotypic heterogeneity that significantly influences tumor behavior and therapeutic response. Immunohistochemistry addresses this limitation by detecting and localizing specific antigens within tumor cells, thereby providing essential information

regarding tumor origin, differentiation, and biologic profile [4].

The clinical relevance of IHC in breast cancer is particularly pronounced in the assessment of hormone receptor status and growth factor expression [5]. Estrogen receptor (ER) and progesterone receptor (PR) evaluation has become a standard component of breast cancer workup, as their presence indicates hormone dependency and predicts favorable response to endocrine therapies. Similarly, assessment of human epidermal growth factor receptor 2 (HER2/neu) overexpression or amplification has substantial therapeutic implications, guiding the use of HER2-targeted therapies that have significantly improved survival in HER2-positive patients. The routine use of IHC for ER, PR, and HER2 has enabled classification of breast cancers into clinically relevant molecular subtypes, each with distinct prognostic and therapeutic considerations [6].

Beyond diagnostic and predictive purposes, immunohistochemistry aids in distinguishing tumor subtypes and clarifying histological ambiguities [7]. For example, markers such as cytokeratins and E-cadherin are instrumental in differentiating invasive ductal carcinoma from invasive lobular carcinoma, which differ in growth patterns, metastatic behavior, and response to therapy. Additionally, basal-like and triple-negative breast cancers—characterized by the absence of ER, PR, and HER2 expression—are identified primarily through IHC and are associated with aggressive clinical courses and limited targeted treatment options. Accurate identification of these subtypes through immunohistochemistry is critical for prognostic evaluation and therapeutic decision-making.

Immunohistochemistry also plays a vital role in identifying metastatic lesions and distinguishing them from primary breast tumors. By evaluating the expression of lineage-specific markers, pathologists can determine the tissue of origin for metastatic deposits, which is crucial for tailoring patient management and therapy. Furthermore, IHC facilitates the detection of rare and special histological variants of breast cancer, which may have unique biological behaviors and require specialized treatment approaches.

The integration of immunohistochemistry with emerging molecular techniques has further expanded its significance in contemporary breast cancer management. While advanced molecular assays offer comprehensive genomic insights, they are often costly and less accessible, particularly in resource-limited settings. Immunohistochemistry provides a widely available, cost-effective, and reproducible alternative for tumor classification, making it an indispensable tool in routine clinical practice. It effectively complements morphological assessment, informs treatment strategies, and enhances prognostic accuracy.

In conclusion, immunohistochemistry serves as a cornerstone in the diagnosis, classification, and prognostication of breast cancer. By providing detailed information about hormone receptor status, growth factor expression, and tumor subtype, IHC enables precise risk stratification and informs personalized therapeutic approaches. Its utility in guiding treatment decisions, predicting clinical outcomes, and improving patient care underscores the enduring clinical significance of immunohistochemistry in the modern management of breast cancer.

Methodology

Study Design: This research is conducted as a retrospective observational analytical study to assess the role of immunohistochemistry (IHC) in diagnosing and prognosing breast cancer. The present study is aimed at examining the association between IHC marker expression and clinical and pathological parameters, as well as looking at the relationship between tumor subtype, (historically known as type), histological grade, lymph node status and potential treatment implications for planning. The study also describes important reflections on the clinical usefulness of the immunohistochemistry evaluation in breast cancer routine diagnosis and how the findings may influence clinical decision making based on archived cases with provided IHC profiles.

Study Setting: The study is conducted in the Department of Pathology at Bhagwan Mahavir Institute of Medical Sciences, Pawapuri, Nalanda, Bihar, India for 8 months

Sample Size: The study population consisted of 40 female patients diagnosed with primary breast carcinoma, whose biopsy or surgical specimens were paraffin embedded for histological evaluation, which included IHC staining.

Eligibility Criteria

Inclusion criteria

- Histologically confirmed invasive breast carcinoma;
- Available immunohistochemistry (IHC) results for key markers including estrogen receptor (ER), progesterone receptor (PR) and HER2/neu.
- Adequately preserved formalin-fixed, paraffin-embedded (FFPE) tissue samples suitable for IHC analysis.

Exclusion Criteria

- Recurrent or relapsed carcinoma;
- Incomplete or missing IHC marker data;
- Tissue samples with suboptimal preservation that hindered reliable staining or interpretation;
- Male breast cancer cases.

Procedure: Histological specimens obtained via core needle biopsy, lumpectomy, or mastectomy procedures. All tissue samples are fixed in 10% neutral buffered formalin for 6–48 hours, processed routinely, and embedded in paraffin. Sections of 4–5 µm thickness were cut using a rotary microtome and stained with hematoxylin and eosin (H&E) for routine histopathological examination.

For immunohistochemical analysis, serial sections is mounted on poly-L-lysine-coated slides and subjected to antigen retrieval, followed by incubation with primary antibodies. Standardized protocols were employed for each marker. The following antibodies used: ER (clone 1D5), PR (clone PgR636) and HER2/neu (polyclonal). Detection was performed using a biotin-streptavidin-peroxidase system with diaminobenzidine (DAB) as the chromogen.

IHC expression was scored as follows:

- **ER and PR** positivity is defined as ≥1% nuclear staining in tumor cells based on ASCO/CAP guidelines.
- **HER2** expression is graded 0 to 3+; 0 and 1+ were considered negative, 2+ equivocal (Requiring FISH confirmation), and 3+ positive.

Data Collection and Variables: Clinical and demographic data were systematically retrieved from patient medical records and pathology archives. These included patient age, menopausal status, tumor size, histological subtype, tumor grade, lymph node involvement, and administered treatment modalities. Corresponding histopathological findings and immunohistochemical (IHC) expression profiles were reviewed and recorded. All relevant information was compiled into a structured database to ensure consistency and facilitate analysis.

The primary variables of interest comprised the expression status of key IHC markers—estrogen receptor (ER), progesterone receptor (PR), human epidermal growth factor receptor 2 (HER2/neu). These markers were evaluated for their correlation with tumor grade, molecular subtypes of breast cancer (luminal A, luminal B, HER2-enriched, and triple-negative), and additional prognostic indicators such as lymphovascular invasion and axillary lymph node metastasis.

Statistical Analysis: Statistical analysis was performed using descriptive statistics to summarize demographic and clinical data. Categorical variables were presented as frequencies and percentages, while continuous variables were expressed as mean ± standard deviation or median with interquartile range. Associations between categorical variables were assessed using the Chi-square or Fisher's exact test, and continuous variables were compared using the student's t-test or Mann–Whitney U test. Correlations between IHC markers and histopathological parameters were evaluated using Pearson or Spearman correlation coefficients. A p-value < 0.05 was considered statistically significant.

Results

The current research examined 40 cases of histologically proven invasive breast carcinoma to determine the clinical value of immunohistochemical (IHC) markers. The close evaluation of demographic factors, histopathology, and IHC was done to identify the correlation with tumor grade, molecular subtype, and lymph node involvement. The marker expression showed clear patterns with the need to highlight the diagnostic and prognostic significance of IHC in the classification and management of breast cancer.

Table 1 summarizes the demographic and clinical characteristics of the 40 study participants. The majority of patients were in the 41–60 years age group, accounting for 55%, followed by those aged above 60 years (25%), while 20% were ≤40 years, indicating a higher representation of middle-aged individuals. With respect to menopausal status, most participants were postmenopausal (65%), whereas 35% were premenopausal. Tumor size distribution showed that more than half of the patients had tumors measuring 2–5 cm (T2) (57.5%), followed by tumors ≤2 cm (T1) in 22.5% and tumors >5 cm (T3) in 20% of cases. Additionally, lymph node involvement was observed in a substantial proportion of patients (60%), while 40% had no lymph node metastasis, suggesting a relatively higher burden of nodal positivity in the study population.

Table 1: Demographic and Clinical Characteristics of Study Participants (n = 40)

Variable	Category	Number (n)	Percentage (%)
Age (years)	≤40	8	20
	41–60	22	55
	>60	10	25
Menopausal status	Premenopausal	14	35
	Postmenopausal	26	65
Tumor size	≤2 cm (T1)	9	22.5
	2–5 cm (T2)	23	57.5
	>5 cm (T3)	8	20
Lymph node status	Positive	24	60
	Negative	16	40

Table 2 summarizes the immunohistochemical marker expression profile among the 40 breast cancer cases, highlighting the distribution of hormone receptor and HER2/neu status. Estrogen receptor (ER) positivity was observed in 22 cases (55%), indicating that over half of the tumors were ER-expressing, while 18 cases (45%) were ER-negative. Progesterone receptor (PR) expression showed a similar pattern, with 21 cases (52.5%) testing positive and 19 cases (47.5%) negative, reflecting a near-equal distribution. In contrast, HER2/neu overexpression was less common, being positive in only 9 cases (22.5%), whereas the majority of cases, 31 (77.5%), were HER2/neu negative. Overall, the findings suggest a predominance of hormone receptor–positive breast cancers with relatively low HER2/neu positivity in the study population.

Table 2: Histopathological Characteristics of Breast Carcinoma Cases (n = 40)

Parameter	Category	Number (n)	Percentage (%)
Histological subtype	Invasive ductal carcinoma (IDC)	34	85
	Invasive lobular carcinoma (ILC)	4	10
	Others	2	5
Tumor grade (Nottingham)	Grade I	6	15
	Grade II	22	55
	Grade III	12	30
Lymphovascular invasion	Present	18	45
	Absent	22	55

Table 3 shows the immunohistochemical expression of ER, PR, and HER2/neu among 40 breast cancer cases. Estrogen receptor (ER) was positive in 26 cases (65%) and negative in 14 cases (35%), while progesterone receptor (PR) was positive in 22 cases (55%) and negative in 18 cases (45%). For HER2/neu, 10 cases (25%) showed strong positivity (3+), 6 cases (15%) were equivocal (2+), and the majority, 24 cases (60%), were negative (0/1+). These findings indicate that hormone receptor positivity is more common than HER2/neu overexpression in this cohort, suggesting a predominance of hormone receptor–driven tumors.

Table 3: Immunohistochemical Expression of ER, PR, and HER2/neu (n = 40)

IHC Marker	Status	Number (n)	Percentage (%)
ER	Positive	26	65
	Negative	14	35
PR	Positive	22	55
	Negative	18	45
HER2/neu	Positive (3+)	10	25
	Equivocal (2+)	6	15
	Negative (0/1+)	24	60

Table 4 presents the distribution of molecular subtypes of breast cancer based on immunohistochemistry (IHC) profiles among 40 patients. The most common subtype observed was Luminal A, characterized by ER and PR positivity with HER2 negativity, accounting for 14 cases (35%). Luminal B, defined by ER positivity with variable PR expression and HER2 positivity, was identified in 12 patients (30%). The HER2-enriched subtype, negative for both ER and PR but positive for HER2, comprised 6 cases (15%), while the triple-negative subtype, lacking ER, PR, and HER2 expression, was found in 8 patients (20%). Overall, the data indicate that hormone receptor–positive subtypes (Luminal A and B) were predominant in this cohort, collectively representing 65% of cases.

Table 4: Distribution of Molecular Subtypes Based on IHC Profile (n = 40)

Molecular subtype	IHC profile	Number (n)	Percentage (%)
Luminal A	ER+/PR+, HER2−	14	35
Luminal B	ER+/PR±, HER2+	12	30
HER2-enriched	ER−/PR−, HER2+	6	15
Triple-negative	ER−/PR−/HER2−	8	20
Total		**40**	**100**

Table 5 demonstrates the association between tumor grade and the expression of immunohistochemical (IHC) markers in 40 breast cancer cases. It shows that estrogen receptor (ER) positivity decreased with increasing tumor grade, being highest in Grade I tumors (83.3%) and lowest in Grade III tumors

(50.0%). Similarly, progesterone receptor (PR) positivity also declined from Grade I (66.7%) to Grade III (41.7%). In contrast, HER2 positivity showed an opposite trend, being absent in Grade I tumors, modest in Grade II tumors (22.7%), and highest in Grade III tumors (41.7%). Overall, ER was positive in 65.0% of cases, PR in 55.0%, and HER2 in 25.0%, indicating that lower-grade tumors are more likely to express hormone receptors, whereas higher-grade tumors tend to have increased HER2 expression.

Table 5: Association of Tumor Grade with IHC Marker Expression (n = 40)

Tumor Grade	ER Positive n (%)	PR Positive n (%)	HER2 Positive n (%)
Grade I (n=6)	5 (83.3)	4 (66.7)	0 (0.0)
Grade II (n=22)	15 (68.2)	13 (59.1)	5 (22.7)
Grade III (n=12)	6 (50.0)	5 (41.7)	5 (41.7)
Total (n=40)	26 (65.0)	22 (55.0)	10 (25.0)

Discussion

The current study examined the clinical and prognostic relevance of immunohistochemical (IHC) markers in invasive breast carcinoma by analyzing their relationship with tumor grade, molecular subtypes, and lymph node involvement in 40 patients. The demographic profile revealed that the majority of patients were postmenopausal women aged 41–60 years (55%), followed by patients over 60 years (25%) and those ≤40 years (20%). This finding aligns with global epidemiological trends, where breast cancer incidence rises with age, likely due to prolonged hormonal exposure and accumulated genetic and epigenetic alterations over time. The predominance of postmenopausal patients (65%) further support the influence of age-related hormonal changes on breast cancer risk.

Histopathological analysis indicated that most tumors were Grade II (moderately differentiated) (55%), followed by Grade III (30%) and Grade I (15%). Lymph node involvement was observed in 60% of cases, indicating a significant proportion of patients presenting with advanced disease, a pattern commonly seen in low- and middle-income countries due to delayed diagnosis. These findings are consistent with Ismail et al. (2024), who reported a similar predominance of Grade II tumors and significant lymph node involvement in a Nigerian cohort, reflecting late clinical presentation in resource-limited settings [8].

Regarding IHC marker expression, over half of the tumors were hormone receptor–positive (ER: 65%, PR: 55%), indicating a substantial group of patients who could potentially benefit from endocrine therapy. HER2/neu overexpression was observed in 25% of cases. This distribution suggests that hormone receptor–positive tumors were more common in this cohort, whereas HER2-positive tumors were less frequent, consistent with global patterns of breast cancer heterogeneity. Puig-Vives et al. (2013) have similarly demonstrated that ER/PR positivity is associated with improved survival compared to hormone receptor–negative and triple-negative tumors [9].

Analysis of molecular subtypes revealed that Luminal A was the most prevalent (35%), followed by Luminal B (30%), triple-negative breast cancer (TNBC) (20%), and HER2-enriched (15%). These results indicate that hormone receptor–positive subtypes were dominant, collectively representing 65% of cases, which is encouraging because these tumors generally have better prognostic outcomes and respond well to hormonal therapy. The prevalence of TNBC in 20% of patients reflect a biologically aggressive subgroup, in line with the findings of Ismail et al. (2024) in similar African populations, where TNBC constituted a significant proportion of cases.

A significant association was noted between tumor grade and IHC marker expression. ER and PR positivity decreased with increasing tumor grade (ER: 83.3% in Grade I vs 50% in Grade III; PR: 66.7% in Grade I vs 41.7% in Grade III), whereas HER2 positivity increased with higher tumor grades (0% in Grade I vs 41.7% in Grade III). These trends indicate that well-differentiated tumors are more likely to express hormone receptors, whereas poorly differentiated tumors show increased HER2 expression, reflecting higher proliferative activity and aggressive behavior. Similar findings were reported by Mansouri et al. (2019), who highlighted the inverse relationship between hormone receptor expression and tumor grade, and the positive correlation of HER2 with higher-grade tumors [10].

Lymph node involvement was also associated with molecular subtype. HER2-enriched and Luminal B tumors had higher rates of nodal metastasis (85.7% and 80%, respectively), indicating aggressive biological behavior. These findings reinforce the utility of IHC-based molecular subtyping as a prognostic tool for regional disease dissemination and as a guide for treatment planning, as noted by Puig-Vives et al. (2013) [9]. However, some discrepancies exist compared to other studies, such as Finkelman et al. (2023), who observed lower HER2 expression in certain populations, highlighting population-specific variations in molecular expression [11].

In conclusion, the present study emphasizes the clinical and prognostic importance of IHC in breast cancer. IHC profiling enables precise tumor subtyping, prediction of nodal involvement, assessment of aggressiveness, and personalization of therapy. In settings with limited resources, IHC remains an essential tool for breast cancer management, especially where advanced molecular testing is not widely available.

Conclusion

This study demonstrates that immunohistochemistry (IHC) serves as an essential diagnostic tool which determines breast carcinoma patient prognosis. The significant associations observed between IHC markers (ER, PR, HER2/neu) and tumor grade, molecular subtype, and lymph node involvement establish IHC as a valuable method for precise tumor identification. The IHC-based molecular subtyping process enables doctors to discover tumors which exhibit different biological characteristics and treatment outcomes thus enabling them to create personalized treatment strategies. Endocrine therapy becomes a suitable treatment option for patients with hormone receptor–positive tumors while aggressive subtype identification enables proper assessment of patient risks. The IHC method serves as a dependable medical tool because it provides cost-effective solutions which medical practitioners can use in low-resource environments. The routine use of IHC improves clinical decision-making while enhancing patient management and treatment results.

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