International Journal of Medical and Pharmaceutical Research

Online ISSN-2958-3683 | Print ISSN-2958-3675
Frequency: Bi-Monthly
Available online on: https://ijmpr.in/

Research Article

IMMUNOHISTOCHEMICAL MARKERS IN CARCINOMA BREAST: EVALUATION AND CORRELATION WITH CLINICOPATHOLOGICAL FEATURES

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Received: 02-08-2025
Accepted: 24-08-2025
Available online: 22-09-2025

ABSTRACT

Background: Breast carcinoma is the most common malignancy in women and shows considerable heterogeneity in its clinical and pathological presentation. Immunohistochemical (IHC) markers such as estrogen receptor (ER), progesterone receptor (PR), and HER2/neu are essential for prognostication and treatment planning. This study was undertaken to evaluate the expression of IHC markers in breast carcinoma and to correlate them with clinicopathological parameters.

Methods: A cross-sectional observational study was conducted on 100 histopathologically confirmed cases of breast carcinoma who underwent breast conservation surgery or modified radical mastectomy in the Department of General Surgery, Maharana Bhupal Government Hospital and RNT Medical College, Udaipur, between February 2024 and July 2025. Clinical details, tumor characteristics, and histopathological findings were recorded. IHC analysis for ER, PR, and HER2/neu was performed, and results were correlated with age, tumor size, histological grade, and lymph node status. Data were analyzed using SPSS version 16, with $p < 0.05$ considered statistically significant.

Results: The mean age of presentation was 52.2 years, with most patients (53%) in the 51–60 years age group. Invasive ductal carcinoma was the most common histological type (81%), and the majority of tumors (68%) measured 2–5 cm. Stage IIB was the most frequent presentation (41%), followed by stage IIIB (23%). ER positivity was observed in 54% of cases, PR in 45%, and HER2/neu overexpression in 32%. ER and PR expression were significantly associated with older age, tumors measuring 2–5 cm, grade II tumors, and 1–3 positive lymph nodes. HER2/neu overexpression was strongly correlated with younger age, tumors >5 cm (84.3%), grade III morphology, and 4–9 positive nodes ($p < 0.001$). A strong inverse relationship was observed between hormone receptor negativity and HER2/neu positivity ($p < 0.0001$). No significant association was found between IHC markers and histological variants ($p = 0.95$).

Conclusion: ER and PR expression correlated with favorable clinicopathological features, while HER2/neu positivity was associated with aggressive disease characteristics. IHC profiling is essential for accurate prognostication and tailored treatment in breast carcinoma.

Keywords: *Breast carcinoma, Immunohistochemistry (IHC), Estrogen receptor (ER), Progesterone receptor (PR), HER2/neu.*

INTRODUCTION

Breast cancer remains the most common malignancy among women worldwide and continues to be a leading cause of cancer-related mortality. According to GLOBOCAN 2020 estimates, it accounts for approximately 2.3 million new cases and 685,000 deaths annually, making it a major global health burden.[1] In India, breast cancer has surpassed cervical cancer as the most prevalent malignancy among women, with an increasing incidence in urban populations and a

disturbing trend toward younger age at diagnosis.[2,3] The age-standardized incidence rate in India is about 25.8 per 100,000 women, and the mortality rate is 12.7 per 100,000 women.[4]

Breast carcinoma is a heterogeneous disease with varied clinical presentation, histological types, and outcomes. Although invasive ductal carcinoma not otherwise specified (IDC-NOS) accounts for nearly 70–80% of cases, other subtypes such as invasive lobular, mucinous, and medullary carcinomas are also encountered.[5] Prognosis has traditionally been assessed using parameters such as tumor size, lymph node status, histological grade, and margin involvement, with the Nottingham modification of the Bloom–Richardson grading system being widely used.[6] However, advances in molecular biology have highlighted that histopathological feature alone cannot fully explain tumor behavior and patient outcomes.

Immunohistochemistry (IHC) has emerged as a simple, reproducible, and cost-effective surrogate for molecular profiling, especially in resource-limited settings. It enables the detection of clinically relevant biomarkers, most notably estrogen receptor (ER), progesterone receptor (PR), human epidermal growth factor receptor 2 (HER2/neu), and Ki-67 proliferation index.[7,8] These markers are now an integral part of breast cancer evaluation, guiding both prognosis and therapeutic decisions. The American Society of Clinical Oncology/College of American Pathologists (ASCO/CAP) guidelines recommend routine IHC assessment of these markers in all invasive breast carcinomas.[9]

ER and PR positivity is associated with favorable prognosis and predicts response to endocrine therapy. In contrast, HER2/neu overexpression, seen in about 15–25% of cases, is associated with aggressive disease but can be effectively targeted with monoclonal antibodies such as trastuzumab.[10,11] The Ki-67 index further stratifies tumors into low- and high-proliferation categories, reflecting biological aggressiveness.[12] Based on IHC profiles, breast cancers are categorized into molecular subtypes—luminal A, luminal B, HER2-enriched, and triple- negative—each with distinct therapeutic and prognostic implications.[13]

Several studies have demonstrated correlations between IHC markers and clinicopathological variables. ER and PR expression are often linked with older age, smaller tumor size, and lower histological grade, while HER2 positivity is more frequently observed in younger patients, higher grades, and larger tumors.[14,15] Triple-negative breast cancer (TNBC), lacking ER, PR, and HER2 expression, is more prevalent in younger women in India and is associated with aggressive behavior and poorer outcomes.[16] These clinicopathological associations highlight the importance of integrating IHC markers into routine practice to enable better risk stratification and treatment planning.

Given the regional variations in incidence, age at presentation, and molecular profiles, it is crucial to generate local data that reflect the Indian population. The present study aims to evaluate the expression of IHC markers (ER, PR, and HER2/neu) in breast carcinoma and to analyze their correlation with clinicopathological parameters such as age, tumor size, histological grade, and lymph node involvement. Such data can provide valuable insights for individualized patient management and improved outcomes.

MATERIALS AND METHODS

This hospital-based cross-sectional observational study was conducted on 100 consecutive patients with breast carcinoma who underwent breast conservation surgery (BCS) or modified radical mastectomy (MRM) in the Department of General Surgery, Maharana Bhupal Government Hospital and RNT Medical College, Udaipur, Rajasthan. The study was carried out over a period of 18 months, from February 2024 to July 2025, after obtaining approval from the Institutional Ethical Committee and informed written consent from all participants.

Study Design and Setting

The study was designed as a cross-sectional observational study and conducted in the Department of General Surgery, RNT Medical College and MB Hospital, Udaipur.

Sample Size

The sample size was calculated using the formula:

$$n = (Z1-\alpha/2)2\ (100-P)\ P/d2$$

Where $Z1-\alpha/2=1.96Z\_\{1-\alpha/2\}=1.96Z1-\alpha/2=1.96$ (for 95% confidence, $\alpha = 0.05$), P = 70.37% (ER positivity rate in small tumors of 3–5 cm, as reported by Yadav P et al., Int Surg J 2022), and d = 10% absolute precision. The minimum sample size required was 80. Considering a 10% dropout rate, 88 patients were needed. A total of 100 patients fulfilling the eligibility criteria were ultimately included.

Inclusion Criteria

- Patients with histopathologically confirmed carcinoma breast.
- Patients undergoing surgical intervention in the form of BCS or MRM.
- Patients (or their relatives) who provided informed written consent.

Exclusion Criteria

- Patients with no identifiable primary tumor.
- Cases of diffuse Paget's disease of the nipple.
- In-situ malignant breast disease.
- Patients unwilling to participate.

Data Collection

All eligible patients admitted with a breast lump or breast pain and diagnosed as carcinoma breast on clinical, radiological, and histopathological evaluation were enrolled. Detailed history, clinical examination, laboratory investigations, radiological findings, operative details, and outcomes were recorded in a standardized proforma. Immunohistochemical (IHC) analysis was performed on tumor specimens to assess expression of relevant markers.

Statistical Analysis

Data were entered into Microsoft Excel (version 10) and analyzed using SPSS (version 16). Results were compiled and tabulated according to study objectives. Appropriate statistical tests were applied, and a p-value of <0.05 was considered statistically significant.

RESULT

1. Age distribution:

In present study, age of presentation ranged from 30-80years. Most common age group was found to be between 51-60years in 53%cases. And Mean age of presentation was 52.2years.

Table1. Age distribution of patients with breast carcinoma

Age group (years)	No. of patients	Percentage
≤40	21	21%
41–50	18	18%
51–60	53	53%
61–70	5	5%
>70	3	3%

2. SIZE OF TUMOUR

The majority of tumors (68%) measured 2–5 cm in diameter, while 30% were >5 cm. Only 2% of cases presented with tumors <2 cm. Most tumors were located in the upper outer quadrant (67%), followed by the upper inner quadrant (20%) and central region (12%).

Table2. Tumor size distribution

Tumor size	No. of patients	Percentage
<2 cm	2	2%
2–5 cm	68	68%
>5 cm	30	30%

3. STAGING OF BREAST CANCER:

In our study, the majority of patients 41 (41%) presented in stage IIB of breast carcinoma, which includes T2N1M0 and T3N0M0 according to the AJCC TNM staging system (8th edition). The next most common stage was IIIB, seen in 23% of patients, comprising T4N0M0, T4N1M0, and T4N2M0. A total of 12 patients (12%) were diagnosed at stage IIA, which included T2N1M0, T1N1M0, and T2N0M0. Only 2 patients (2%) presented in stage I disease.

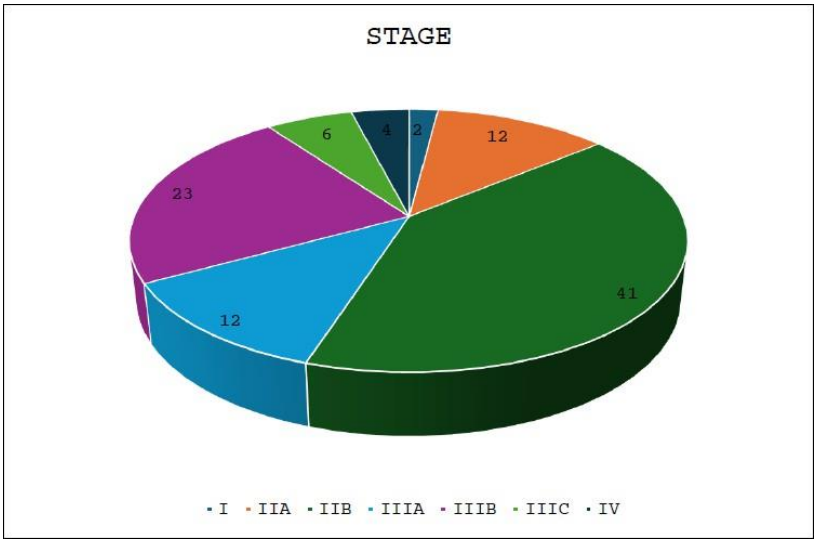

Fig 1: STAGING OF BREAST CANCER ACCORDING TO AJCC 8th EDITION

4. POSITIVE LYMPH NODE ON AXILLARY LND AND HPE OF CARCINOMA BREAST:

In present study it shown that majority of patient 46 (46%) had around 1-3 positive axillary lymph nodes on HPE, followed by 29% patients having 4-9 positive lymph nodes and only 10% having > 10 positive lymph nodes. There were around 15 patients (15%) who did not have any positive lymph nodes on HPE.

Table 3: POSITIVE LYMPH NODE ON AXILLARY LND AND HPE OF CARCINOMA BREAST

LYMPH NODE (LNs)	NO. OF PATIENTS	PERCENTAGE
NO POSITIVE LNs	15	15%
1-3POSITIVE LNs	46	46%
4-9POSITIVE LNs	29	29%
>10POSITIVE LNs	10	10%

5. HISTOPATHOLOGICAL VARIANT ON HPE AFTER SURGERY:

In histopathological examination, 81 cases (81%) had invasive duct carcinoma, 5 cases (5%) had invasive lobular carcinoma, 5 patients had invasive papillary carcinoma (5%), 6 patients had medullary type (6%) and 2 patient had inflammatory carcinoma (2%), 1 patient had mixed type (both ductal and lobular components).

Table 4: HISTOPATHOLOGICAL VARIANT ON HPE AFTER SURGERY

VARIANT	NO. OF PATIENTS	PERCENTAGE
INVASIVE DUCTAL CARCINOMA	81	81%
INVASIVE LOBULAR CARCINOMA	5	5%
PAPILLARY TYPE	5	5%
MEDULLARY	6	6%
INFLAMMATORY	2	2%
MIXED	1	1%

6. GRADE OF TUMOUR ACCORDING TO RB SCORE WITH NOTTINGHAM MODIFICATION:

In present study, around 56 patients (56%) had tumors of grade II according to RB score with Nottingham modification on HPE. 32% had tumour belonging to grade III and rest, 12% had grade I.

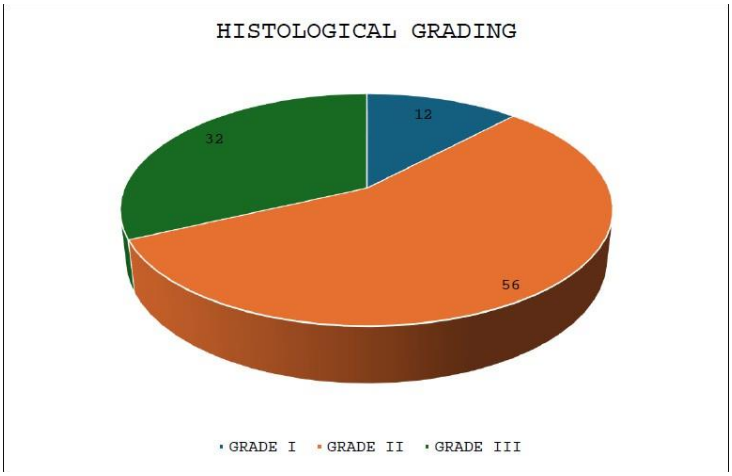

FIG 2: GRADE OF TUMOUR ACCORDING TO RB SCORE WITH NOTTINGHAM MODIFICATION

7. IMMUNOHISTO CHEMICAL MARKERS IN CARCINOMA BREAST:

In our study, estrogen receptor (ER) positivity was observed in 54% of cases, progesterone receptor (PR) positivity in 45%, and HER2/neu over expression in 32% of cases. This indicates a comparatively higher expression of hormone receptors (ER and PR) in the study population.

Table 5: IMMUNOHISTO CHEMICAL MARKERS IN CARCINOMA BREAST

IHC MARKER	NO. OF PATIENTS	PERCENTAGE
ER+	54	54%
PR+	45	45%
HER2NEU+	32	32%

8. CORRELATION OF AGE AT PRESENTATION WITH IHC MARKERS IN CARCINOMA BREAST:

Our analysis demonstrated a significant correlation between different immunohistochemical (IHC) markers and age at presentation, with the p-value indicating a highly statistically significant association. Shown in table 6 and fig 3.

Table 6: CORRELATION OF AGE AT PRESENTATION WITH IHC MARKERS IN CARCINOMA BREAST

VARIABLE	ER+ (n=54)		PR+ (n=45)		Her2Neu+ (n=32)		p-value
GRADE OF TUMOUR	N	%	N	%	N	%	
I	12	22.2%	8	17.7%	0	0%	<0.001
II	40	74%	35	77.8%	15	46.8%	
III	2	3.7%	2	4.7%	17	53.1%	

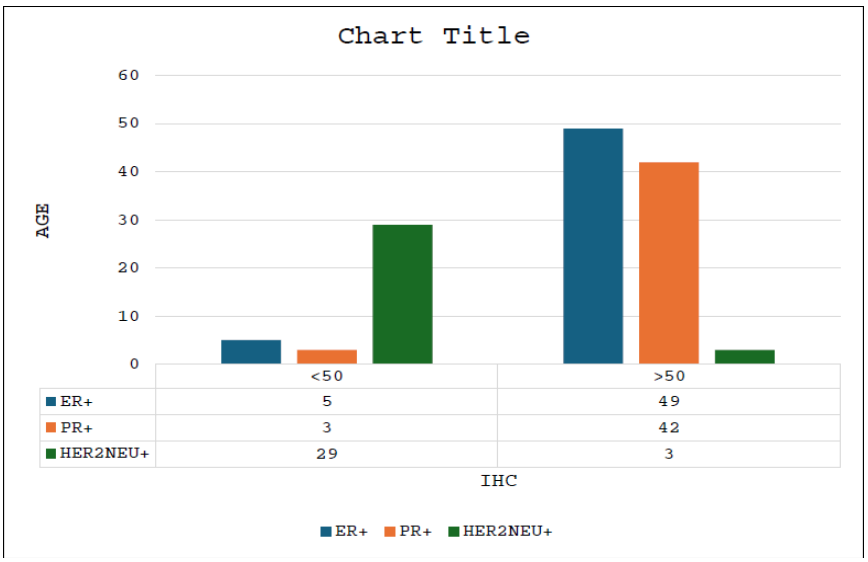

FIG 3: CORRELATION OF AGE AT PRESENTATION WITH IHC MARKERS IN CARCINOMA BREAST

9. CORRELATION OF GRADE OF TUMOUR WITH IHC MARKERSIN CARCINOMA BREAST:

In fig 4 & table 7, shows correlation of grade of tumour based on RB score with IHC markers, where p value is highly significant statistically calculated using chi square tests.

Table 7: CORRELATION OF GRADE OF TUMOUR WITH IHC MARKERSIN CARCINOMA BREAST:

VARIABLE	ER+ (n=54)		PR+ (n=45)		Her2Neu+ (n=32)		p-value
SIZE OF TUMOUR	N	%	N	%	N	%	
<2cm	2	3.7%	0	0%	0	0%	<0.001
2-5cm	50	92.6%	43	95.5%	5	15.6%	
>5cm	2	3.7%	2	4.4%	27	84.38%	

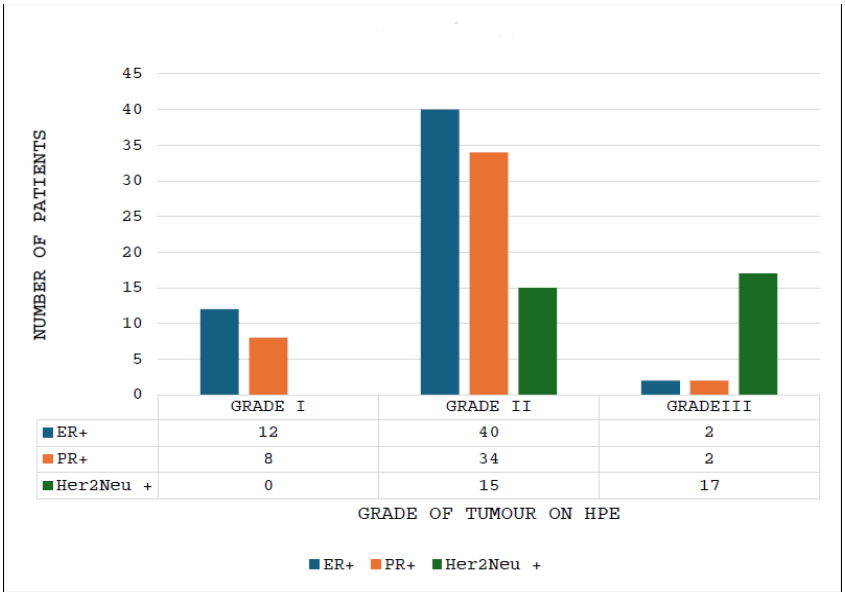

Fig 4: CORRELATION OF GRADE OF TUMOUR WITH IHC MARKERSIN CARCINOMA BREAST

10. CORRELATION OF NUMBER OF POSITIVE LYMPH NODES (LNs) WITH IHC MARKERS IN CARCINOMA BREAST:

In our study, positive correlation was seen between positive lymph nodes and the immunohistochemical receptor status (ER, PR, and Her2Neu), with a p-value of< 0.00001 as determined by the Chi-square test.

TABLE 8: CORRELATION OF NUMBER OF POSITIVE LYMPH NODES (LNS) WITH IHC MARKERS IN CARCINOMA BREAST

VARIABLE	ER+ (n=54)		PR+ (n=45)		Her2Neu+ (n=32)		p-value
NO. OF POSITIVE LNs ON HPE	N	%	N	%	N	%	
NO POSITIVE LN	15	27.78%	7	15.5%	0	0%	**<0.00001**
1-3POSITIVE LN	39	72.2%	37	82.2%	8	0%	
4-9POSITIVE LN	0	0%	1	2.2%	23	71.8%	
≥10POSITIVE LN	0	0%	0	0%	1	3.12%	

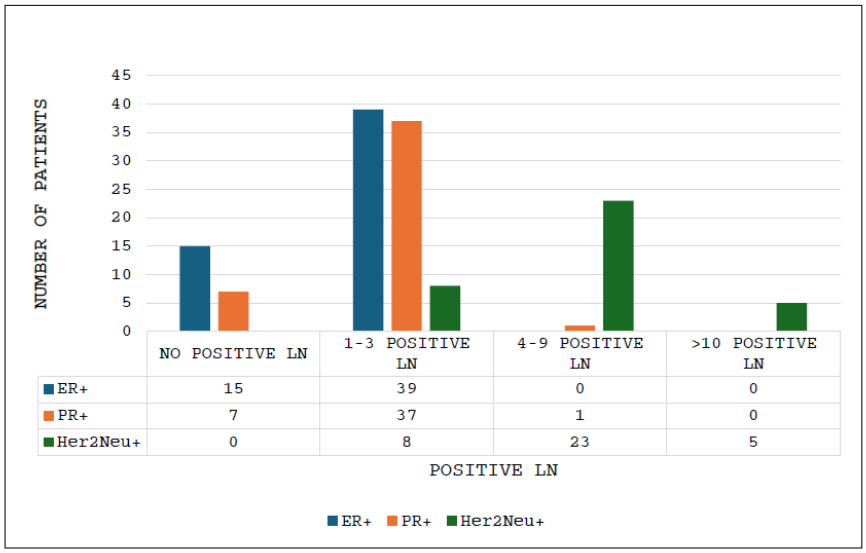

Fig 5: CORRELATION OF NUMBER OF POSITIVE LYMPH NODES (LNS) WITH IHC MARKERS IN CARCINOMA BREAST

11. CORRELATION OF HISTOPATHOLOGICAL VARIANT WITH IHC MARKERS IN CARCINOMA BREAST:

In the present study, no statistically significant association was observed between histopathological variants and immunohistochemical markers (ER, PR, and Her2Neu),
with a p value of 0.95.

Table 9: CORRELATION OF HISTOPATHOLOGICAL VARIANT WITH IHC MARKERS IN CARCINOMA BREAST

VARIABLE	ER+ (n=54)		PR+ (n=45)		Her2Neu+ (n=32)		p-value
VARIANT ON HPE	N	%	N	%	N	%	
INVASIVE DUCTAL CARCINOMA (IDC)	50	92.6%	41	91%	29	90.62%	0.95
INVASIVE LOBULAR CARCINOMA (ILC)	0	0%	0	0%	4	12.5%	
PAPILLARY TYPE	0	0%	2	4.4%	1	3.12%	
MEDULLARY	4	7.4%	0	0%	0	0%	
INFLAMMATORY	0	0%	1	2.2%	1	3.12%	

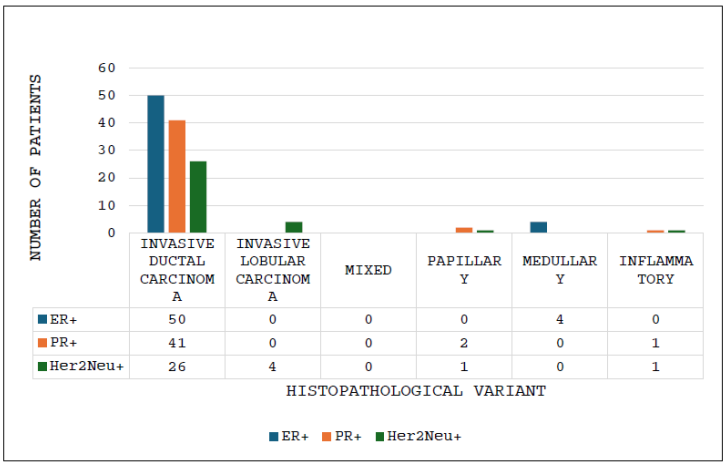

Fig 6: CORRELATION OF HISTOPATHOLOGICAL VARIANT WITH IHC MARKERS IN CARCINOMA BREAST

12. CORRELATION BETWEEN IHC MARKERS IN PRESENT STUDY:

A strong inverse correlation was observed between HER2/neu overexpression and hormone receptor status ($p < 0.0001$). The majority of hormone receptor–negative cases showed HER2/neu positivity: 65% of ER-negative and 50% of PR-negative tumors expressed HER2/neu which is represent in table 10 & fig 7:

Table 10: CORRELATION BETWEEN IHC MARKERS IN PRESENT STUDY

Her2Neu	ER+	ER-	PR+	PR-
POSITIVE	2	30	4	28
NEGATIVE	52	16	41	27
TOTAL	54	46	45	55
p-VALUE	<0.0001			

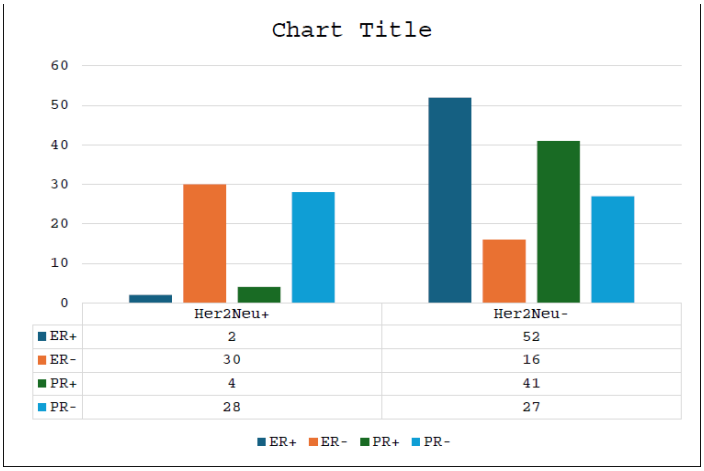

FIG 7: CORRELATION BETWEEN IHC MARKERS IN PRESENT STUDY

DISCUSSION

Breast cancer is a heterogeneous disease with variable presentation and prognosis, and the role of immunohistochemical (IHC) markers in predicting tumor behavior and guiding treatment has been well established.[7,8] In our study of 100 patients, we evaluated ER, PR, and HER2/neu expression and correlated them with age, tumor size, histological grade, and lymph node status.

The mean age of presentation was 52.2 years, with most patients in the 51–60 years group. This finding is consistent with Indian studies showing a peak incidence in the fifth decade, often younger than the Western population.[2,3,14] Postmenopausal women comprised the majority of our cases, and hormone receptor positivity (ER and PR) was significantly higher in women aged ≥51 years. Similar trends have been reported by Yadav et al. and Shet et al., where older age was associated with higher ER/PR positivity.[14,16] Conversely, HER2/neu expression was more common in younger patients, a finding supported by Ravikumar et al.[15]

In terms of tumor size, most tumors in our series measured 2–5 cm, with HER2/neu overexpression significantly associated with tumors >5 cm ($p < 0.001$). This correlation between increasing size and HER2/neu positivity reflects the aggressive biology of HER2-enriched tumors, as described in earlier studies (10,11,15). ER and PR expression, on the other hand, were predominantly observed in tumors measuring 2–5 cm, suggesting a relatively less aggressive course.

Histological grading showed that the majority of tumors were grade II, with ER and PR positivity highest in grade II carcinomas, while HER2/neu expression was predominantly seen in grade III tumors. These associations agree with findings from Indian and Western literature, where hormone receptor positivity is linked with lower grade tumors, and HER2/neu with high- grade morphology and poor prognosis.[6,14,15]

Lymph node status remains an important prognostic factor in breast cancer. In our study, ER and PR expression correlated with 1–3 positive lymph nodes, whereas HER2/neu positivity was strongly associated with 4–9 positive nodes ($p < 0.00001$). This emphasizes the aggressive potential of HER2-positive tumors, in line with earlier reports by Ravikumar et al. and Ross & Fletcher.[11,15]

A significant inverse relationship between hormone receptors and HER2/neu was observed in our study, with HER2/neu

positivity seen in 65% of ER-negative and 50% of PR-negative tumors ($p < 0.0001$). This mutual exclusivity has been described in previous studies and underlies the distinct biological subgroups of breast cancer—luminal (ER/PR-positive), HER2- enriched, and triple-negative—each with different therapeutic strategies and outcomes .[12,13]

Our findings shown that ER and PR positivity is common in older women, smaller tumors, lower grade, and fewer nodal metastases, correlating with a more favorable prognosis. In contrast, HER2/neu positivity is strongly associated with younger age, larger tumor size, higher grade, and extensive nodal involvement, indicating aggressive disease. These correlations reaffirm the importance of IHC profiling in breast carcinoma, particularly in resource-limited settings where molecular testing is not routinely feasible.

CONCLUSION

In this study, immunohistochemical profiling of breast carcinoma revealed a relatively high expression of hormone receptors (ER and PR), while HER2/neu positivity was observed in nearly one-third of cases. ER and PR expression were significantly associated with older age, smaller tumor size, lower histological grade, and limited nodal involvement, indicating a more favorable prognosis. In contrast, HER2/neu overexpression correlated with younger age, larger tumors, higher grade, and advanced nodal status, reflecting aggressive tumor biology.

The inverse relationship between hormone receptor expression and HER2/neu positivity highlights the distinct molecular subgroups of breast cancer, each with prognostic and therapeutic implications. Our findings emphasize the critical role of immunohistochemistry in routine evaluation, particularly in resource-limited settings, for guiding individualized management and improving patient outcomes.

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