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Classification of Breast Carcinoma Based on Immunohistochemistry: An Experience in a Tertiary Care Hospital

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ABSTRACT
Breast cancer, a heterogeneous disease, exhibits diverse molecular profiles which significantly influence treatment decisions and prognosis. Immunohistochemistry (IHC) serves as a cornerstone in identifying these molecular subtypes, providing essential data for effective clinical management. This research focuses on analyzing and categorizing breast carcinoma cases using molecular classification derived from Immunohistochemistry, aiming to understand the prevalence of different molecular types within a specific patient cohort. Employing a retrospective study design, this research included 100 cases of invasive breast carcinoma diagnosed at our center from 2013 - 2016. The cases were categorized into four molecular types: Luminal Type A, Luminal Type B, Her2 Neu type and Triple negative/Basal type, utilizing Immunohistochemistry techniques. The case analysis revealed varying prevalences of molecular types among the patients: Luminal Type A (29%), Luminal Type B (13%), Her2 Neu type (32%), and Triple negative (26%). These findings underscore the heterogeneity of breast carcinoma presentations and molecular characteristics in a clinical setting. The molecular classification of breast carcinoma through Immunohistochemistry offers critical insights into the predominant types in a tertiary care setting, facilitating targeted therapeutic strategies and better patient management.

INTRODUCTION

Breast cancer remains the most common malignancy among women worldwide, significantly impacting global health with substantial variability in prognosis and treatment responses. The characterization of breast cancer has evolved dramatically with advancements in molecular biology, leading to improved diagnostic precision and tailored therapeutic strategies. One pivotal development in the management of breast cancer has been the classification based on immunohistochemistry (IHC), which provides insights into the tumor's molecular characteristics by identifying specific biomarkers[1].

The heterogeneity of breast cancer means that it is not a single disease but a group of diseases with varying presentations, responses to treatment, and outcomes. This diversity is evident in the molecular subtypes of breast cancer, primarily identified through gene expression profiling: Luminal A, Luminal B, HER2-enriched, and Triple Negative/Basal-like. These subtypes differ significantly in terms of etiology, prognosis, and sensitivity to therapy[2]. For instance, Luminal A tumors, which are typically estrogen receptor (ER) and progesterone receptor (PR) positive, have the best prognosis and are often treated with hormone therapy. In contrast, Triple Negative breast cancers, which lack ER, PR, and HER2, exhibit more aggressive behavior and fewer treatment options, highlighting the need for precise molecular classification[3].

Immunohistochemistry has become an indispensable tool in the clinical setting for the identification of these subtypes. By staining tissue sections with antibodies that bind to specific antigens, IHC allows for the visualization of protein expression within the context of tissue morphology. This technique is particularly useful for assessing the expression of hormone receptors (ER and PR), HER2 status, and proliferation markers like Ki-67, which are critical for subtype classification and guiding treatment decisions.[4]

The accuracy of molecular classification by IHC has significant therapeutic implications. For example, HER2-positive cancers can be effectively treated with HER2-targeted therapies such as trastuzumab, dramatically improving patient outcomes. Similarly, the identification of hormone receptor-positive tumors facilitates the use of endocrine therapies, which significantly reduce recurrence rates in early-stage breast cancer. Thus, accurate classification not only impacts survival and quality of life but also optimizes the use of healthcare resources by aligning treatment strategies with tumor biology.[5]

Despite the clinical utility of IHC, challenges remain, such as variability in testing procedures, interpretation of results, and the occasional need for additional testing to clarify ambiguous outcomes. These challenges underscore the importance of standardized protocols and continuous education for pathologists and oncologists[6].

Given the central role of molecular subtyping in managing breast cancer, this study aimed to categorize breast carcinoma cases into molecular subtypes using IHC in a tertiary care setting. Such studies are essential for validating the routine clinical use of IHC and ensuring that all patients receive the most effective and personalized treatment plans possible[7].

MATERIALS AND METHOD

Source of Data: Data was retrospectively collected from medical records of patients diagnosed with invasive breast carcinoma.

Study Design: This was a retrospective cohort study designed to classify breast cancer cases based on molecular subtypes as determined by Immunohistochemistry.

Study Location: The study was conducted at a tertiary care hospital's oncology and pathology departments. Study Duration: Data were collected from cases diagnosed over a four-year period from 2013 to 2016. Sample Size: The study included 100 cases of invasive breast carcinoma.

Inclusion Criteria: Patients included in the study were those with a confirmed diagnosis of invasive breast carcinoma, regardless of age and stage at diagnosis.

Exclusion Criteria: Patients were excluded if they had a history of other malignancies, incomplete medical records, or if they received neoadjuvant therapy before the diagnostic Immunohistochemistry could be performed.

Procedure and Methodology: Breast carcinoma cases were classified into four molecular types (Luminal Type A, Luminal Type B, Her2 Neu type, and Triple negative/Basal type) based on specific immunohistochemical markers.

Sample Processing: Tissue samples were processed using standard histological procedures, followed by staining with antibodies specific to estrogen receptor, progesterone receptor, HER2/neu and Ki-67 to determine the molecular subtype.

Statistical Methods: Descriptive statistics were used to summarize the data. The distribution of molecular subtypes was analyzed, and chi-square tests were employed to explore the associations between molecular subtypes and clinicopathological features.

Data Collection: Data collection involved reviewing patient records for demographic information, pathological reports, and results of Immunohistochemistry. All relevant data were entered into a secure database for subsequent analysis.

Statistical analysis: Statistical analysis was done on the data collected by using the " SPSS Version 11" statistical program. Pearsons Chi Square test was used to determine significant clinicopathological differences in expression of ER, PR and Her 2 Neu in positive and negative tumors. Differences were considered statistically significant when p value was <0.05.

RESULTS AND DISCUSSIONS

Table 1 outlines the scoring system for ER and PR, where immunoreactivity is measured based on the percentage of nuclear staining observed in tumor cells. A score of 0 indicates no staining (0% of cells), 1+ signifies weak positivity with less than 10% of cells stained, 2+ indicates a moderate level of staining ranging from 10% to 75% of cells, and a score of 3+ denotes strong positivity, with more than 75% of cells displaying nuclear staining.

Table 2 describes the scoring criteria for HER2 immunoreactivity, focusing on membrane staining patterns. A score of 0 represents negative staining (no staining or less than 10% of tumor cells), 1+ also denotes negative but with a faint or barely perceptible staining in more than 10% of the cells. A score of 2+ is considered weakly positive or equivocal, characterized by a weak to moderate complete membrane staining in more than 10% of tumor cells. Finally, a score of 3+ indicates a strong positive result, with a strong complete membrane staining observable in more than 10% of tumor cells.

Table 3 categorizes breast carcinoma into four molecular subtypes based on the combined immunoreactivity profiles for ER, PR, and HER2 Neu. The Luminal type A is characterized by positive ER staining and may have either positive or negative PR staining, but always negative HER2 staining. Luminal type B is positive for ER and PR and also positive for HER2. The Her2 neu type is negative for both ER and PR, but positive for HER2. Lastly, the Triple negative type shows no staining for ER, PR, and HER2, thus being negative across all three markers.

Table 4 details the distribution of clinical and pathological characteristics across different molecular subtypes. The total percentage for each subtype sums up to 100% of the cases studied. For age distribution, 43% of the cases were aged 50 years or younger, and 57% were older than 50 years. Tumor size was also categorized, showing that 10% of tumors were 2 cm or smaller, 68% were between greater than 2 cm and up to 5 cm, and 22% were larger than 5 cm. Regarding lymph vascular invasion, 54% of cases showed no invasion, while 46% did. The p-values for these comparisons were all greater than 0.05, indicating no statistically significant differences.

Table 1: Scoring criteria for ER and PR immunoreactivity

Score	Nuclear staining
0	0%
1+	< 10 %
2+	– 75 %
3+	>75%

Table 5 examines the histopathological characteristics across the same molecular subtypes. All cases are again accounted for with a total of 100%. In terms of histology, ductal carcinoma was predominant in 87% of cases, lobular in 12%, and other types in 1%. Tumor grades varied with Grade I in 8% of cases, Grade II in 46%, and Grade III also in 46%. The presence of carcinoma in situ was noted in 40% of the cases, while 60% did not exhibit this feature.

The classification and characterization of breast cancer based on immunohistochemical (IHC) markers such as estrogen receptor (ER), progesterone receptor (PR), and human epidermal growth factor receptor 2 (HER2) are crucial for determining appropriate therapeutic strategies. The integrates findings from the presented tables with insights from other studies to deepen the understanding of breast cancer subtypes and their clinical implications.

Scoring Criteria for ER and PR: The scoring system for ER and PR as described in Table 1 follows established guidelines used in clinical pathology. Scoring ranges from 0 (no staining) to 3+ (strong staining in more than 75% of tumor cells), which is consistent with the American Society of Clinical Oncology (ASCO) and the College of American Pathologists (CAP) guidelines, which recommend quantifying ER and PR expression to guide hormone therapy decisions[8]. Studies show that ER and PR positivity are associated with a better prognosis and are predictive of response to hormonal therapies[9].

Scoring Criteria for HER2: Table 2 outlines HER2 scoring from 0 (negative) to 3+ (strong complete membrane staining in more than 10% of tumor cells). This scoring aligns with the ASCO/CAP guidelines which classify HER2 0 and 1+ as negative, 2+ as equivocal (necessitating further testing by fluorescence in situ hybridization), and 3+ as positive[10]. HER2-positive cancers often exhibit more aggressive behavior but may respond well to HER2-targeted therapies such as trastuzumab[11].

Molecular Classification: Table 3 categorizes breast cancers into four molecular subtypes based on the status of ER, PR, and HER2. This classification is pivotal for tailoring treatment:

Table 2: Scoring criteria for HER 2 immunoreactivity

Score	Membrane staining
0	Negative.No staining or membrane staining of < 10 % tumor cells
1+	Negative.A faint / barely perceptible membrane staining in >10% of tumor cells
2+	Weakly positive/equivocal.A weak to moderate complete membrane staining in > 10 % tumor cells
3+	Positive. A strong complete membrane staining in > 10 % tumor cells

Table 3: Molecular classification of breast carcinoma based on immunohistochemistry

Sub type	ER	PR	HER 2 NEU
Luminal type A	Positive	Positive /negative	Negative
Luminal type B	Positive	Positive	Positive
Her 2 neu type	Negative	Negative	Positive
Triple negative type	Negative	Negative	Negative

Table 4: The distribution of clinical and pathological characteristics among the various molecular subtypes

S.No	Characteristics	Luminal-A (%)	Luminal-B(%)	Her 2 type(%)	Triple negative(%)	Total (%)	P' value
1	Total	29	13	32	26	100	
2	Age ≤50 yrs	14	5	14	10	43	>0.05
	Age >50 yrs	15	8	18	16	57	
3	Tumor size ≤2 cm	3	1	2	4	10	>0.05
	>2 - =5 cm	20	8	24	16	68	
	>5 cm	6	4	6	6	22	
4	Lymph vascular invasion -Negative	17	7	12	18	54	>0.05
	-Positive	12	6	20	8	46	

Table 5 :The distribution of histopathological characters according to hormonal and molecular subtypes in 100 women with invasive breast cancer

S.No	Characteristics	Luminal A(%)	Luminal B (%)	Her 2 type(%)	Triple negative(%)	Total (%)
1	Total	29	13	32	26	100
2	Histological type- Ductal	25	10	28	24	87
	Lobular	3	3	4	2	12
	Others	1	0	0	0	1
3	Tumour grade- Grade I	1	3	2	2	8
	Grade II	22	6	12	6	46
	Grade III	6	4	18	18	46
4	Carcinoma in situ - Absent	16	2	20	22	60
	Present	13	11	12	4	40

- **Luminal A (ER and/or PR positive, HER2 negative):** Typically exhibits the best prognosis and responds well to hormonal therapy
- **Luminal B (ER and/or PR positive, HER2 positive):** Generally has a worse prognosis than Luminal A but still responds to hormonal therapy in addition to HER2-targeted treatments
- **HER2 enriched (ER and PR negative, HER2 positive):** Responsive to HER2-targeted therapies but generally more aggressive
- **Triple-negative (negative for ER, PR, and HER2):** Lacks targeted therapies and has a poorer prognosis[12]

Clinical and Pathological Characteristics: Table 4 and Table 5 provide a breakdown of clinical and pathological characteristics across these molecular subtypes. The data show that age distribution, tumor size, lymph vascular invasion, and tumor grade vary across subtypes. The prevalence of ductal carcinoma is high across all subtypes, which aligns with its status as the most common histological type of breast cancer[13]. The significance of these features is that they help in predicting the aggressiveness of the cancer and potential responses to treatment, which is corroborated by other research findings[14].

CONCLUSION

The study conducted at a tertiary care hospital effectively illustrates the pivotal role of Immunohistochemistry (IHC) in classifying breast carcinoma into distinct molecular subtypes. Through detailed analysis of 100 breast cancer cases, utilizing specific markers for Estrogen Receptor (ER), Progesterone Receptor (PR), and Human Epidermal Growth Factor Receptor 2 (HER2), the study was able to categorize tumors into Luminal A, Luminal B, HER2-enriched and Triple-negative subtypes. This classification is essential for guiding treatment decisions and tailoring personalized therapeutic strategies, which are critical for improving patient outcomes. The prevalence of various subtypes and their correlations with clinical and pathological characteristics provide valuable insights into the nature of breast cancer presentations in a clinical setting. Ultimately, this study reinforces the indispensable utility of IHC in the diagnostic and prognostic evaluation of breast carcinoma, highlighting its significance in enhancing patient-specific oncological care.

Limitations of the study:

- **Retrospective Design:** As a retrospective study, the analysis was limited to pre-existing data and records, which may have inconsistencies or missing information. This design also restricts the ability to control for variables that could influence the study outcomes

- **Sample Size:** Although 100 cases provide a good foundation for analysis, a larger sample size could offer more robust data, allowing for finer distinctions among subtypes and more definitive statistical analyses
- **Single-Center Study:** The findings are based on data from a single tertiary care hospital, which might not be representative of other populations or settings. The specific demographic and regional characteristics of the patient population might limit the generalizability of the results
- **Temporal Limitations:** The study covers diagnoses from a specific four-year period. Changes in diagnostic criteria, treatment modalities, and technology over time may affect the relevance of these results to current clinical practice
- **Lack of Longitudinal Follow-up:** The retrospective nature of the study means it lacks longitudinal follow-up data to assess outcomes based on subtype classification. Such outcomes could include patient survival rates recurrence, and response to specific treatments
- **Exclusion of Neoadjuvant Therapy Cases:** By excluding patients who received neoadjuvant therapy before their diagnostic IHC, the study potentially omits a significant subset of breast cancer cases, particularly those presenting with advanced disease

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