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Molecular Subtypes of Breast Cancer: Deciphering their links to tumor aggression and spread

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Abstract

Breast cancer is a heterogeneous disorder in both molecular alterations and clinical behavior. Several factors such as histological grade, type and size of tumor, lymph node metastasis, estrogen receptor (ER), progesterone receptor (PR) and human epidermal growth factor receptor 2 (HER2/neu), influence the prognosis and response to the treatment of cancer.

This study was conducted in a tertiary care hospital, Bengaluru from 2020 to 2023. 180 cases of invasive breast carcinomas were molecularly classified according to the St. Gallen Consensus 2011 using ER, PR, HER-2, and Ki-67 markers. These were correlated with other conventional prognostic parameters and analyzed statistically.

The study highlights the diversity of breast cancer molecular subtypes. Luminal B was the predominant subtype. TNBC emerged as a significant determinant of aggressive tumor behavior, presenting at younger ages, exhibiting larger tumor sizes, higher rates of lymph node metastasis and lymphovascular invasion. Therefore, routine molecular subtyping for all cases of breast carcinoma is essential, providing invaluable insights into clinical outcomes and guiding personalized treatment decisions.

Keywords: Molecular classification, Her 2- positive, luminal A, luminal B, subtypes of breast cancer, triple-negative breast cancer.

Introduction

Breast cancer (BC) is a commonest and leading cause of deaths in women due to cancer [1]. According to Globocan data 2020, in India, BC accounted for 13.5% of all cancer cases and 10.6% of all deaths [2].

Hormonal analysis and molecular subtyping are used as an important predictive and prognostic factor in women with carcinoma of the breast. Prognosis depends on clinical, pathological, and molecular factors. These include histological type, histological grade, lymphovascular invasion, lymph node metastases, and the status of hormonal receptors- Estrogen receptor (ER), progesterone receptor (PR), and human epidermal growth factor (HER2) status of the tumor. Emphasis is laid on Ki 67 for distinguishing Luminal A and B subtypes [3].

Nearly 75% of invasive breast cancers are ER positive, PR follows ER expression, rarely found in ER negative tumors and often indicating a functional ER pathway. Both ER and PR serve as diagnostic and prognostic biomarkers, with higher PR levels linked to better outcomes and lower levels linked to a more aggressive disease course.

HER2 overexpression is linked with metastatic and recurrent breast cancers, showing an increase by 50% to 80%. 15% of invasive breast cancers show HER2 gene amplification and protein overexpression. This trigger increased activation of proto-oncogenic signaling pathways, resulting in unregulated growth of cancer cells. HER2 positive tumors have shorter disease-free survival and an important marker to predict response to chemotherapy with doxorubicin and trastuzumab.

Ki 67, a marker of cell proliferation, guides treatment, follow-up, and potentially predicts outcomes. High levels correlate with lower survival.

Targeted therapy drives the need for molecular classification in breast cancer. Identifying subtype-specific genes like RASDF7 (Luminal A), DHRS11 (HER2+), and ADSSL1 (TNBC) offers promise for new prognostic markers and targeted therapies [4].

Current practice in India relies on classifying breast cancer based on ER, PR, and HER2/neu expression in biopsies. However, the biological significance and clinical relevance of this standard approach to molecular classification remain unclear in the Indian context. This study aims to address this gap by investigating the association between IHC based molecular subtypes and existing prognostic parameters to establish the validity and value of this classification system for Indian breast cancer patients [1].

Methods

The present study was conducted in a tertiary care hospital, Bengaluru from 2020 to 2023. 180 cases of invasive breast carcinomas were subjected to routine staining and immunohistochemistry (IHC) with estrogen receptors (ER), progesterone receptors (PR), Her2/neu and Ki 67. The specimens were evaluated both histopathologically and immunohistochemically by Allred scoring for ER, PR, HER-2 markers. According to the St. Gallen Consensus 2011, the breast cancer cases were classified into molecular subtypes - Luminal A (ER+/PR+/HER2-/low Ki-67); Luminal B (ER+/PR+/HER2-/+/high Ki-67); HER2-overexpression (ER-/PR-/HER2+) and triple negative breast cancers/TNBCs (ER-/PR-/HER2-). Low Ki 67 is defined as <14% and high Ki 67 as >14% [1]. These were correlated with other conventional prognostic parameters such as age at time of diagnosis, tumor size based on histopathology, Scarff Bloom Richardson (SBR) grade, histopathological subtype, lymph nodal involvement, lymphovascular invasion (LVI), perineural invasion (PNI) and ductal carcinoma in-situ (DCIS).

Informed consent was taken from the patients involved in the study.

Statistical analysis: Data was entered into Microsoft excel data sheet and was analyzed using SPSS 22 version software. Categorical data was represented in the form of frequencies and proportions. Chi-square test or Fischer's exact test (for 2x2 tables only) was used as test of significance for qualitative data.

P value (Probability that the result is true) of <0.05 was considered as statistically significant after assuming all the rules of statistical tests.

Statistical software: MS Excel, SPSS version 22 (IBM SPSS Statistics, Somers NY, USA) was used to analyze data.

Results

A total of 180 cases of breast carcinomas, 87 modified radical mastectomies, 32 wide local excisions and 61 biopsies were included in the present study. Among the various histological types of breast cancer, invasive ductal carcinoma (figure 1) was the most common type at 95%. The rest of the cases were other histological types that included 3 cases of medullary, 2 mucinous, 2 papillary,1 invasive lobular and 1 neuroendocrine type.

42% of the breast carcinoma patients were aged <50 years while 58% were >50 years. At the time of diagnosis, the average age of the patient was 53±5 years (age ranging from 29 to 81 years).

Positive ER (figure 2) and PR (figure 3) immunostaining were seen in 85% and 83% of the cases respectively. Her-2 (figure 4) immunostaining was found positive in 31% of cases.

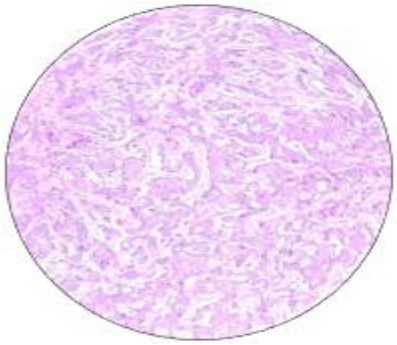

Fig.1: H and E,40X, showing invasive ductal carcinoma.

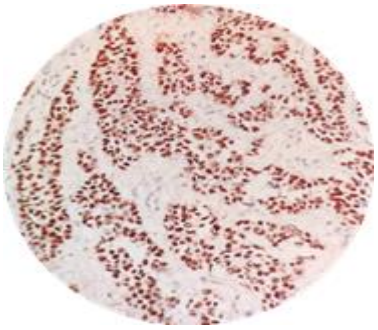

Fig.2: IHC,40X, ER showing nuclear positivity.

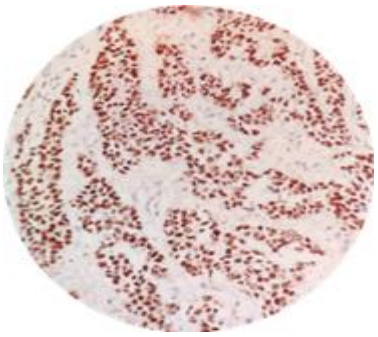

Fig. 3: IHC,40X, PR showing nuclear positivity.

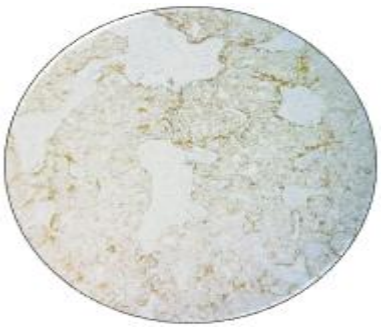

Fig. 4: IHC,40X, Her-2 neu showing membranous positivity.

Luminal B was the most common molecular subtype (43%) followed by Triple negative (25%), luminal A (16%) and Her-2/neu overexpression (16%).

The average size of the tumor was 3.7cm. Large size tumors (>5cms) were more commonly seen in TNBCs.

Most of the diagnosed cancer cases exhibited poor differentiation (55%), with TNBCs displaying the highest proportion of poorly differentiated tumors (80%) (Table 1).

Table 1: Comparison of molecular subtypes with Scarff Bloom Richardson (SBR) grade.

Molecular Subtype	Grade 1		Grade 2		Grade 3		Total	p-value
Luminal A	5	17%	15	52%	9	31%	100	<0.05
Luminal B	6	8%	34	44%	37	48%	100	
HER2-overexpression	1	3%	9	31%	18	66%	100	
TNBC	1	2%	8	18%	36	80%	100	

In terms of clinical staging, stage II was the most prevalent (40%) among the patients. However, there were distinct distribution patterns across the molecular subtypes. Luminal A tumors were predominantly diagnosed at Stage I (46%), while Luminal B tumors were most frequently observed at Stage II (44%). Notably, Stage III tumors were more commonly associated with TNBCs, representing 63% of cases. These observed trends were found to be statistically significant (Table 2).

Table 2: Comparison of molecular subtypes with clinical staging of breast cancer

Molecular Subtype	Stage 1		Stage 2		Stage 3		Total	p-value
Luminal A	22	46%	15	31%	11	23%	100	<0.05
Luminal B	21	29%	32	44%	19	27%	100	
HER2- overexpression	8	19%	18	44%	16	38%	100	
TNBC	14	19%	14	19%	48	63%	100	

TNBCs were notably more prone to lymph node metastasis, accounting for 40% of total cases and a higher incidence of lymphovascular invasion (LVI-35%) (Table 3).

Perineural invasion (PNI-57%) and perinodal spread (50%) were observed more frequently in the Luminal B subtype. Additionally, Ductal carcinoma in-situ (DCIS- 40%) showed a stronger association with the Luminal B subtype. However, these findings did not reach statistical significance (Table 3).

Table 3: Comparison of molecular subtypes with Clinicopathological prognostic factors of breast cancer

	Luminal A		Luminal B		HER2-overexpression		TNBCs		Total	p-value
LVI										
Absent	12	25%	13	28%	15	32%	7	15%	100	<0.05
Present	8	12%	21	30%	16	23%	25	35%	100	
Lymph node metastases										<0.05
Absent	15	35%	13	32%	12	28%	2	5%	100	

Present	11	14%	16	20%	19	25%	31	40%	100	
PNI										
Absent	22	22.70%	34	35.10%	14	14.40%	27	27.80%	100	0.389
Present	3	14.30%	12	57.10%	2	9.50%	5	19.00%	100	
DCIS										
Absent	21	21.20%	38	38.40%	11	11.10%	29	29.30%	100	0.299
Present	4	20%	8	40%	5	25%	3	15%	100	

Patients with metastatic lymph nodes had elevated levels of Ki-67(74%) and 91% of TNBCs had high Ki-67, indicating higher proliferation rate in these tumors and poorer prognosis.

Discussion

Breast cancer presents as a complex and diverse illness. Relying solely on histopathology for assessment proves insufficient in assessing its behavior. To comprehensively evaluate prognosis and outcomes, we must integrate clinical observations, pathological findings, and molecular insights. This holistic approach provides a better understanding of the disease's trajectory and aids in tailored management strategies.

St. Gallen Consensus have classified the breast cancer into four molecular subtypes- Luminal A, Luminal B, triple negative and Her2 overexpression which are classified based on ER, PR, Her-2 neu and Ki 67 molecular expression [5].

Luminal A subtype breast tumors are low-grade, slow-growing, and have the most favorable prognosis with lower relapse incidence and higher survival rates. These tumors show high responsiveness to hormone therapy with tamoxifen or aromatase inhibitors and limited benefits from chemotherapy. They more commonly metastasize to bones.

Luminal B tumors are of higher grade and worse prognosis compared to Luminal A. They may exhibit intermediate/high histologic grade. It constitutes 10–20% of luminal tumors. They respond better to both hormone therapy and chemotherapy compared to Luminal A. They show frequent bone and visceral metastases.

HER2-positive subtype, constituting 10–15% of breast cancers. These tumors exhibit faster growth compared to luminal types, but prognosis has improved with the advent of HER2-targeted therapies with drugs like trastuzumab, T-DM1, pertuzumab, and tyrosine kinase inhibitors. They show a high response rate to chemotherapy but have a predilection for bone metastasis, and visceral relapses are more frequent compared to other subtypes.

TNBCs lack expression of ER, PR and HER2, representing about 20% of all breast cancers. TNBCs are characterized by their aggressiveness, BRCA2 mutation, early relapse, and tendency to present at advanced stages, with a high proliferation rate and genomic instability. It is further subdivided into basal and non-basal types, based on expression of cytokeratins (CK)5/6 and EGFR1 [4].

In our study, we observed that the luminal B emerged as the predominant subtype, aligning with findings reported by Mittal et al [1]. Conversely, Pereira et al [5] found TNBCs to be the most prevalent subtype in their study. Al-Thoubaity FK et al [6] and Cheng et al [7], in their respective research, identified luminal A as the predominant molecular subtype within the cohort of breast cancer patients examined.

In our study, we found that 42% of breast carcinoma patients were under the age of 50, with an average presentation age of 53 years. However, we observed no statistically significant association between molecular subtypes and age (P = 0.6).

24.7% of TNBCs occurred in patients under the age of 50. This contrasts with the study by Pereira et al [5] and Carey et al [8], reported a higher incidence of TNBC (34%) in the same age group, consistent with observations among White and African American populations. However, Gupta et al [9] and Lin et al [10] found that younger women presented more commonly with luminal A followed by triple negative molecular subtype.

Tumor size analysis revealed a predominant occurrence of tumors within the 2-5 cm range, consistent with findings from the Mittal et al [1] study. Notably, TNBC exhibited a higher incidence of larger tumors (>5 cm), echoing trends observed in similar Indian studies [9,10]. Furthermore, Carey et al [8] identified that women with triple-negative tumors were 2.5 times more likely to present with poorly differentiated tumors, emphasizing the aggressive nature of this subtype.

When comparing histological grade with molecular subtypes, it was evident that the majority of luminal A and luminal B subtype cases fell into grade II, indicating a moderate level of differentiation. Conversely, HER2/neu and TNBC subtypes were predominantly grade III, suggesting a higher degree of tumor aggressiveness within these molecular subtypes. These findings were statistically significant ($p<0.05$).

Consistent with our findings, Pereira et al. [5] and Alwan et al. [11] observed in their respective studies that a significant proportion of clinically advanced tumors (Stage III and IV) were associated with HER2-positive tumors. This underscores the potential link between HER2 positivity and tumor progression to advanced stages, as also seen in our investigation.

We found that lymph node metastasis was the most prevalent in TNBCs, occurring in 40% of cases, followed by Her2 positive tumors (25%). This was statistically significant ($p<0.05$). Pereira et al [5] reported a notable lymph node metastasis rate of 67.4% among Her-2 positive cases, whereas Mittal et al [1] identified the highest incidence of lymph node metastasis in TNBCs and luminal B subtype. Moreover, research by Inic et al [12] revealed a significant association between high Ki 67 expression and lymph nodal metastasis, indicating a potential biomarker for predicting this pathological feature.

In our study, we observed lymphovascular invasion in 39% of all cases, with 35% of triple-negative subtype cases showing this feature followed by luminal B (30%), which was statistically significant ($p<0.05$). In contrast, Mittal et al [1] found a higher overall LVI prevalence of 47%, with 44% attributed to the triple-negative subtype. In the study by Liao et al [13], the luminal B and HER2 positive subtypes exhibited the highest incidence of LVI positivity and lymph node involvement. Their findings emphasized the prognostic significance of LVI in overall and recurrence-free survival among breast carcinomas.

Perineural invasion was detected in 18% of the cases, with the Luminal B subtype exhibiting the highest incidence at 57%. This was statistically not significant. Hosoya et al [14] reported a PNI incidence of 14.1% and highlighted PNI as an independent adverse prognostic factor for distant metastasis-free survival (DMFS).

Regarding ductal carcinoma in situ (DCIS), it was present in 16.8% of cases in our study, with the Luminal B subtype showing the highest prevalence at 40%.

However, these findings did not reach statistical significance. Tamimi RM et al [15] found that Luminal B and HER2 molecular phenotypes were more frequent among DCIS cases. Conversely, Al-Thoubaity et al [6] noted a lower prevalence of carcinoma-in-situ in HER2 positive tumors.

Conclusion

The study highlights the diversity of breast cancer molecular subtypes. Luminal B was the predominant subtype. TNBC emerged as a significant determinant of aggressive tumor behavior, presenting at younger ages, exhibiting larger tumor sizes, higher rates of lymph node metastasis and lymphovascular invasion. Therefore, routine molecular subtyping for all cases of breast carcinoma is essential, providing invaluable insights into clinical outcomes and guiding personalized treatment decisions.

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