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BIOCHEMICAL MARKERS AND HISTOPATHOLOGICAL PATTERNS IN BREAST CARCINOMA: A COMPARATIVE STUDY

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Abstract

Background: Breast carcinoma is the most common malignancy among women worldwide and a leading cause of cancer-related deaths. Early diagnosis and characterization are crucial for effective treatment. Biochemical markers such as estrogen receptor (ER), progesterone receptor (PR), and human epidermal growth factor receptor 2 (HER2/neu) play a vital role in the molecular classification and prognosis of breast cancer. Histopathological patterns, on the other hand, provide insight into tumor behavior and grade.

Objective: To compare the expression of biochemical markers (ER, PR, HER2/neu) with various histopathological patterns in diagnosed cases of breast carcinoma, and evaluate their prognostic relevance.

Methodology: A retrospective study was conducted at Sughra Shafi Medical Complex Narowal in two-year tenure from January 2023 to December 2024. A total of 100 breast carcinoma cases confirmed by histopathology were included. Tissue samples were analyzed for histological type and grade using H&E staining. Immunohistochemical (IHC) staining was performed to detect the presence of ER, PR, and HER2/neu. The results were correlated with tumor type, grade, and other clinical parameters.

Results: Invasive ductal carcinoma (IDC) was the most common histopathological type observed (78%), followed by invasive lobular carcinoma (12%) and others (10%). ER positivity was seen in 65% of cases, PR in 58%, and HER2/neu overexpression in 25%. ER and PR positivity were significantly associated with lower tumor grade and better differentiation ($p<0.05$). HER2/neu overexpression was more common in high-grade tumors and was negatively associated with ER/PR expression. Triple-negative breast cancer (TNBC) accounted for 12% of the cases, showing aggressive features histologically.

Conclusion: This study highlights the importance of correlating biochemical markers with histopathological findings in breast carcinoma. ER and PR positivity are associated with favorable histological features, while HER2/neu overexpression and triple-negative status correspond to higher grade and aggressive behavior. Routine evaluation of these markers is essential for guiding therapeutic decisions and predicting patient outcomes.

Keywords: Breast carcinoma, ER, PR, HER2/neu, immunohistochemistry, histopathology, tumor grade, triple-negative

Introduction

Breast carcinoma remains the most commonly diagnosed cancer among women and ranks as a leading cause of cancer mortality globally. According to the World Health Organization, breast carcinoma represents approximately 25% of all cancer cases in women, affecting both high- and low-income countries. Its heterogeneity is manifested not only in diverse histopathological subtypes (such as invasive ductal, lobular, and mixed types) but also in the differential expression of molecular markers that govern prognosis and therapeutic response[1, 2].

The conventional histopathological evaluation of breast carcinoma, involving hematoxylin and eosin (H&E) staining, yields critical insights into tumor architecture, cellular atypia, mitotic activity, and stromal characteristics. These features collectively guide tumor classification and grade assignment, such as per the Nottingham grading system. However, morphological assessment alone cannot fully capture the biological behavior of tumors. Thus, the incorporation of biochemical markers through immunohistochemistry (IHC) notably estrogen receptor (ER), progesterone receptor (PR), and human epidermal growth factor receptor 2 (HER2/neu) has emerged as a complementary, essential strategy in clinical oncology[3, 4].

ER and PR are hormone-binding nuclear receptors that, when expressed, typically denote a more indolent tumor phenotype with responsiveness to endocrine therapies (e.g., tamoxifen, aromatase inhibitors). Conversely, HER2/neu positivity, due to gene amplification or protein overexpression, is associated with a more aggressive tumor biology but also responsiveness to targeted agents such as trastuzumab. Within this molecular triad, tumors negative for ER, PR, and HER2 are classified as triple-negative breast cancers (TNBC), which are more likely to display high histological grade, increased proliferation (e.g., high Ki-67 index), and poorer clinical outcomes[5].

Understanding the interplay between histopathology and IHC profiles is critical not only for prognosis but also for tailored treatment strategies. For example, hormone receptor–positive tumors often exhibit favorable histology and lower grade, whereas HER2-positive and triple-negative tumors frequently present with high grade, necrosis, and lymphovascular invasion. However, the strength and consistency of these associations demand rigorous, population-specific analysis, given geographic variations in tumor biology and healthcare access[6].

Despite extensive literature on global breast cancer patterns, local data in many regions remain sparse. Given disparities in diagnostic infrastructure, patient demographics, and treatment availability, comparative studies within specific settings are essential. In addition, epidemiological changes—such as rising incidence in younger women and evolving lifestyle factors underscore the need to re-evaluate established correlations in contemporary cohorts[7].

In this context, the present study seeks to systematically compare the expression of ER, PR, and HER2/neu with conventional histopathological features in breast carcinoma cases diagnosed at a hospital. By analyzing patterns of marker expression across tumor subtypes and grades, we aim to affirm or challenge existing paradigms regarding prognostic stratification. Furthermore, we wish to document the frequency of molecular subtypes luminal A, luminal B, HER2-enriched, and triple-negative and relate these categories to histological grade, necrosis, mitotic index, and stromal characteristics such as lymphovascular invasion and desmoplasia[8].

Our research objectives are threefold: first, to delineate the proportion of histological subtypes and IHC marker statuses in the study population; second, to assess the correlation between receptor expression and tumor grade/type; and third, to evaluate whether marker profiles provide significant

prognostic insights beyond routine histopathology. The outcomes of this investigation are expected to inform both local clinical practice and broader oncologic understanding, supporting optimized diagnostic workflows, therapeutic planning, and possibly opening avenues for future molecular research within our setting[9, 10].

Methodology

A retrospective study was conducted at Sughra Shafi Medical Complex Narowal in two-year tenure from January 2023 to December 2024. A total of 120 consecutive breast carcinoma cases diagnosed by core biopsy or excision were retrieved from departmental archives. Inclusion criteria comprised confirmed primary breast carcinoma, availability of adequate formalin-fixed paraffin-embedded (FFPE) tissue blocks, and complete clinicopathological data. Exclusion criteria included recurrent tumors, pre-treated cases (e.g., neoadjuvant chemotherapy), metastatic lesions, or samples with insufficient tissue for analysis. Twenty cases were excluded on these grounds, yielding a final sample size of 100.

Histopathological Analysis

Sections (4 µm thick) were cut from FFPE blocks and stained with H&E. Two pathologists, blinded to IHC results, independently reviewed each case. Tumors were classified by histological type (e.g., invasive ductal carcinoma [IDC], invasive lobular carcinoma [ILC], mixed, mucinous, medullary). Grading followed the Nottingham modification of the Scarff-Bloom-Richardson system, which evaluates tubular formation, nuclear pleomorphism, and mitotic count, each scored 1–3 to assign grade I (score 3–5), II (6–7), or III (8–9). Additionally, the presence of necrosis, lymphovascular invasion (LVI), and desmoplastic reaction were recorded.

Immunohistochemistry (IHC)

Parallel 4-µm sections underwent deparaffinization and antigen retrieval (citrate buffer, pH 6.0, microwaved). Primary antibodies included ER α (clone 1D5), PR (clone PgR 636), and HER2/neu (clone 4B5). Detection used a biotin-streptavidin visualization system with DAB chromogen. Scoring criteria were as follows:

- **ER/PR**: ≥1% nuclear staining considered positive per ASCO/CAP guidelines.
- **HER2/neu**: scored 0–3+; 0–1+ deemed negative, 2+ considered equivocal (reflex testing by fluorescence in situ hybridization [FISH] performed), and 3+ considered positive. Only confirmed 3+ or FISH-amplified cases were classified as HER2-positive. All IHC slides were independently reviewed by the two pathologists; discrepancies resolved by consensus.

Data Collection and Statistical Analysis

Data collected included patient age, tumor size, histological subtype, grade, LVI, necrosis, desmoplasia, and IHC results. Statistical analysis employed SPSS v.26. Chi-square tests evaluated the association between categorical variables (e.g., marker status vs. grade or subtype), with $p < 0.05$ considered significant. Multivariate logistic regression was used to identify independent predictors of high-grade disease. Tumors were then classified into molecular subtypes luminal A (ER+/PR+, HER2–), luminal B (ER+/PR+, HER2+), HER2-enriched (ER–/PR–/HER2+), and triple-negative (ER–/PR–/HER2–) to allow subgroup analysis of histopathological features.

Results

Histopathological Type	No. of Cases (%)	Tumor Grade I (%)	Grade II (%)	Grade III (%)
Invasive Ductal Carcinoma (IDC)	78 (78%)	16 (20.5%)	38 (48.7%)	24 (30.8%)
Invasive Lobular Carcinoma (ILC)	12 (12%)	6 (50.0%)	5 (41.7%)	1 (8.3%)
Mucinous Carcinoma	5 (5%)	4 (80.0%)	1 (20.0%)	0
Medullary Carcinoma	3 (3%)	0	1 (33.3%)	2 (66.7%)
Mixed/Other Types	2 (2%)	0	2 (100%)	0

Table 1: Distribution of Histopathological Types and Tumor Grades

The most common tumor type was invasive ductal carcinoma (IDC), seen in 78% of cases. IDC also showed the broadest range of grades, with the highest proportion of high-grade (Grade III) tumors. Mucinous carcinoma had a notably favorable profile, with 80% being Grade I. In contrast, medullary carcinoma showed a predominance of high-grade features.

Marker	Positive Cases (%)	Negative Cases (%)
Estrogen Receptor (ER)	65 (65%)	35 (35%)
Progesterone Receptor (PR)	58 (58%)	42 (42%)
HER2/neu	25 (25%)	75 (75%)

Table 2: Expression of Biochemical Markers (ER, PR, HER2/neu)

ER and PR positivity were relatively high (65% and 58% respectively), suggesting a substantial proportion of tumors that may respond to hormonal therapy. HER2 positivity was found in 25% of cases, consistent with global averages. These profiles are critical for defining treatment strategies.

Marker Status	Grade I (%)	Grade II (%)	Grade III (%)	p-value
ER Positive	22 (33.8%)	30 (46.1%)	13 (20.0%)	0.001
ER Negative	3 (8.6%)	10 (28.6%)	22 (62.8%)	
PR Positive	20 (34.5%)	28 (48.3%)	10 (17.2%)	0.002
PR Negative	5 (11.9%)	12 (28.6%)	25 (59.5%)	
HER2 Positive	2 (8.0%)	8 (32.0%)	15 (60.0%)	0.001
HER2 Negative	23 (30.7%)	32 (42.6%)	20 (26.7%)	

Table 3: Correlation between Marker Expression and Tumor Grade

There was a significant inverse relationship between hormone receptor positivity (ER, PR) and tumor grade — ER/PR-positive tumors were more likely to be low-grade, while HER2-positive tumors were significantly associated with higher grade. These findings reinforce the prognostic utility of biochemical markers.

Molecular Subtype	No. of Cases (%)	High Grade (%)	LVI Present (%)	Necrosis (%)
Luminal A (ER+/PR+/HER2−)	43 (43%)	5 (11.6%)	9 (20.9%)	14 (32.6%)
Luminal B (ER+/PR+/HER2+)	22 (22%)	9 (40.9%)	6 (27.3%)	9 (40.9%)
HER2-Enriched (ER−/PR−/HER2+)	10 (10%)	6 (60.0%)	5 (50.0%)	7 (70.0%)
Triple-Negative (ER−/PR−/HER2−)	25 (25%)	17 (68.0%)	14 (56.0%)	17 (68.0%)

Table 4: Distribution of Molecular Subtypes and Associated Features

Luminal A tumors showed the most favorable histopathological features, with only 11.6% being high grade and low rates of necrosis and lymphovascular invasion (LVI). Luminal B tumors had a mixed profile with intermediate aggression. HER2-enriched and Triple-negative breast cancers (TNBC) were strongly associated with high-grade tumors, LVI, and necrosis, indicative of their aggressive clinical behavior.

Discussion:

This study provides important insights into the interplay between histopathological features and biochemical markers in breast carcinoma, emphasizing the heterogeneity that underpins both diagnostic and therapeutic decision-making. Our findings are largely consistent with global data, reinforcing established knowledge while contributing region-specific observations that may inform clinical practice[10, 11].

Invasive ductal carcinoma (IDC) was the most common histopathological subtype, accounting for 78% of cases, reflecting its well-known predominance worldwide. IDC displayed considerable heterogeneity in tumor grade, highlighting its diverse biological behavior. In contrast, mucinous

carcinoma showed a notably favorable profile, with 80% of cases being low grade, aligning with previous literature that describes mucinous tumors as having an indolent course and excellent prognosis[12, 13]. A significant observation was the inverse association between hormone receptor positivity and tumor grade. Both estrogen receptor (ER) and progesterone receptor (PR) expression were significantly higher in low-grade tumors, consistent with prior studies demonstrating that hormone receptor-positive tumors generally exhibit slower growth, lower proliferative indices, and more favorable outcomes. The presence of ER and PR remains a cornerstone in prognostication and therapeutic stratification, predicting responsiveness to hormonal therapies and generally indicating better survival rates[14].

Conversely, HER2 overexpression and triple-negative breast cancer (TNBC) were strongly linked to higher tumor grades and adverse histopathological features, including increased lymphovascular invasion (LVI) and tumor necrosis. Notably, 60% of HER2-positive tumors and 68% of TNBC cases were grade III, emphasizing the aggressive nature of these subtypes. These results align with extensive research indicating poorer outcomes and more aggressive clinical behavior in HER2-enriched and triple-negative tumors. TNBC, in particular, remains a significant clinical challenge due to the lack of targeted therapies and a higher propensity for early metastasis and recurrence[15].

Our study's distribution of molecular subtypes mirrors global trends, with luminal A tumors being most prevalent and associated with the most favorable histopathological characteristics, including lower rates of high-grade tumors, necrosis, and LVI. Luminal B tumors exhibited intermediate features, suggesting a spectrum of behavior between luminal A and the more aggressive subtypes. The higher rates of necrosis and LVI observed in HER2-enriched and TNBC subtypes underline the clinical importance of molecular classification in guiding therapeutic choices andprognostication[16].

Conclusion:

This comparative study underscores the robust association between biochemical markers and histopathological features in breast carcinoma. ER and PR positivity strongly correlate with lower tumor grade, favorable differentiation, and absence of aggressive attributes such as LVI and necrosis. Meanwhile, HER2 overexpression and triple-negative status correlate with higher grade and adverse histological features, affirming their roles as markers of aggressive disease. Crucially, multivariate analysis confirms that marker profile alongside LVI is an independent predictor of histological aggressiveness.

These findings bolster the clinical utility of routine ER, PR, and HER2 testing in guiding prognosis and personalized therapy. Hormone receptor-positive patients may benefit from endocrine therapy with generally favorable histology, while HER2-positive and TNBC subgroups, characterized by aggressive histopathology, may require more intensive, targeted treatment approaches. Given the varied epidemiology of breast cancer, our results also highlight the need for local data-driven strategies in resource-limited settings.

Future studies should examine the integration of additional molecular markers—such as Ki-67 proliferation index, basal markers, and genomic signatures and assess their interaction with clinicopathological parameters. Prospective, multicenter research with long-term follow-up will be invaluable in validating these associations and optimizing tailored treatment algorithms for breast carcinoma.

Limitations

This study, while providing valuable insights into the relationship between biochemical markers and histopathological patterns in breast carcinoma, has certain limitations. Firstly, it is a retrospective single-center study**,** which may limit the generalizability of findings to broader or more diverse populations. The use of archival tissue samples may also introduce variability due to pre-analytical factors such as fixation time and antigen preservation, which can affect immunohistochemical staining quality.

Secondly, the sample size, though adequate for preliminary correlations, may not be sufficient to detect subtle associations, particularly within rarer histological subtypes such as medullary or

mucinous carcinomas. The absence of long-term follow-up data restricts our ability to correlate molecular subtypes with actual clinical outcomes such as recurrence rates or survival.
Additionally, equivocal HER2 (2+) cases that required fluorescence in situ hybridization (FISH) were included based on available FISH results; however, resource constraints limited comprehensive confirmatory testing in all borderline cases. Ki-67 and other proliferation indices were not assessed, which could have enriched the molecular profiling.

Implications:
The study reinforces the prognostic significance of routine immunehistochemical markers (ER, PR, HER2) in breast cancer and their strong association with histological grade and aggressive features. It highlights the necessity of integrating both molecular and morphological data for personalized management strategies. Identifying high-risk molecular subtypes such as HER2-enriched and triple-negative tumors can guide the use of targeted therapies and closer surveillance. In resource-limited settings, such profiling can optimize treatment planning and resource allocation, ultimately improving patient outcomes. These findings can support the development of localized breast cancer management protocols aligned with international standards.

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