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A study on molecular subtype of breast carcinoma using ER, HER2, PR, and its relation with Ki67

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Abstract

Background: Using molecular biomarkers of breast cancer (BC) receives optimal treatment. Established biomarkers like estrogen receptor (ER), progesterone receptor (PR), HER2, and Ki67 may play significant roles in the sub-categorization of BC to predict the prognosis and specific therapy for each patient.
This study aims to characterize breast carcinoma using molecular markers (ER, PR, and HER2 neu), identify their sub-types, and attain its relation with Ki67.

Methods: This cross-sectional (retrospective) study was conducted over four years at the Department of Pathology, SVS Medical College, Mahabubnagar, from April 2018 to March 2022. Fifty diagnosed cases of breast carcinomas were subjected to routine staining and immunohistochemistry (IHC) with ER, PR, HER 2 neu, and Ki67 using tissue microarray and classified into molecular subtypes.

Results: In this study, 32% of participants were in the 50-59 age group. The right breast was involved in 52% of participants. The central quadrant was involved in 44%, UOQ 32%, UIQ 16%, and LOQ 8%. Moderately differentiated tumors exist in 40%, poorly differentiated in 36%, and well-differentiated in 24%. 84% of patients were ER-positive, 70% were PR-positive, and 72% were HER2/neu-positive. 28% of patients with positive Ki-67 were grade I, 22% were grade II, and 50% were grade III. Her2/neu contributed to 12% and triple-negative BC seen in 4%. The association between the ER status and Ki-67 positivity status is not statistically significant, with a P-value of 0.13. The association between the PR status and Ki-67 positivity status is statistically significant, with a P-value of 0.002. The association between the Her2/neu status and Ki-67 positivity status is also statistically significant, with a P-value of <0.0001.

Conclusion: Most cases were Ki67 positive and HER2 neu positive. Triple-negative cases showed a high Ki67 index. Ki67 plays a pivotal role in making treatment decisions. The Ki67 index is independent and can be used independently without being correlated with other indices, and Ki67 should be evaluated routinely in breast carcinoma cases.

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Highlights

What is current knowledge?

Breast Carcinoma prognosis varies with tumor size, axillary lymph node status, histologic grade, histologic type and their biological markers like progesterone receptor (PR), estrogen receptor (ER), HER2/neu expression pattern.

What is new here?

Percentage of PR positivity was more than ER. HER2/neu overexpression is associated with poor prognosis and high-grade features. Molecular subtyping using immunohistochemistry of PR, ER, HER2/neu, Ki67 provides its usage in prognostic significance and predictive information of about targeted therapies.

Introduction

Breast cancer (BC) is the most prevalent and leading cause of mortality in women, with over 100,000 cases identified each year (1). According to the World Health Organization, cancer kills 7.6 million people globally each year, with BC accounting for 502,000 of BC alone.

Prognostic information is critical in assessing the likely prognosis of the disease and planning future management. Clinical characteristics such as menopausal status, tumor size, histologic type, histologic grade, and pathological stage are important prognostic indicators. Furthermore, other characteristics are prognostic and predictive of response to therapy (2). These variables include estrogen receptor (ER), progesterone receptor (PR), HER2/neu, and Ki-67.

BCs are traditionally classified using the WHO classification, the most recent version from 2012 (3). One significant disadvantage of this classification is that most breast tumors fall into one of two major histological classifications: invasive or invasive lobular carcinoma (4). This means that this categorization cannot define the broader heterogeneity of BC with diverse molecular and clinical features. As the concept of heterogeneity in BC has gained acceptance, a new molecular categorization has been proposed that may provide better targets for therapy. Perou & Sorliein proposed this in 2000 (5). BC molecular subtypes are defined as Luminal A (ER+/PR+/HER2-/low Ki-67), Luminal B (ER+/PR+/HER2-/+/high Ki-67), HER2 overexpression (ER-/PR-/HER2+), and triple-negative BC/TNBC (ER-/PR-/HER2-). Basal-like TNBCs are TNBCs that are positive for basal markers (CK5/6) (6).

Three biomarkers, ER, PR, and HER2/neu, are commonly tested to estimate the molecular category of BC. However, Ki67 can be an independent factor in predicting tumor grade.

This study aims to characterize BC using molecular markers such as ER, PR, and HER2, identify subtypes, and determine its relationship with Ki67.

Methods

Patients

This cross-sectional (retrospective) study was conducted over four years (April 2018 to March 2022) at the Department of Pathology, SVS Medical College, Mahabubnagar. Fifty diagnosed cases of breast carcinoma based on radiology and clinical parameters were enrolled.
The Institutional Ethical Committee, SVS Medical College, Mahabubnagar, approved the study procedure.

Inclusion and exclusion criteria

Patients with all relevant clinical history and investigations presenting with BC and patients willing to consent were included in the study. Patients with benign breast lesions, inflammatory lesions, phylloids tumors, and sarcomas were excluded.

Two sections of 4-5µ thickness were prepared from the corresponding paraffin blocks, one on the albumin-coated slide for H&E staining and another on poly- L-lysine-coated slide for immunohistochemistry. Invasive breast Carcinoma histological typing and histological grading (I, II, and III) were done according to the Nottingham system using the scale assigned to three features such as tubular formation (1-3), nuclear atypia (1-3), and mitoses (1-3).

Ready-to-use antibodies were used such as Anti-ER Rabbit monoclonal antibody (Biogenex company), Anti-PR (CloneEp2) Rabbit monoclonal antibody (Biogenex company), Anti-c-erbB2/Her2-neu (CloneCB11) Mouse monoclonal antibody (Biogenex company), and Ki-67 (CloneMiB-1) mouse monoclonal antibody (Biogenex company). IHC with ER, PR, HER2, and Ki67 were done. The association between age, laterality, tumor grade, ER, PR, HER2/neu, and

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Ki67 expression were studied.

Statistical analysis

Results related to continuous variables were represented as Mean±SD (Min-Max), and categorical variables were expressed as frequency (%). Student t-test was used to compare inter-group variation for continuous variables. The chi-square test assessed the association between the two categorical variables. Statistical analysis was performed using a statistical package for Social Science Program Version 25.0 (SPSS, IBM, US) for Windows. The P value is significant if less than 0.05.

Results

Among the study population, the majority belonged to the age group of 50-59 years, accounting for 32%, followed by 60-69 years (26%) and 40-49 years (24%), respectively. 14% of the population were 30-39 years old, and 4% were of >70 years. The right breast was involved in 52% and the left in 48%. Pain was one of the common symptoms in 44% of participants before diagnosis. Among the study cases, the central quadrant was involved in 44%, followed by UOQ in 32% and UIQ in 16%. LOQ was involved in 8%.

Nottingham histologic score invasive duct cell carcinoma (IDCC)-6 observed in 24% of cases, IDCC-8 in 20%, 12% each in IDCC-9 and IDCC-4, respectively.

Among the study cases, moderately differentiated tumors contributed 40%, poorly differentiated to 36%, and well-differentiated to 24%.

Tumor grading & TNM staging:

Among the study cases, 40% contributed to grade II, 36% to grade III, and 24% to grade I (Table 1). The number of lymph nodes involved is illustrated in (Table 2).

Table 1. TNM staging of study population

TNM staging	Frequency	Percentage
T1cN1	1	2
T1cN1a	1	2
T1N0	1	2
T1N1	1	2
T1Nx	3	6
T2N0	2	4
T2N1a	10	20
T2N2a	5	10
T2N3a	4	8
T2Nx	12	24
T3N0	1	2
T3N1a	4	8
T3N2a	1	2
T3N3a	2	4
T3Nx	1	2
T4bN3a	1	2
Grand Total	50	100

Table 2. Number of lymph nodes involved

No. of lymph nodes involved	Frequency	Percentage
Zero	18	36
1-5	21	42
6-10	5	10
11-15	2	4
16-20	0	0
21-25	4	8
Total	50	100

Table 3. Molecular subtypes

Molecular Subtype	**Frequency**	**Percentage**
Her2	6	12
Luminal A	12	24
TNBC	2	4
Grand Total	**50**	**100**

Molecular subtypes of breast Carcinoma are described in (Table 3). Among the study cases, 84% were ER-positive, 70% were positive for PR, and 72% were positive for HER2/neu. For Ki-67 positivity, 28% of cases were of grade I, 22% were of grade II, and 50% were of grade III (Table 4).

Table 4. The relationship between ER and Ki-67

ER	Ki-67			Total
	G1	G2	G3	
Negative	0	3	5	8
Positive	14	8	20	42
Total	14	11	25	50

Among the study cases, the luminal type B subtype was predominant with 60%, followed by luminal type A (24%). Her2 contributed to 12%, and triple-negative BC was seen in 4%. No significant difference was noted between ER and Ki-67 of various grading. No correlation was observed between Her2 positivity and Ki-67 of its expression grading. The correlation between the ER status and Ki-67 status of positivity is statistically significant (P=0.002). The correlation between the Her2 status and Ki/67 status of positivity is statistically significant (P = 0.0001) (Tables 5 and 6).

Table 5. The relationship between PR and Ki-67

PR	Ki-67			Total
	G1	G2	G3	
Negative	0	7	8	15
Positive	14	4	17	35
Total	14	11	25	50

Table 6. Relationship between Her2/neu and Ki-67

Her2/neu	Ki-67			Total
	G1	G2	G3	
Negative	12	1	1	14
2+	2	3	2	7
3+	0	7	22	29
Total	14	11	25	50

The distribution of breast carcinoma and its basic histochemical characteristics are described in (Figure 1). Immunohistochemical analysis with ER, PR, and Her2 markers and their expression are shown in (Figure 2). Immunohistochemical analysis of breast carcinoma of HER2 cells and its correlation with Ki-67 is depicted in (Figure 3).

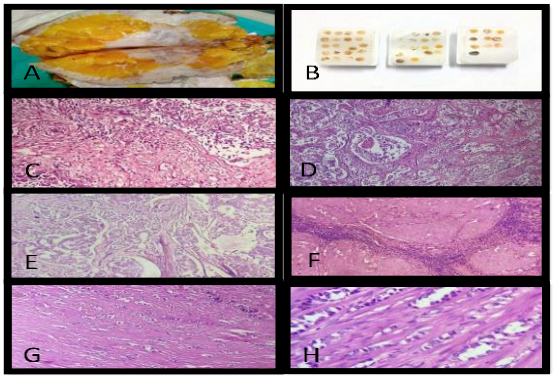

Figure 1. a. Cut section showing tumor mass in mastectomy specimen, b. Tissue microarray blocks, c. IDCC-NOS grade I (100x), d.IDCC-NOS grade II, e.IDCC-NOS grade III (100x), f. Medullary carcinoma breast (100x), g. Invasive lobular breast carcinoma (100x), h. Invasive lobular breast carcinoma (400x).

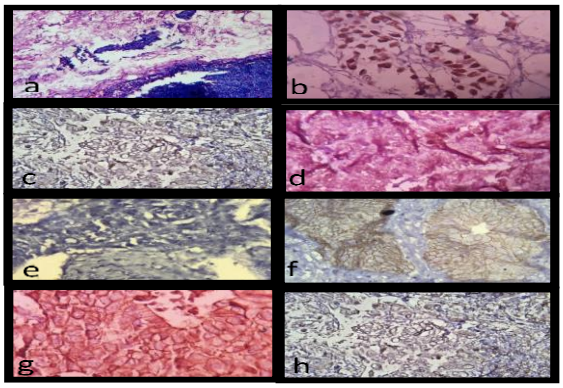

Figure 2. Immunohistochemical analysis: a. Lymph node involvement inbreast carcinoma (100x), b. ER-positive, c. ER-negative, d. PR-positive, e. Her2/neu1+, f. Her2/neu2+, g. Her2/neu3+, h. PR-negative

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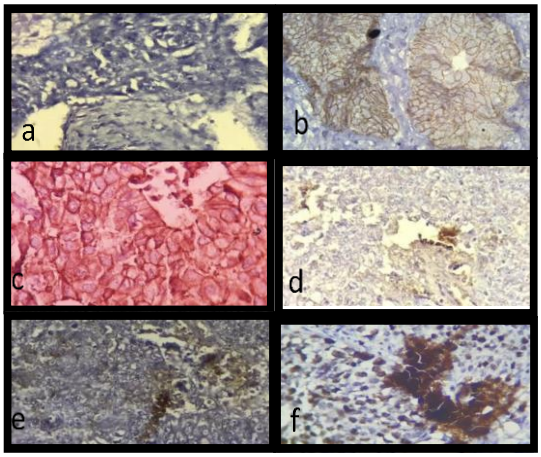

Figure 3. Immunohistochemical analysis: a. Her2/neu 1+ b. Her2/neu 2+ c. HER2/neu 3+ d. Ki-67 grade I e. Ki-67 grade II g. Ki-67 grade III

Discussion

The present study was conducted on molecular characterization of breast carcinoma and its expression to predict the BC sub-types to elucidate the correlation between these factors and between the factors and the subtypes of BC.

In the present study, among the study population, 32% were of the age group of 50- 59 years, followed by 60-69 years (26%) and 40-49 years (24%), respectively. Other studies' participants were mainly younger than age 50 years. Hence, BCC patients were younger in Asian countries than in European countries. Al Naumi HA et al. (7) showed that forty-three cases (53.75%) were younger than 50.

In the present study, the right breast was involved in 52% and the left in 48%, with almost equal distribution. Mittal A et al. (8) showed that the left breast was the most commonly affected in forty-eight cases (69%).

In this study, the central quadrant was involved in 44%, followed by UOQ in 32% and UIQ in 16%. LOQ was involved in 8%.

In the present study, 40% of patients had moderately differentiated tumors, 36% had poorly differentiated tumors, and 24% had well-differentiated tumors. 40% of patients were diagnosed with grade II (moderately differentiated), 36% with grade III (poorly differentiated), and 24% with grade I (well differentiated).

Mittal A et al. showed that grade I, grade II, and grade III tumor differentiation was observed in 25%, 56%, and 19% of the study population.

The present study showed that 84% of patients were ER-positive, whereas Al Naumi HA et al. found ER to be positive in sixty-two cases (77.5%). Arpita J et al. (9) showed ER was positive in thirty-two cases (49.23%).

The present study shows that PR was positive in 70%. Al Naumi HA et al. showed that PR was positive in fifty-four cases (67.5%). Arpita J et al. noted PR positivity in twenty-three cases (35.38%).

The present study showed that Her2/neu was positive in 72%. Arpita J et al. showed that Her2/Neu positivity was seen in 15.38% of cases, and Her2/Neu was negative in 84.62%.

The present study showed that 28% of patients were of gI, 22% were of gII, and 50% were of gIII for Ki-67 positivity. Al Naumi HA et al. showed high expression (≥20% of nuclei were positive) in 45% of patients, and low expression was observed in 55% of cases.

In the present study, among the study population, the luminal type B subtype was predominant with 60%, followed by luminal type A (24%). Her2 contributed to 12%, and triple-negative BC was seen in 4%. Mittal A et al. revealed that the expression of Luminal A was positive in 14 patients (22%), Luminal B in 18 (28%), and Her2 and triple-negative in 16 (25%) each. Arpita J et al. depicted the expression of Luminal A in 32.31%, Luminal B in 18.46%, Her2 overexpression in 13.84%, and triple-negative in 35.39%, respectively.

The present study's association between the ER and Ki-67 positivity is insignificant (P=0.13). The association between the PR and Ki-67 positivity is statistically significant (P=0.02). The association between the Her2 and Ki-67 positivity is statistically significant (P<0.0001).

Al Naumi HA et al. and Khanna M et al. (10) studies also showed a significant association.

In our study, Triple Negative and HER2 positive and Ki67 higher expression represents the worst prognosis of breast carcinoma. ER+/PR+/HER2-/Ki67(less expression) represents the better prognosis of breast carcinoma. Patients with a low Ki67 index displayed a better survival rate than those with a higher Ki67 index.

The current study's limitations include that it was conducted only in a limited sample size due to its timeliness, and the study results can be generalized only after experimentation in a multi-centric setup.

Conclusion

Most of the breast carcinoma cases were Ki67 and Her2 neu positive. The PR and Ki-67 positivity association is statistically significant in breast carcinoma. The association between the Her2/neu and Ki-67 positivity is statistically significant and shows very high significance. The Ki67 index is independent and can be used independently without being correlated with other indices, and Ki67 should be evaluated routinely in BC subjects.

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Ethical statement

The study was performed per the Declaration of Helsinki, and written informed consent was obtained from all participants. The study received approval from the Ethics Committee of S.V.S Medical College, Mahabubunagar (No: IEC/SVS/Path/2018/01).

Conflicts of interest

The authors declared no conflict of interest in this study.

Author contributions

SZ, SH, FM, & SD analyzed and interpreted the patient's data. BS, ShK & RF performed the histological examination and was a major contributor in writing the manuscript. SK, RF & NF were supervised the total work and edited the manuscript. All authors approved the final manuscript.

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