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Study Of Breast Carcinoma On The Basis Of Molecular Classification And Its Correlation With Clinicopathological Features

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Abstract
Background: *Breast cancer is the most frequently diagnosed cancer in women globally. Molecular classification based on immunohistochemistry markers i.e. ER, PR, HER2 has become an essential tool in guiding treatment strategies and predicting outcomes.*
Aim & Objective: *To diagnose and classify breast cancer cases on the basis of immunohistochemistry markers and correlate them with clinicopathological features.*
Materials & Methods: *This hospital-based cross-sectional study included patients diagnosed with breast cancer who underwent either modified radical mastectomy or wide excision. Specimens were processed as per standard histopathology protocol and histopathological diagnosis was given. IHC staining for ER, PR and HER2 were done with molecular classification of these cases.*
Observation & Results: *148 cases of breast carcinoma were studied. The most frequently affected age group was 40–49 years, most cases had IBC-NST, Luminal A subtype was the most prevalent. Intermediate tumor sizes were frequently observed. Higher percentage of Grade 3 tumors in all molecular subtypes was noted. The trend of increasing tumor necrosis from Luminal A to Basal-like subtypes was noted.*
CONCLUSIONS: *Molecular subtypes exhibited variable patterns with respect to age, tumor size, nodal status and provides valuable prognostic and therapeutic insights beyond conventional histopathology.*
Keywords: *Breast carcinoma, Clinicopathological features, Immunohistochemistry, Molecular classification*

INTRODUCTION:

Breast cancer is the most frequently diagnosed cancer in women globally and a leading cause of cancer-related mortality. Traditional classification systems based on tumor morphology often fall short in capturing the biological complexity and clinical behaviour of breast cancer. Patients with similar histological types may exhibit widely different prognosis and treatment responses. Recent advances in molecular biology and gene expression profiling have led to a shift in how breast cancer is understood and classified. These insights have identified several molecular subtypes of breast carcinoma, each with distinct clinical characteristics and therapeutic responses. [1] However, gene expression profiling is expensive and not readily available in many settings. In such contexts, immunohistochemistry (IHC) offers a practical alternative for molecular classification, using markers such as Estrogen Receptor (ER), Progesterone Receptor (PR), Human Epidermal growth factor Receptor 2 (HER2). IHC-based molecular classification has become an essential tool in guiding treatment strategies and predicting outcomes. [2] Nevertheless, there remains limited population-based research, particularly in India and other developing countries, correlating these molecular subtypes with clinicopathological parameters. So, the present study was conducted with an aim to diagnose and classify breast cancer cases on the basis of immunohistochemistry markers (i.e. ER, PR and HER2) and an objective to correlate the breast cancer molecular subtypes with clinicopathological features.

MATERIALS & METHODS

This hospital-based cross-sectional study was conducted in the Department of Pathology and Molecular biology & Genetics at a tertiary care centre from March 2023 to December 2024.
Minimum estimated sample Size was 81 cases

$n = Z^2(p*q)/L^2$

Where, n = minimum cases for this study, Z = Constant = 2, p = Prevalence,
q =100 – Prevalence, L = Precision error (10%), p = 28% [3], q = 72 %

Therefore,

n = 4 (28*72) = 81

The study included patients diagnosed with breast cancer who underwent either modified radical mastectomy (MRM) or wide local excision (WLE), while excluding those with recurrent disease or those with only tru-cut biopsy specimens. Specimens were grossed and processed as per standard histopathology protocol. The slides were evaluated on the basis of recent 2019 WHO classification of breast tumors. [4] Microscopic grading of breast cancer was done on the basis of Nottingham modification of Bloom-Richardson System taking into consideration: Tumor tubule or gland formation, Nuclear pleomorphism and Mitotic count (per 10 high power fields). [4] The sections were further examined for number of lymph nodes involved, tumor necrosis and vascular emboli. Immunohistochemical staining for ER, PR and HER2 were done in the department of Molecular Biology and Genetics. IHC stained slides were evaluated on the basis of ASCO/CAP guideline [5, 6] for molecular classification of breast cancers cases and was done as Luminal A, Luminal B, HER2 enriched and Basal Like. On the basis of this molecular classification, correlation was done with clinical and histopathological features like age and sex of patient, tumor laterality, duration, histological type of cancer, histologic grades, tumor size, lymph node status, tumor necrosis and vascular emboli.

Ethical consideration: This study was approved by Institutional Ethical Committee of the institute. Written informed consent of each participant was taken prior to the study.

Data collection and analysis: Data was collected by case record form and entered into MS excel 2016. Data analysis was done in SPSS Software version 26.

RESULTS

A total of 148 cases of breast carcinoma were studied within a period of March 2023 to December 2024, which was a hospital based cross sectional study. The youngest patient was 30 years old and the oldest was 84 years of age. The mean age at diagnosis was 54.5 ± 13.2 years. The most frequently affected age group was 40–49 years i.e. 49 cases (33.2%), followed by 50–59 years 30 cases (20.3%), indicating a predominance of breast cancer in perimenopausal women in the Indian context. The study population comprised 146 females (98.6%) and 2 males (1.4%), confirming the female predominance of breast carcinoma. Laterality showed an equal distribution, with 74 cases (50%) each involving the right and left breasts. A majority of patients 111/148 (75%) reported to the hospital within six months of symptom onset, suggesting rapid growth of tumor. The duration of lump in all patients ranged from 15 days to 1.5 years. Modified radical mastectomy was performed in 130 cases (88%), while wide local excision was undertaken in 18 cases (12%), reflecting the generally advanced presentation.

Most cases 99 (66.9%) had Invasive breast carcinoma - no special type (IBC-NST), followed by 17 (11.5%) cases had Mixed invasive breast carcinoma with Invasive lobular carcinoma. The study comprises six cases of Invasive lobular carcinoma (ILC), including three cases of the classical subtype and three cases of the pleomorphic subtype. (Table 2)

Majority of tumors i.e. 117 cases (79.2%) measured between 2 and 5 cm, while only 5 cases (3.3%) were less than 2 cm and 26 cases (17.5%) were larger than 5 cm. Tumor grading revealed a high proportion of Grade 3 tumor: 96 cases (64.9%) followed by Grade 2: 46 cases (31.1%) and Grade 1: 6 cases (4.1%). Lymph node assessment revealed metastatic involvement in 49.4% of cases, with 32 cases (21.6%) showing metastasis in 1–3 lymph nodes and 41cases (27.8%) in more than 3 lymph nodes, while 75 cases (50.6%) showed no lymph node involvement. Tumor necrosis was present in 68 cases (45.9%), and vascular emboli in 73 cases (49.3%). (Table 3)

Luminal A subtype was the most prevalent, observed in 59 cases (39.9%), followed by the Basal-like subtype in 48 cases (32.4%). HER2-enriched subtype and Luminal B subtypes were less common, accounting for 26 cases (17.6%) and 15 cases (10.1%), respectively. (Table 1)

Among the 49 cases in age group of 40-49 years, 19 (38.8%) cases were Luminal A, 13 (26.5%) cases were Basal like, 11 (22.4%) cases were HER2 enriched, and 6 (12.2%) cases were luminal B breast cancer. On applying chi square test, there was no correlation found between age group and molecular classification.
Total 117 cases had tumor size of between 2-5 cm, which constituted 47/59 (79.7%) cases of luminal A, 35/48 (72.9%) cases of Basal like, 21/26 (80.8%) cases of HER 2 enriched and 14/15 (93.3%) case of luminal B breast carcinoma. On applying chi square test, there was no correlation found between tumor size and molecular subtype.
In this study among 148 cases, Invasive Breast Carcinoma of No Special Type (IBC-NST) was mostprevalent 99 cases (66.9%), showing a heterogeneous molecular profile: Luminal A 38/99 cases (38.4%), Basal-like 35/99 cases (35.4%), HER2-enriched 19/99 cases (19.2%), and Luminal B 7/99 cases (7.1%). On applying chi square test, there was no correlation found between Histological diagnosis and molecular subtype.
Total 96 cases had Grade 3 tumor, which constituted 37/48 (77.1%) cases of Basal like, 30/59 (50.8%) cases of luminal A, 19/26 (73.1%) cases of HER 2 enriched and 10/15 (66.7%) case of luminal B breast carcinoma. On applying chi square test, there was no correlation found between grade of tumor and molecular subtype.
Total 75 cases had no lymph node metastasis, which constituted 28/59 (47.5%) cases of luminal A, 25/48 (52.1%) cases of Basal like, 14/26 (53.8%) cases of HER 2 enriched and 8/15 (53.3%) case of luminal B breast carcinoma. On applying chi square test, there was no correlation found between lymph node metastasis and molecular subtype.
Total 68 cases had presence of tumor necrosis, which constituted 26/48 (54.2%) cases of Basal like, 13/26 (50.0%) cases of HER 2 enriched, 6/15 (40.0%) case of luminal B and 23/59 (39.0%) cases of luminal A breast carcinoma. On applying chi square test, there was no correlation found between tumor necrosis and molecular subtype.
Total 73 cases had presence of vascular emboli, which constituted 24/59 (40.7%) cases of luminal A, 23/48 (47.9%) cases of Basal like, 14/26 (53.8%) cases of HER 2 enriched, and 12/15 (80.0%) case of luminal B breast carcinoma. On applying chi square test, there was no correlation found between vascular emboli and molecular subtype.

DISCUSSION

Molecular subtypes of breast cancer

Among the molecular subtypes, Luminal A subtype was the most prevalent, observed in 59 cases (39.9%), followed by the Basal-like subtype in 48 cases (32.4%). HER2-enriched subtype and Luminal B subtypes were less common, accounting for 26 cases (17.6%) and 15 cases (10.1%), respectively which aligns with other studies [7, 8] and literature.

Age group wise distribution of cases

In this study, the peak incidence of breast cancer was noted in the 40–49-year age group (33.2%), These findings are consistent with previous reports from South and Southeast Asia, where breast cancer is often diagnosed at a younger age compared to Western populations. [9-11]
The presence of 10.1% of cases in the 30–39-year group in the current study is noteworthy, as it reflects the burden of early-onset breast cancer, which has been associated with more aggressive tumor subtypes and poorer outcomes, particularly in triple-negative and HER2 enriched tumors which is similar to study from south India. [12]
Younger women had higher proportions of HER2-enriched subtypes (57.69%) which aligned with Fatma et al. [13] (58.82%) and Devdas et al. [8] (66.67%), whereas older women had more Luminal A (62.71%) and Basal-like (triple-negative) tumors (58.33%), these observations differed from studies by Fatma et al. [13] and Devdas et al. [8] which highlights the importance of individual and regional variations.
Gender wise distribution of cases
Of the 148 patients, 146 (99%) were female and 2 (1%) were male, which is consistent with existing literature showing that breast cancer is a disease of women. [3, 13]
Distribution of cases as per Laterality of breast cancer

This study found an equal distribution of right and left breast cancer cases (50% each), studies also observed a nearly equal distribution in other Indian populations. [9, 14] While some studies have reported a mild predilection for left-sided breast cancer. [15]

Duration of breast cancer among cases

Duration of symptoms in majority of patients (111, i.e. 75%) was less than 6 months; This is indicative of a relatively early clinical presentation, similar trend was also observed in previous study. [8]

Distribution of cases based on Size of tumor

Most patients in our study had intermediate tumor sizes (2–5 cm) (117, i.e. 79.05%), which are associated with a moderately high risk of lymph node involvement and distant metastasis similar observation was also noted by other studies. [13, 14, 16]

Fatma et al. [13] and Mittal et al. [14] also had majority of their tumor sizes between 2–5 cm for Luminal B, HER2 enriched and Basal like subtype, but for luminal A the proportion of tumors less than 2 cm in our study (3.39%) was notably lower than that reported by Fatma et al. [13] (50.92%). This discrepancy may reflect differences in the clinical settings or the stage at diagnosis in different populations.

However, no significant association between tumor size and molecular subtypes was observed in the present study or in previous studies, as indicated by non-significant p-values across all studies.

Distribution of cases based on Histopathological diagnosis

IBC-NST was the most frequent histopathological subtype in our study population, accounting for 66.9% of cases. This is in line with the general consensus. [8, 9, 17]. IBC-NST showed a heterogeneous molecular profile these findings were in concordance with Devadass et al. [8]

Invasive breast carcinoma (no special type) mixed with invasive lobular carcinoma or invasive mucinous carcinoma exhibited the Luminal A subtype, consistent with their hormone receptor-positive phenotype. Metaplastic carcinoma was primarily Basal-like, aligning with its known triple-negative status and aggressive behaviour, these findings are consistent with those of Barodawala et al. [9]

Distribution of cases based on Grade of tumor

The present study observed the highest proportion of Grade 3 tumor 96 cases (64.9%), which was slightly higher compared to other studies [14, 18], which had majority of Grade 2 tumors indicating a higher incidence of aggressive tumor in the present study.

Higher percentage of Grade 3 tumors in all molecular subtypes in our study can be related to overall greater number of cases in Grade 3 category. Variability in tumor grade distributions across different molecular subtypes in different studies [7, 13, 14] could be due to population and regional differences.

Lymph node metastasis seen in breast cancer cases

50.6% of patients showed no lymph node involvement, the findings were consistent with study from Saudi Arabia. [13]

In our study for the Luminal A subtype, 47.46% of patients showed no lymph node metastasis, which is relatively similar to the 44.24% observed by Fatma et al. [13]

For the Luminal B, HER2 enriched and Basal like subtype number of patients with no lymph node metastasis, were comparable to those observed by Carey et al. [15]

Distribution of cases based on Tumor necrosis

Tumor necrosis was observed in 45.9% of the cases, when compared to other studies tumor necrosis in the present study is less frequent [19]; this difference may be explained by the patient population and tumor subtypes.

The trend of increasing tumor necrosis from Luminal A to Basal-like subtypes was noted in the present study and Ebrahimi et al. [20], reflecting known differences in tumor aggressiveness. However, the association was not statistically significant ($p = 0.416$), consistent with Ebrahimi et al. ($p = 0.197$).

Distribution of cases based on presence of Vascular emboli

Our study showed a relatively high rate of vascular emboli (49.3%) compared to other studies. [21, 22] These differences might be attributed to the methodological variations, the use of different diagnostic techniques, or variations in tumor biology across populations.

Frequency of vascular emboli was more in Luminal B subtypes compared other studies [14, 23] but P value was statistically not significant.

CONCLUSIONS

Breast carcinomas exhibit considerable molecular diversity. Invasive breast carcinoma of no special type shows a heterogeneous molecular distribution, whereas invasive lobular carcinomas and invasive mucinous carcinomas are primarily of the luminal A subtype. Most metaplastic carcinomas exhibit basal-like subtype. Luminal A is the most common molecular subtype.

More aggressive molecular subtypes, particularly the basal-like type, are associated with high-grade tumors. Tumor necrosis is least observed in luminal A and most frequent in basal-like subtype, reflecting increasing aggressiveness across molecular subtypes.

Higher frequency of clinical presentation as high-grade tumors, tumor size >2cm and basal-like subtype underscores the importance of early detection through screening. Molecular subtypes exhibited variable patterns with respect to age, tumor size, and nodal status.

Molecular subtyping stands out as a vital component of modern oncology and provides valuable prognostic and therapeutic insights beyond conventional histopathology offering more accurate prognostication and therapeutic direction, ultimately improving patient care and outcomes.

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Table 1. Distribution of cases based on Molecular classification of breast cancer

Molecular subtype	Frequency	Percentage
Luminal A	59	39.9%
Luminal B	15	10.1%
HER2 enriched	26	17.6%
Basal like	48	32.4%
Total	148	100%

Table 2. Distribution of Histological diagnoses among the molecular subtypes of breast carcinoma

Histological diagnosis	All cases n=148 (%)	Luminal A n=59 (%)	Luminal B n=15 (%)	HER2-enriched n=26 (%)	Basal-like n=48 (%)	p-value
IBC-NST	99 (66.8%)	38 (64.4%)	7 (46.7%)	19 (73.1%)	35(72.9%)	0.235 NS
IBC+ILC	17 (11.4%)	9 (15.3%)	3 (20.0%)	1 (3.8%)	4 (8.3%)	
IBC-NED	12 (8.1%)	3 (5.1%)	3 (20.0%)	3 (11.5%)	3 (6.2%)	
ILC	6 (4.0%)	3 (5.1%)	1 (6.7%)	1 (3.8%)	1 (2.1%)	
METC	5 (3.4%)	0 (0.0%)	0 (0.0%)	1 (3.8%)	4 (8.3%)	
IBC+MUC	4 (2.8%)	3 (5.1%)	0 (0.0%)	0 (0.0%)	1 (2.1%)	
IBC+ACC	2 (1.4%)	1 (1.7%)	0 (0.0%)	1 (3.8%)	0 (0.0%)	
ACC	1 (0.7%)	1 (1.7%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	
IBC+EPC	1 (0.7%)	0 (0.0%)	1 (6.7%)	0 (0.0%)	0 (0.0%)	
IBC+NEC	1 (0.7%)	1 (1.7%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	

(IBC-NST: Invasive breast carcinoma (no special type), IBC+ILC: Mixed invasive breast carcinoma with invasive lobular carcinoma, IBC-NED: Invasive breast carcinoma with focal neuroendocrine differentiation, ILC: Invasive lobular carcinoma, METC: Metaplastic carcinoma, IBC+MUC: Mixed invasive breast carcinoma with mucinous carcinoma, IBC+ACC: Mixed invasive breast carcinoma with adenoid cystic carcinoma, ACC: Adenoid cystic carcinoma, IBC+EPC: Invasive breast carcinoma with encapsulated papillary carcinoma, IBC+NEC: Invasive breast carcinoma with focal neuroendocrine carcinoma)

Table 3. Distribution of clinicopathological features among the molecular subtypes of breast carcinoma

Characteristics	All cases n=148 (%)	Luminal A n=59 (%)	Luminal B n=15 (%)	HER2-enriched n=26 (%)	Basal-like n=48 (%)	p-value
Tumor size (in cm)						
<2	5 (3.4%)	2 (3.4%)	0 (0.0%)	0 (0.0%)	3 (6.2%)	0.603 NS
2-5	117 (79.1%)	47 (79.7%)	14 (93.3%)	21 (80.8%)	35 (72.9%)	
>5	26 (17.5%)	10 (16.9%)	1 (6.7%)	5 (19.2%)	10 (20.8%)	
Grade of tumor						

1	6 (4.1%)	3 (5.1%)	0 (0.0%)	2 (7.7%)	1 (2.1%)	0.08
2	46 (31.1%)	26 (44.1%)	5 (33.3%)	5 (19.2%)	10 (20.8%)	1
3	96 (64.9%)	30 (50.8%)	10 (66.7%)	19 (73.1%)	37(77.1%)	NS
No. of lymph nodes involved						
0	75 (50.7%)	28 (47.5%)	8 (53.3%)	14 (53.8%)	25 (52.1%)	0.29
1-3	32 (21.6%)	15 (25.4%)	4 (26.7%)	5 (19.2%)	8 (16.7%)	2
>3	41 (27.7%)	16 (27.1%)	3 (20.0%)	7 (26.9%)	15 (31.3%)	NS
Tumor necrosis						
Present	68(45.9%)	23 (39.0%)	6 (40.0%)	13 (50.0%)	26 (54.2%)	0.41
Absent	80(54.1%)	36 (61.0%)	9 (60.0%)	13 (50.0%)	22 (45.8%)	6
						NS
Vascular emboli						
Present	73(49.3%)	24 (40.7%)	12 (80.0%)	14 (53.8%)	23 (47.9%)	0.41
Absent	75(50.7%)	35 (59.3%)	3 (20.0%)	12 (46.2%)	25 (52.1%)	4
						NS

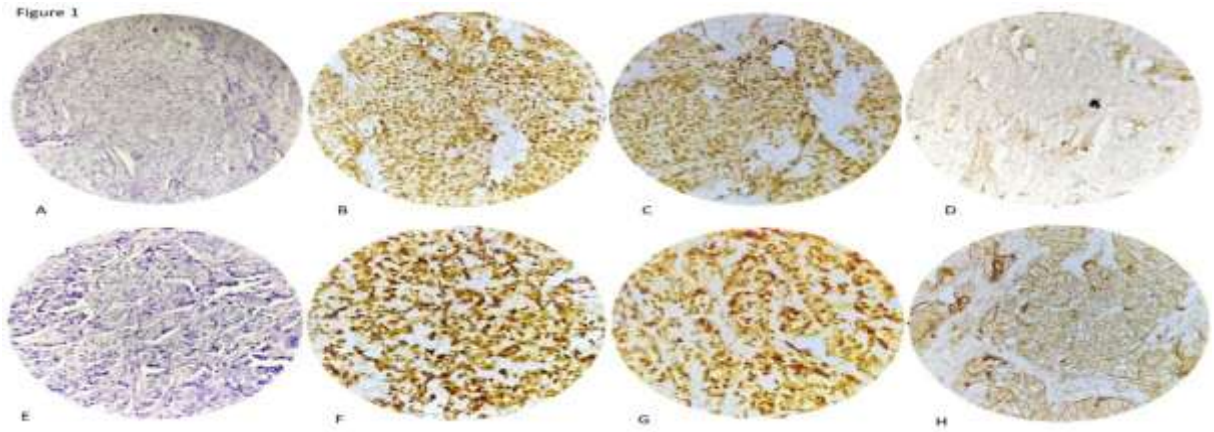

Figure 1
Invasive breast carcinoma - no special type: Luminal A
A. H&E (400x), B. ER positive (400x), C. PR positive (400x), D. HER2 negative (400x)
Invasive breast carcinoma - no special type: Luminal B
E. H&E (400x), F. ER positive (400x), G. PR positive (400x), H. HER2 positive (400x)

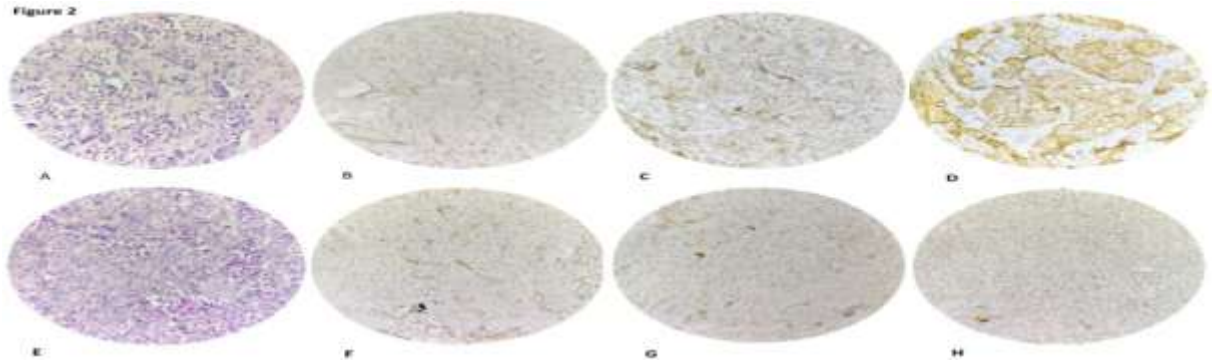

Figure 2
Invasive breast carcinoma - no special type: HER2 Enriched
A. H&E (400x), B. ER negative (400x), C. PR negative (400x), D. HER2 positive (400x)
Invasive breast carcinoma - no special type: Basal-like