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Immunohistochemical Surrogate Molecular Subtyping of Breast Carcinoma and Its Clinicopathological Correlation: A Cross-Sectional Study

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ABSTRACT

Background and aim: Breast carcinoma is a biologically heterogeneous malignancy with distinct molecular subtypes that differ in prognosis and therapeutic response. Immunohistochemistry (IHC)-based surrogate molecular classification using estrogen receptor (ER), progesterone receptor (PR), HER2, and Ki-67 provides a practical alternative to gene expression profiling. This study evaluated the distribution of molecular subtypes and their association with clinicopathological parameters.

Material and methods: A retrospective hospital-based cross-sectional study was conducted on 108 histopathologically confirmed cases of primary breast carcinoma diagnosed between August 2022 and July 2024 at a tertiary care center in Vadodara, Gujarat. Tumors were graded using the modified Scarff-Bloom-Richardson (Nottingham) grading system. Immunohistochemistry for ER, PR, HER2, Ki-67, and CK5/6 was performed according to established guidelines. Molecular subtype analysis was performed in 97 cases after excluding 11 HER2-equivocal cases without confirmatory fluorescence in situ hybridization.

Results: Invasive carcinoma of no special type was the predominant histological subtype, and Grade II tumors were the most common. Luminal B was the predominant molecular subtype, followed by triple-negative breast cancer, Luminal A, and HER2-enriched tumors. Molecular subtype showed significant associations with histological subtype, ER, PR, HER2, and Ki-67 expression, but not with age, tumor size, histological grade, lymphovascular invasion, perineural invasion, or lymph node status.

Conclusions: Luminal B was the predominant molecular subtype in this cohort. IHC-based surrogate molecular classification is a practical approach for routine breast cancer subtyping and provides clinically relevant prognostic stratification in resource-limited settings. Larger multicenter studies with long-term follow-up are needed to validate these findings.

1. Introduction

Breast cancer is the most frequently diagnosed malignancy and one of the leading causes of cancer-related mortality among women worldwide. According to Globocan 2022 estimates, approximately 2.3 million new cases and 670,000 deaths were reported globally, accounting for nearly 11.6% of all newly diagnosed cancers. The incidence of breast cancer continues to rise, particularly in low- and middle-income countries, posing a substantial public health challenge owing to delayed diagnosis and disparities in access to healthcare services.[1] Breast carcinoma is a biologically heterogeneous disease that exhibits considerable variation in histopathological characteristics, molecular alterations, therapeutic response, and clinical outcomes. Conventional prognostic factors, including tumor size, lymph node status, histological grade, lymphovascular invasion, and hormone receptor status, remain fundamental in clinical practice. However, advances in molecular pathology have significantly improved the understanding of breast cancer biology and enabled more individualized therapeutic strategies.[2] Gene expression profiling has identified intrinsic molecular subtypes of breast cancer, including Luminal A, Luminal B, HER2-enriched, and Basal-like tumors. Although molecular assays provide comprehensive biological characterization, their routine clinical application remains limited in many developing countries because of high cost and limited availability. Consequently, immunohistochemistry (IHC)-based surrogate molecular classification using estrogen receptor (ER), progesterone receptor (PR), HER2, and Ki-67 has become the most practical and widely accepted alternative for routine diagnostic practice.[3] Luminal A tumors are typically hormone receptor-positive, HER2-negative, and exhibit low proliferative

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activity, resulting in favorable clinical outcomes. In contrast, Luminal B tumors demonstrate higher proliferative indices and are associated with more aggressive biological behavior and poorer prognosis than Luminal A tumors. Depending on HER2 expression, Luminal B tumors may be categorized as HER2-positive or HER2-negative. The St. Gallen International Expert Consensus recommended a Ki-67 cut-off value of 14% for distinguishing Luminal A from Luminal B tumors in routine clinical practice.[4, 5] HER2-enriched breast carcinomas are characterized by HER2 overexpression or gene amplification and are associated with aggressive clinical behavior; however, the introduction of HER2-targeted therapy has substantially improved patient outcomes.[6] Triple-negative breast cancer (TNBC), defined by the absence of ER, PR, and HER2 expression, represents a biologically aggressive subtype associated with early recurrence, visceral metastasis, and limited targeted therapeutic options. Basal-like tumors, frequently identified by the expression of basal cytokeratins such as CK5/6, constitute a major subgroup of TNBC and generally exhibit an unfavorable prognosis.[7, 8] The distribution of molecular subtypes varies considerably across different populations because of ethnic, geographic, environmental, and socioeconomic factors. Compared with Western populations, Indian patients with breast carcinoma frequently present at a younger age, with larger tumor size, higher histological grade, and a greater proportion of biologically aggressive molecular subtypes. Despite the increasing burden of breast cancer in India, comprehensive data regarding the distribution of IHC-based molecular subtypes and their clinicopathological associations remain limited, particularly from Western India. Therefore, the present study was undertaken to evaluate the histomorphological spectrum of breast carcinoma, determine the distribution of molecular subtypes using immunohistochemical surrogate markers (ER, PR, HER2, and Ki-67), and analyze their association with clinicopathological characteristics among patients diagnosed at a tertiary care center in Vadodara, Gujarat.

2. Material and methods

Study design and setting

This retrospective hospital-based cross-sectional study was conducted in the Department of Pathology, Government Medical College, Vadodara, Gujarat, India, with Institutional Ethics Committee approval (Approval No. IECBHR/261-2023). Consecutive eligible cases diagnosed between August 2022 and July 2024 were included to minimize selection bias.

Study population

The study included 108 consecutive female patients with histopathologically confirmed primary breast carcinoma. Cases were retrieved from the departmental histopathology records and included trucut biopsies, lumpectomy specimens, simple mastectomy specimens, and modified radical mastectomy specimens received during the study period.

Inclusion criteria

- Histopathologically proven cases of primary breast carcinoma
- Adequate tissue is available for histopathological examination and immunohistochemistry.
- Only treatment-naïve patients with primary breast carcinoma were included in the study.

Exclusion criteria

- Poorly preserved tissue samples
- Benign breast lesions
- Biopsy specimens lacking malignant cells
- Patients who had received neoadjuvant chemotherapy prior to tissue sampling were excluded because treatment may alter histomorphological features and biomarker expression.

Clinical and histopathological evaluation

Relevant clinical data, including age, laterality, tumor size, lymph node status, and other gross findings, were obtained from the requisition forms accompanying the specimens. Tissue samples were fixed in 10% neutral buffered formalin, routinely processed, embedded in paraffin, and sectioned at 3–4 μm thickness. Sections were stained with hematoxylin and eosin (H&E) and examined microscopically. All cases were evaluated for histological type, tumor grade, tumor necrosis, lymphovascular invasion, perineural invasion, and lymph node involvement. Histopathological evaluation was performed according to the WHO Classification of Breast Tumours.[9] Tumor grading was carried out using the modified Scarff-Bloom-Richardson (Nottingham) grading system. (Table 1)

Table 1. Modified bloom richardson scoring.

SR. NO.	Features	Subcategory	Score
1.	Tumor tubule or gland formation	> 75% of tumor	1
		10 - 75% of tumor	2
		< 10% of tumor	3
2.	Nuclear pleomorphism	Minimal variation in nuclear size and shape	1
		Moderate variation in nuclear size and shape	2
		Marked variation in nuclear size and shape	3
3.	Mitotic count (per 10 high-power fields)	0 to 9/10 hpf	1
		10-19/10 hpf	2
		More than equal to 20/10 hpf	3
	Total Score	Grade I (well differentiated)	3-5
		Grade II (moderately differentiated)	6 or 7
		Grade III (poorly differentiated)	8 or 9

Immunohistochemistry

Tissue processing

Representative tumor blocks were selected for immunohistochemical analysis. Formalin-fixed paraffin-embedded tissue sections (2–3 μm thick)

were stained using antibodies against estrogen receptor (ER), progesterone receptor (PR), HER2, Ki-67, and CK5/6 according to the manufacturer's instructions. Appropriate positive and negative controls were included with each staining batch. Immunoreactivity was assessed using established international guidelines.

Deparaffinization and rehydration

Sections were deparaffinized in xylene and rehydrated through graded isopropyl alcohol (99%, 90%, 70%) followed by washing in running tap water. Slides were maintained in phosphate-buffered saline (PBS, pH 7.4) and were not allowed to dry at any stage.

Antigen retrieval

Heat-induced epitope retrieval (HIER) was performed using Tris-EDTA buffer (pH 9.0 for nuclear markers and pH 8.0–9.0 for cytoplasmic/membranous markers) in a microwave oven. Slides were heated at 900 W for 20 minutes, then at 540 W for 20 minutes, and allowed to cool to room temperature for approximately 1 hour before washing in PBS.

Immunostaining procedure

Endogenous peroxidase activity was blocked using 3% hydrogen peroxide for 10 minutes. After PBS washing, sections were incubated with a protein-blocking reagent for 10 minutes. Primary antibodies were applied and incubated 1 hour for cytoplasmic/membranous markers and 1.5 hours for nuclear markers) in a humid chamber. Following PBS washes, sections were treated sequentially with super enhancer (20 minutes) and polymer-HRP (30 minutes). Visualization was achieved using freshly prepared diaminobenzidine (DAB) chromogen for 10 minutes. Slides were then rinsed in distilled water, counterstained with hematoxylin, dehydrated through graded alcohol, cleared in xylene, and mounted with DPX.

Quality control

Internal quality control was ensured by including appropriate positive and negative control tissues in each staining batch. All staining procedures were performed according to the manufacturer's recommendations under standardized laboratory conditions. Immunohistochemical analysis was performed on representative tumor blocks using the following antibodies: estrogen receptor (ER, Biogenex, clone EP1), progesterone receptor (PR, Path in situ, clone EP2), HER2/neu (Path in situ, clone EP3), Ki-67 (Biogenex, clone MKI67/2462), and CK5/6 (Biogenex, clones EP67 & EP24). Appropriate positive and negative controls were included with each batch. ER and PR positivity were identified as nuclear staining, HER2/neu positivity as membranous staining, Ki-67 as nuclear staining, and CK5/6 as cytoplasmic staining.

Interpretation of IHC results

ER and PR expression were evaluated using the Allred scoring system,[11] which combines proportion and intensity scores to yield a total score ranging from 0 to 8. Scores ⩾3 were considered positive. Interpretation of ER and PR expression was performed in accordance with the ASCO/CAP recommendations. Tumors demonstrating nuclear staining in at least 1% of invasive tumor cells were considered hormone receptor-positive. (Tables 2 and 3)

Table 2. Allred system proportion score.

Score for Proportion of Positive Nuclei	Percentage of Stained Tumor Cell Nuclei
0	No staining
1	<1%
2	1-10%
3	11-33%
4	34-66%
5	67-100%

Table 3. Allred system intensity score.

Score for Intensity	Intensity of Staining
0	No staining
1	Weak staining
2	Moderate staining
3	Strong staining

HER2 immunohistochemical interpretation was performed according to the ASCO/CAP 2018 guidelines. HER2/neu expression was scored as 0, 1+, 2+, or 3+ in accordance with the American Society of Clinical Oncology/College of American Pathologists (ASCO/CAP) guidelines. Scores of 3+ were regarded as positive, while scores of 0 and 1+ were considered negative. Cases demonstrating equivocal (2+) HER2/neu expression on immunohistochemistry were recommended for confirmation by fluorescence in situ hybridization (FISH), in accordance with ASCO/CAP guidelines.[12] However, due to insufficient follow-up and the unavailability of FISH results, these cases were excluded from the final molecular subtype analysis. The Ki-67 labeling index was calculated as the percentage of positively stained invasive tumor cell nuclei. A cut-off value of 14% was used to distinguish low and high proliferative activity according to the St. Gallen International Expert Consensus (2011), allowing comparison with previously published studies.

Molecular subtyping

Breast carcinomas were classified into molecular subtypes based on immunohistochemical surrogate markers as follows[13]:

- Luminal A: ER and/or PR positive, HER2/neu negative, Ki-67 <14%.
- Luminal B: ER and/or PR positive with either HER2/neu positivity (any Ki-67) or HER2/neu negativity with Ki-67 ⩾14%.
- HER2-enriched: ER negative, PR negative, HER2/neu positive.
- Triple-negative: ER negative, PR negative, HER2/neu negative.

Data were entered into Microsoft Excel and analyzed using IBM SPSS Statistics version 26.0 (IBM Corp., Armonk, NY, USA). Continuous variables were expressed as mean ± standard deviation, whereas categorical variables were summarized as frequencies and percentages. Associations between categorical variables were evaluated using the Chi-square test or Fisher's exact test whenever the expected cell count was less than five. Molecular subtype analysis was performed using complete-case analysis after excluding cases with equivocal HER2 (2+) status lacking confirmatory FISH

results. All statistical tests were two-tailed, and a p-value <0.05 was considered statistically significant. Since no reliable regional estimate of molecular subtype prevalence was available, an anticipated prevalence (p) of 50% was assumed to obtain the maximum sample size for this cross-sectional study. Assuming a 50% prevalence of hormone receptor positivity, with a 95% confidence level (Z = 1.96) and 10% absolute precision, the minimum required sample size was 96 cases. The present study included 108 cases, thereby exceeding the calculated sample size.

4. Results

A total of 108 cases of breast cancer were recorded during the study period from August 2022 to July 2024. Out of 108 cases, 73 cases were received as biopsy, 31 cases as Simple Mastectomy/ Modified radical mastectomy, and 4 cases as lumpectomy specimens. All 108 cases were found in females. The age of patients ranged from the second decade to the eighth decade. In this study, the majority of cases (31.49%) were aged 51-60 years. The mean age of presentation was 55 years. (Fig. 1) Of 108 cases, 59 (55%) involved the left breast. The remaining 49 cases (45%) involved the right breast. Tumor size was applicable in 35 cases, of which most (16 cases, i.e., 45.71%) were>5 cm (T3). 105 cases were diagnosed histologically as Invasive Ductal Carcinoma. Only 2 cases were of Mucinous Carcinoma, and 1 case was of Invasive Lobular carcinoma histologically. (Table 4)

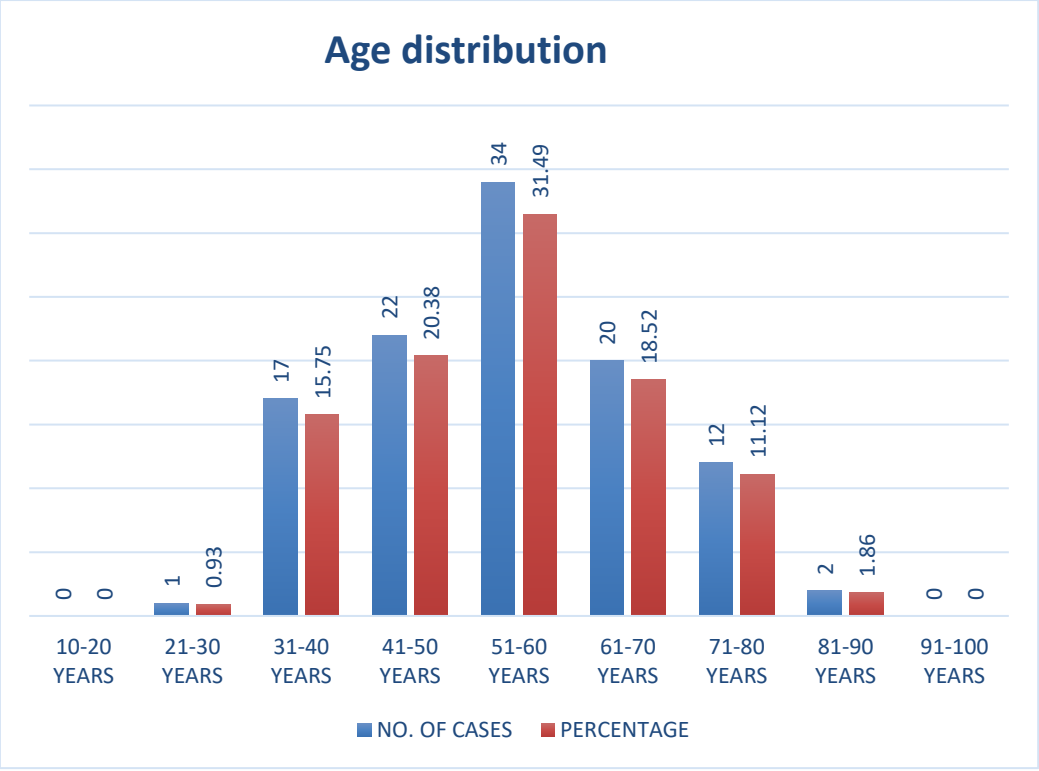

Fig. 1. Age-wise Distribution.

Of 108 cases, 59 (55%) involved the left breast. The remaining 49 cases (45%) involved the right breast. Tumor size was applicable in 35 cases, of which most (16 cases, i.e., 45.71%) were>5 cm (T3). 105 cases were diagnosed histologically as Invasive Ductal Carcinoma. Only 2 cases were of Mucinous Carcinoma, and 1 case was of Invasive Lobular carcinoma histologically. (Table 4 and Fig. 2) ER positivity was observed in 54 (50%) cases, showing characteristic nuclear staining (Figure 3). PR positivity was observed in 39 (36.11%) cases and demonstrated nuclear immunoreactivity in tumour cells (Figure 4). HER2 overexpression (3+) was identified in 36 (33.33%) cases and showed complete circumferential membranous staining (Figure 5). A high proliferative index (Ki-67 ≥14%) was observed in 83 of 108 cases (76.85%), suggesting aggressive tumour biology in a substantial proportion of patients (Figure 6). Representative CK5/6 immunostaining in a basal-like (triple-negative) breast carcinoma is shown in Fig. 7.

Table 4. Clinicopathological profile of breast carcinoma cases.

Clinopathological Findings	
Site-wise Distribution	No of cases
Right	49(45%)
Left	59(55%)
Tumour size	
≤ 2 cm.	6 (17.14%)
>2cm but ≤5 cm	13 (37.14%)
>5cm	16 (45.71%)
Histological Subtype	

Invasive Ductal Carcinoma	105 (97.23%)
Invasive Lobular Carcinoma	1 (0.93%)
Mucinous Carcinoma	2 (1.86%)
Lymphovascular Invasion	
Present	7 (24.14%)
Absent	22 (75.86%)
Perineural Invasion	
Present	3 (10.35%)
Absent	26 (89.65%)
Lymph Node Metastasis	
Present	12 (41.38%)
Absent	17 (58.62%)
Grade	
I	20 (18 %)
II	47 (44 %)
III	41 (38 %)
ER Status	
Positive	54 (50%)
Negative	54 (50%)
PR Status	
Positive	39 (36.11%)
Negative	69 (63.89%)
Her2	
Positive	36 (33.33%)
Negative	61 (56.48%)
Equivocal	11 (10.18%)
Ki 67 Status:	
<14%	25 (23.15%)
≥14%	83 (76.85%)
Total No of cases	108

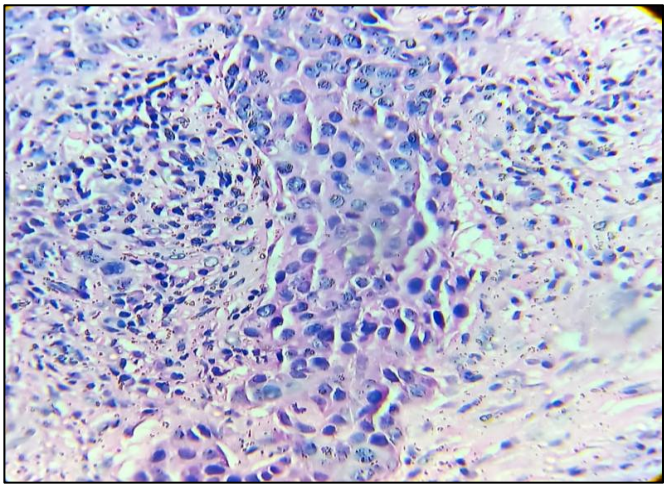

Fig. 2. Photomicrograph of invasive ductal carcinoma (40X, H&E stain).

Lymphovascular invasion was assessable in 29 cases, of which 7 cases (24.1%) showed invasion. Perineural invasion was present in 3 of 29 cases (10.3%). Lymph node metastasis was identified in 12 of 29 modified radical mastectomy specimens (41.4%; 95% CI: 23.5%–59.3%). Lymph node status was not applicable in biopsy and lumpectomy specimens due to the nature of sampling. Regarding tumor grade, Grade II tumors were the most common, comprising 44% of cases (95% CI: 35.1%–53.7%), followed by Grade III tumors at 38% (95% CI: 29.1%–47.1%) and Grade I tumors at 18%. A high proliferative index (Ki-67 ≥14%) was observed in 83 of 108 cases (76.85%; 95% CI: 68.9%–84.8%), suggesting aggressive tumor biology in a substantial proportion of patients. Molecular subtyping was performed in 97 cases with complete immunohistochemical data. Luminal B was the most common molecular subtype, observed in 34 cases (35.05%; 95% CI: 25.6%–44.6%), followed by triple-negative breast cancer in 30 cases (30.93%; 95% CI: 21.7%–40.1%). HER2-enriched subtype was identified in 24 cases (24.74%; 95% CI: 16.1%–33.4%), while Luminal A constituted 9 cases (9.28%; 95% CI: 3.5%–15.1%). Among Luminal B tumors, 22 cases (64.71%) were HER2-negative, and 12 cases (35.29%) were HER2-positive. (Table 5)

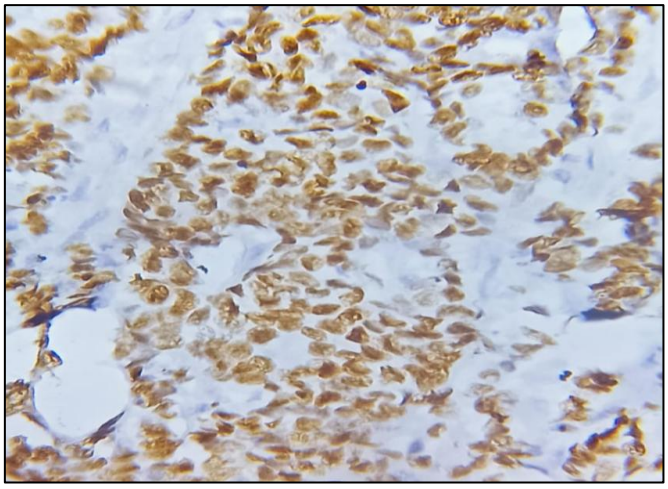

Fig. 3. Photomicrograph of ER nuclear positivity (40X).

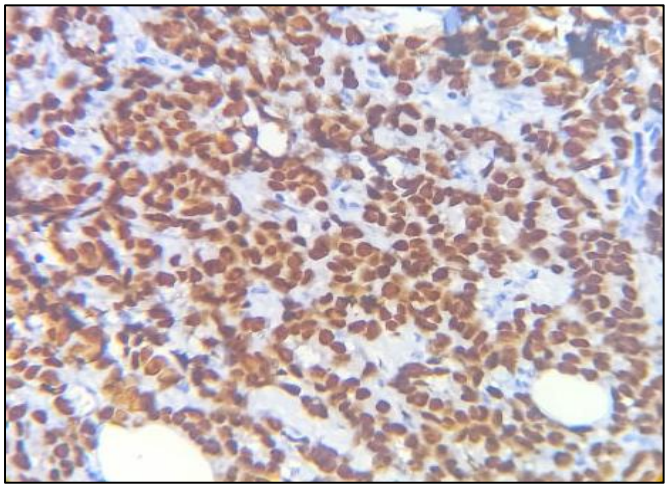

Fig. 4. Photomicrograph of PR nuclear positivity (40X).

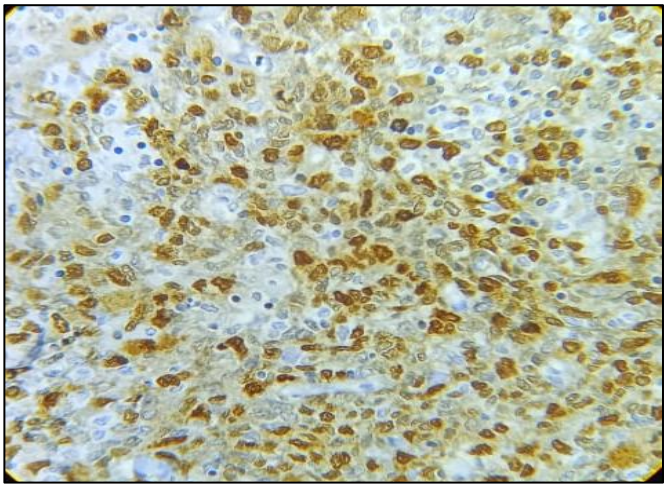

Fig. 6. Photomicrograph of Ki67 85%.

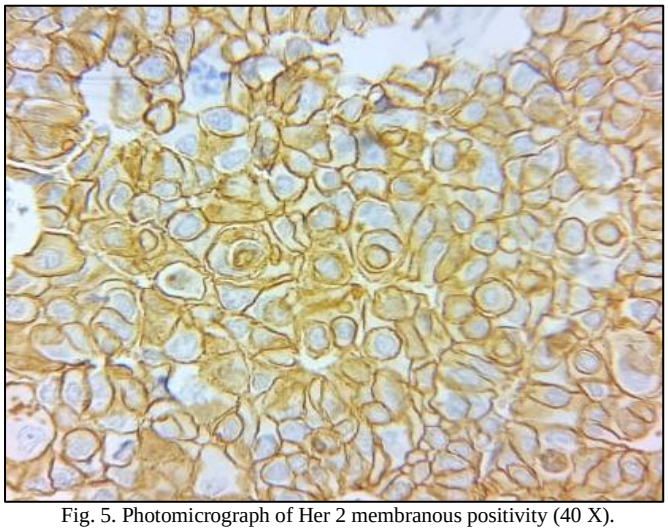

Fig. 5. Photomicrograph of Her 2 membranous positivity (40 X).

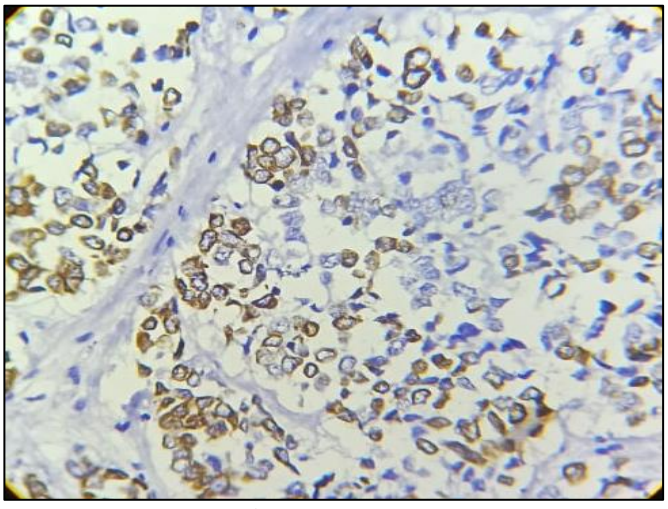

Fig. 7. Photomicrograph of CK 5/6 Positivity (40 X)- Basal-like subtype.

Table 5. Comparison of Clinicopathological features of different molecular subtypes.

Characteristics	Luminal A	Luminal B	Her2 Enriched	Triple Negative	χ^2 (df)	P-value
Age						
≤50 years	0(0%)	11(32.35%)	10(41.67%)	14(46.67%)	7.07 (3)	0.070
>50 years	9(100%)	23(67.65%)	14(58.33%)	16(53.33%)		
Mean age	64.89±9.51	56.88±13.35	55.04±11.84	52.53±14.04		
Tumour size						
≤2 cm	1(16.67%)	1 (8.33%)	3(42.86%)	0(0%)	9.55 (6)	
>2 to ≤5 cm	4(66.66%)	4 (33.33%)	1(14.28%)	4(66.67%)		0.186
>5 cm	1(16.67%)	7 (58.33%)	3(42.86%)	2(33.33%)		
Mean Tumour Size	3.60±1.38cm	5.74±3.10cm	3.79±3.07cm	5.00±3.15cm		
Tumour Grade						
Grade I	5(55.56%)	8(23.53%)	5(20.83%)	2(6.67%)	11.83 (6)	0.080
Grade II	3(33.33%)	15(44.12%)	9(37.5%)	13(43.33%)		
Grade III	1(11.11%)	11(32.35%)	10(41.67%)	15(50%)		
Lymphovascular Invasion						
Present	1(25%)	2(18.18%)	2(28.57%)	1(25%)	0.28 (3)	1.000
Absent	3(75%)	9(81.82%)	5(71.43%)	3(75%)		
Perineural Invasion						
Present					3.37 (3)	0.323

Absent	0(0%)	0(0%)	1(14.29%)	1(25%)		
	4(100%)	11(100%)	6(85.71%)	3(75%)		
Lymph Node Involvement					5.62 (3)	0.144
Present	2(50%)	7(63.64%)	2(28.57%)	0(0%)		
Absent	2(50%)	4(36.36%)	5(71.43%)	4(100%)		
Histological Type						
Invasive Ductal Carcinoma	7(77.78%)	33(97.06%)	24(100%)	30(100%)		
Invasive Lobular Carcinoma	0(0%)	1(2.94%)	0(0%)	0(0%)	21.802 (6)	0.008
Mucinous Carcinoma	2(22.22%)	0(0%)	0(0%)	0(0%)		
ER						
Positive	9(100%)	34(100%)	0(0%)	0(0%)	89.49 (3)	<0.001
Negative	0(0%)	0(0%)	24(100%)	30(100%)		
PR						
Positive	9(100%)	20(58.82%)	0(0%)	0(0%)	61.29 (3)	<0.001
Negative	0(0%)	14(41.18%)	24(100%)	30(100%)		
HER 2						
Positive	0(0%)	12(35.29%)	24(100%)	0(0%)	63.73 (3)	<0.001
Negative	9(100%)	22(64.71%)	0(0%)	30(100%)		
Ki67					30.67 (3)	
<14%	9(100%)	5(14.71%)	7(29.17%)	4(13.33%)		<0.001
≥14%	0(0%)	29(85.29%)	17(70.83%)	26(86.67%)		
Total cases N= 97	9(9.28%)	34(35.05%)	24(24.74%)	30 (30.93%)		

11 cases were HER2 equivocal in IHC, so these cases were excluded from molecular subtyping due to the unavailability of FISH testing. No statistically significant association was found between age (p= 0.070), tumor size (p= 0.186), tumour grade (p= 0.080), lymphovascular invasion (p= 1.000), perineural invasion (p= 0.323), and lymph node involvement (p= 0.144) and the different molecular subtypes. Statistically significant distributions were observed among Histological types (p-value = 0.008), ER status (p-value <0.001), PR status (p-value <0.001), HER2 status (p-value <0.001), and Ki-67 (p-value <0.001) across different molecular subtypes.

4. Discussion

Breast carcinoma is a biologically heterogeneous disease with distinct histopathological and molecular characteristics that influence prognosis and therapeutic decision-making. In the present study, invasive carcinoma of no special type (NST) was the predominant histological subtype, Grade II was the most frequent histological grade, and Luminal B emerged as the most common molecular subtype, followed by triple-negative breast cancer (TNBC). Significant associations were observed between molecular and histological subtypes, ER, PR, HER2, and Ki-67 expression. In contrast, no significant associations were observed with patient age, tumor size, histological grade, lymphovascular invasion, perineural invasion, or lymph node status. These findings emphasize the value of routine immunohistochemical surrogate molecular classification in improving the pathological characterization of breast carcinoma and guiding individualized management. The mean age of patients in the present study was 55 years, with most cases occurring in the fifth and sixth decades of life. Similar age distributions have been reported in previous studies,[13–15] while a younger peak age at presentation was observed in another study.[16] Such differences may reflect variations in demographic characteristics, referral patterns, healthcare accessibility, and regional epidemiological factors. Consistent with previous Indian studies, invasive carcinoma NST was the predominant histological subtype.[13–18] Grade II tumors were also the most common, consistent with previously published findings.[13, 14, 17, 19] Although higher histological grade is generally associated with aggressive tumor biology, no significant association between histological grade and molecular subtype was observed in the present study, suggesting that histological grade and molecular classification provide complementary rather than interchangeable prognostic information. The immunohistochemical profile observed in the present study was comparable to that reported in previous Indian studies. Hormone receptor positivity remains one of the most important prognostic and predictive biomarkers in breast carcinoma because it identifies patients who are likely to benefit from endocrine therapy. HER2 overexpression is associated with a more aggressive biological phenotype but also predicts response to HER2-targeted therapy, whereas Ki-67 reflects tumor proliferative activity and contributes to surrogate molecular classification. Similar distributions of ER, PR, HER2, and Ki-67 expression have been reported in previous studies.[13–15, 19] Minor differences among studies may be explained by variations in patient characteristics, tumor stage at presentation, antibody clones, laboratory protocols, and interpretation criteria, particularly for Ki-67 assessment, which remains one of the least standardized immunohistochemical biomarkers. These findings reinforce the importance of standardized IHC evaluation for accurate molecular classification and appropriate therapeutic stratification. Luminal B was the predominant molecular subtype in the present study, followed by triple-negative breast cancer, Luminal A, and HER2-enriched carcinoma. Comparable findings have been reported in previous studies,[14, 15] whereas another study identified Luminal A as the most frequent subtype.[13] Such variation among studies may reflect differences in patient demographics, referral bias, tumor stage at diagnosis, and the Ki-67 cut-off used for surrogate molecular classification. The relatively high proportion of Luminal B and triple-negative breast cancers

in our cohort suggests a greater burden of biologically aggressive disease within the study population. Furthermore, molecular subtypes demonstrated significant associations with histological subtype and ER, PR, HER2, and Ki-67 expression, confirming the biological validity of surrogate molecular classification. In contrast, no significant association was identified between molecular subtype and age, tumor size, histological grade, lymphovascular invasion, perineural invasion, or lymph node involvement. Similar observations have been reported in several previous studies, although inconsistent results across the literature suggest that these relationships may vary with sample size, patient selection, and population characteristics. Collectively, these findings support the routine use of immunohistochemical surrogate molecular classification as an integral component of breast cancer evaluation because it provides clinically relevant prognostic information and facilitates individualized treatment planning. The present study has several strengths. It provides contemporary data on the distribution of immunohistochemical surrogate molecular subtypes of breast carcinoma in Western India, a region with limited published evidence. In addition, the simultaneous evaluation of histomorphological features alongside ER, PR, HER2, Ki-67, and CK5/6 expression enabled a comprehensive clinicopathological assessment. Nevertheless, several limitations should be acknowledged. This was a single-center study with a relatively small sample size, which may limit the generalizability of the findings. Furthermore, 11 HER2-equivocal cases were excluded from molecular subtype analysis because confirmatory fluorescence in situ hybridization (FISH) was unavailable. The absence of treatment response, recurrence, and long-term survival data also precluded assessment of the prognostic impact of molecular subtypes. Therefore, larger, multicenter, prospective studies incorporating standardized biomarker assessment, confirmatory HER2 testing, and long-term clinical outcomes are warranted to further validate these findings and better define the clinicopathological significance of surrogate molecular subtypes in breast carcinoma.

5. Conclusion

In conclusion, immunohistochemical surrogate molecular classification is a practical and reliable approach for categorizing breast carcinoma in routine pathology practice. In the present study, Luminal B was the predominant molecular subtype and showed significant associations with histological subtype, ER, PR, HER2, and Ki-67 expression. These findings highlight the value of integrating molecular subtyping with histopathological evaluation to improve prognostic stratification and support individualized treatment decisions, particularly in resource-limited settings. Further multicenter studies with larger cohorts are needed to validate these observations.

Conflict of Interest

The authors declared that there is no conflict of interest.

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