

## ASSOCIATION BETWEEN THE EXPRESSION OF ESTROGEN RECEPTOR, PROGESTERONE RECEPTOR, AND HER-2/NEU AND PROGNOSTIC FACTORS IN BREAST CARCINOMA

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**Abstract**

**Background:** Breast cancer is the most commonly occurring malignancy among women all over the world. The aim of this study was to assess the expression of estrogen receptor (ER), progesterone receptor (PR), and HER-2/neu in breast carcinoma and to determine their association with tumor size, histologic grade, lymph node metastasis, and age. **Materials and Methods:** Total of 65 tissue blocks from patients with breast carcinoma who underwent mastectomy between October 2022 to June 2023 at Department of Pathology, GSVM Medical College, Kanpur, UP, India, were included in this study. Pertinent patient data, including age, tumor size, histologic grade, and lymph node status, were obtained from the pathology department. **Result:** The patients had age range of 27 to 67 years. ER and PR expressions were found to be positively correlated with each other and inversely correlated with HER-2/neu expression. Our analysis revealed a significant association between ER and PR expression and low histologic grade, whereas HER-2/neu expression was linked to high histologic grade. Furthermore, HER-2/neu expression was found to be significantly associated with lymph node involvement. However, none of these markers showed any correlation with age or tumor size. Based on our results, it can be concluded that assessing ER, PR, and HER-2/neu expression is essential for determining the prognosis of breast cancer patients and guiding therapeutic decisions. **Conclusion:** Our study provides valuable insights into the role of hormone receptor and HER-2/neu expression in breast carcinoma. Our findings may help clinicians to develop personalized therapeutic approaches for breast cancer patients. This study highlights the need for further research in this area to better understand the underlying molecular mechanisms and to develop more effective treatments for breast cancer.

## INTRODUCTION

Breast cancer is the most prevalent malignancy affecting women worldwide. In 2020, it surpassed lung cancer to become the leading cause of global cancer incidence, with approximately 2.3 million new cases reported, constituting 11.7% of all cancer cases.<sup>[1,2]</sup> Projections indicate that the global burden of breast cancer is expected to exceed 2 million cases by the year 2030.<sup>[1,3]</sup> In India, the incidence of breast cancer has shown a significant increase, rising by nearly 50% between 1965 and 1985.<sup>[4]</sup> In 2016, it was estimated that there were 118,000 new breast cancer cases in India, with females accounting for 98.1% of the cases.<sup>[5]</sup> The number of prevalent cases was reported to be 526,000. Over a span of 26 years, from 1990 to 2016, the age-standardized incidence rate of breast cancer in females increased by 39.1% across all states in the

country.<sup>[6]</sup> According to the Globocan data for 2020, breast cancer accounted for 13.5% of all cancer cases and 10.6% of all cancer-related deaths in India. The cumulative risk of developing breast cancer was estimated to be 2.81.<sup>[7]</sup> These statistics highlight the significant impact of breast cancer on a global scale, with India facing an increasing burden of the disease. Continued efforts in awareness, early detection, and effective treatment strategies are crucial in addressing this public health challenge.<sup>[8]</sup> The early detection, appropriate treatment, and genetic predisposition have a significant influence on the survival rate of patients with breast cancer. Several clinical, pathological, and molecular factors determine the prognosis of the disease, including conventional prognostic indicators such as tumor size, histologic type, grade, and lymph node metastases.<sup>[9,10]</sup> The relevance of estrogen and progesterone receptors (ER, PR), as well as HER-

2/neu, has gained significant importance in recent times, dramatically impacting the management of this malignant condition.<sup>[11]</sup> As ER/PR expression has a proven positive correlation with tumor differentiation as described in figure 1, it is highly recommended to assess the ER and PR status of biopsy specimens before initiating therapy.<sup>[12]</sup> This has become the standard practice in the treatment of breast cancer. Therefore, the evaluation of these receptors is crucial for making the correct treatment decisions for breast cancer patients.<sup>[13]</sup>

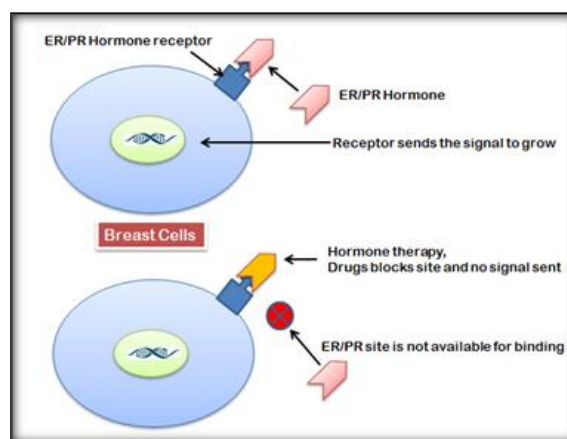
Normal breast development depends on the presence of ovarian steroids. When the balance is disturbed, it can result in abnormal processes, such as epithelial hyperplasia, intraductal carcinoma, and invasive carcinoma.<sup>[14]</sup> Estrogen, a crucial mitogen, has a significant impact by binding to its receptor (ER) and is present in around 50-80% of breast cancers. Endocrine therapies are used to combat the effects of estrogen.<sup>[15]</sup> Therapeutic hormones like Tamoxifen competitively disrupt the ER, which inhibits the transcriptional activation of genes necessary for tumor growth. The presence of hormone receptors (ER and PR) in tumor tissue is closely related to the response to hormone therapy and chemotherapy. Research has shown that approximately 55-60% of women with ER-positive tumors respond positively to hormone therapy, either as an addition or replacement, while only around 8% of women with ER-negative tumors do. Tumors that are well-differentiated are more likely to exhibit positive ER and PR status and typically have a more promising prognosis.<sup>[16,17]</sup>

The progesterone receptor (PR) is a significant surrogate indicator that reflects the functional status of estrogen receptor (ER). It plays a crucial role in predicting the characteristics of breast carcinoma.<sup>[18]</sup> Invasive breast carcinomas exhibit PR in approximately 60-70% of cases, with a higher incidence observed in older and postmenopausal women. The absence of PR expression in tumor cells is linked to a poorer prognosis, making it a valuable prognostic marker. In patients with larger tumor sizes, poorly differentiated morphology, an increased number of axillary lymph node metastases, and higher stage tumors, it is more likely that both ER and PR will exhibit a negative status. Therefore, PR expression can be considered a significant predictive factor when planning treatment and determining prognosis for patients with breast carcinoma.<sup>[19,20]</sup>

The HER-2/neu proto-oncogene, also known as C-erb B2 (HER-2), is situated on chromosome 17. Invasive breast carcinomas exhibit an amplification of this gene and an overexpression of the associated protein (HER-2) in roughly 15% to 25% of cases, which is linked to a poor prognosis. The HER-2/neu gene encodes a transmembrane glycoprotein named p185, which belongs to the family of epidermal growth factor receptors and possesses tyrosine kinase activity.<sup>[21,22]</sup>

The overexpression of HER-2/neu serves as a dependable predictor of response to trastuzumab (Herceptin), a targeted therapy. Nevertheless, it does not provide a positive indication of response to chemotherapy or overall survival. Moreover, HER-2/neu is an independent negative predictor of both overall survival and time to relapse in patients with lymph-node-positive breast cancer. The expression of this protein has been associated with a higher histologic grade, spread to axillary nodes, and an increased involvement of nodes. There is also an inverse correlation between the expression of HER-2/neu and the presence of estrogen receptor (ER) and progesterone receptor (PR).<sup>[23,24]</sup>

This study aimed to investigate the potential relationship between the expression of ER, PR, and HER-2/neu and other important prognostic factors such as tumor size, histologic grade, lymph node metastasis, and age.



**Figure 1: The role of hormone receptors as protective molecular markers for breast cancer**

## MATERIALS AND METHODS

**Study design:** In this particular cross-sectional investigation, we have carefully analyzed tissue sample of 65 patients which were procured from individuals who had undergone mastectomy and were subsequently diagnosed with breast cancer at Department of Pathology, GSVM Medical College, Kanpur, UP, India. The study period covered a duration spanning from October 2022 to June 2023. As part of our data collection process, we took detailed notes of relevant information such as the patients' ages, size of the tumor, histologic grade, and the lymph node status, which were all extracted from the patient files that were kept at the pathology department. The individuals were classified into two distinct age groups: those who were younger than 50 years of age and those who were 50 years of age or older. In addition, the size of the tumor was stratified into two categories: those that were 2 cm or smaller and those that were larger than 2 cm. To assess the histologic grading of the tumors, the Bloom-Richardson scoring system was utilized.

This system classifies tumors into grade I, II, and III based on their characteristics.

#### Immunohistochemistry:

The breast carcinoma cases included in the study underwent immunohistochemical analysis to determine their ER, PR, and HER2/neu hormonal profiles. Thin sections measuring about 4-5  $\mu\text{m}$  were deparaffinized and rehydrated. To inhibit endogenous peroxidase activity, the sections were exposed to a solution of 3% hydrogen peroxide diluted in nine parts of methanol for duration of 30 minutes. Antigen retrieval was performed using citrate buffer (pH 6.0) in a microwave[25].

The sections were then incubated overnight with primary antibodies for ER, PR, and HER2/neu, placed in a moist chamber. The following day, the sections were brought to room temperature, washed, and a secondary antibody (biotinylated anti-mouse polyclonal by Dako) was applied. The sections were re-incubated for 1 hour. To visualize the results, a chromogen (Diaminobenzidine) was added for a period of 1-3 minutes, and the sections were examined under a microscope. Hematoxylin staining was subsequently carried out for 1 minute as a counterstain. The semiquantitative evaluation was carried out for ER, PR, and HER2/neu. The H-score was used for ER and PR, where a score of  $\leq 50$  denoted a negative outcome and a score between 51 and 300 represented a positive result. For HER-2/neu, the DAKO scoring system was employed, with scores of 0, 1+, or 2+ categorized as negative, and a score of 3+ signifying a positive outcome.

**Statistical Analysis:** The data were presented in the form of the mean and standard deviation (SD). To analyze the data, we employed the SPSS software version 20 for Windows (Chicago, Illinois, USA). We utilized the chi-square test and t-test to determine the statistical significance of the findings. The level of statistical significance was set at  $P < 0.05$ . Any result that met this criterion was considered to be statistically significant. It is noteworthy that the use of these tests enabled us to present an accurate account of the research data in a scientifically rigorous manner.

## RESULTS

In the present investigation, we conducted an analysis of 65 paraffin tissue blocks that were procured from patients who had been diagnosed with breast carcinoma. The age of the patients was determined with a range of 27 to 67 years. The age group that was most frequently observed among the various age groups was found to be between 35 and 45 years, which accounted for cases (38%). Further, 75.34% of patients were below the age of 50.

On conducting histologic grading, we found that the majority of tumors (78.44%) belonged to grade II. The size of the tumors varied between 0.45 cm and 17.50 cm, and the average tumor size was found to be  $4.55 \pm 3.6$  cm. We also assessed the involvement

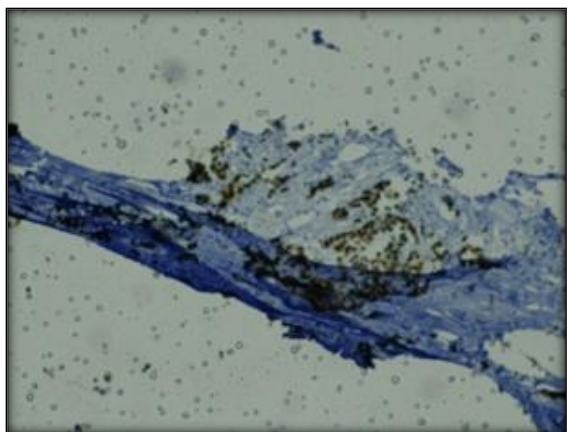
of lymph nodes and found that out of the 65 patients, lymph node metastasis was observed in 80.75% individuals (Figure 2,3 & 4).

Table 1 provides an overview of the clinical characteristics of all 65 women who were included in the study and were diagnosed with breast carcinoma. The table includes information on the age distribution of the patients, their histologic grade, tumor size, and lymph node involvement. The results from this study provide insights into the clinical characteristics of breast carcinoma and may serve as a basis for developing effective treatment strategies for this disease. This study adds to the existing body of knowledge on breast carcinoma and contributes to the ongoing efforts to improve the diagnosis and treatment of this disease. The findings from this study may also have implications for the development of new diagnostic and therapeutic modalities for breast carcinoma. Therefore, this study has significant implications for both clinical practice and research in the field of oncology.

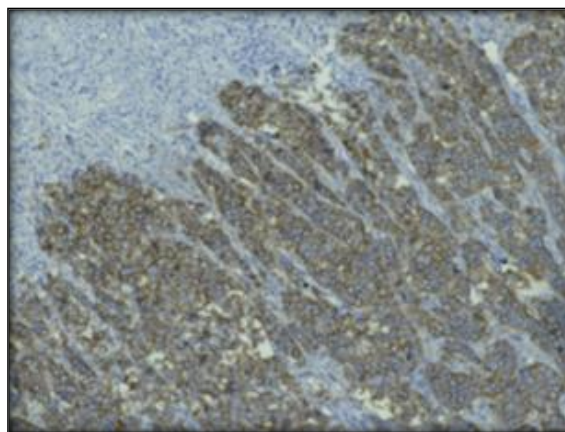
The results of our study showed a significant and positive correlation between the expression of estrogen receptor (ER) and progesterone receptor (PR) with a p-value of 0.01. Conversely, the expression of HER-2/neu exhibited an inverse relationship with ER and PR expression with a p-value of 0.001. Our findings also showed that HER-2/neu expression had a statistically significant correlation with lymph node metastasis with a p-value of 0.05. However, no such correlation was found for ER and PR.

Moreover, we discovered that ER and PR expression had a significant correlation with histologic grades I and II with a p-value of 0.05. In contrast, HER-2/neu expression demonstrated a significant correlation with histologic grade III with a p-value of 0.01. Interestingly, there was no significant correlation between the expression of these biomarkers and age or tumor size.

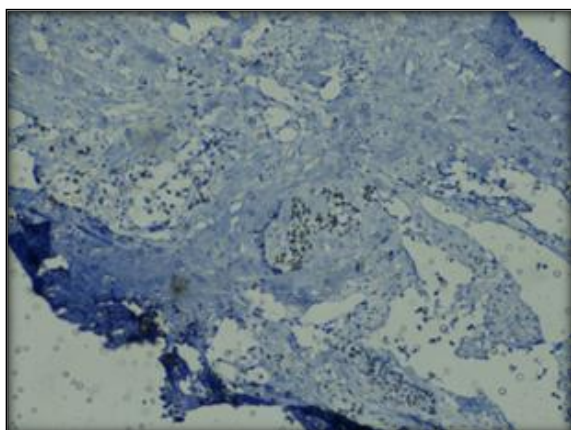
Results also suggest a complex interplay between ER, PR, and HER-2/neu expression in relation to lymph node metastasis and histologic grade, highlighting the importance of these biomarkers as prognostic factors in breast cancer. It is worth noting that age and tumor size did not significantly influence the expression of these biomarkers in the studied cohort. These findings provide a better understanding of the underlying biology of breast cancer and may guide the development of targeted therapies for patients with specific biomarker expression profiles.



**Figure 2: Immunohistochemical staining of breast cancer tissue showing positivity for ER**



**Figure 4: Immunohistochemical staining of breast cancer tissue showing positivity for HER2/neu**



**Figure 3: Immunohistochemical staining of breast cancer tissue showing positivity for PR**

**Table 1: Clinical characteristics of breast carcinoma investigated in present study**

Clinical characteristics		Ratio
Lymph node	Positive	80.75%
	Negative	19.25%
Tumor size(cm)	≤2	39.19%
	>2	60.81%
Age(years)	<50	75.34%
	≥50	24.66%
Tumor grade	I	10.11%
	II	78.44%
	III	11.45%

## DISCUSSION

Our study encompassed an examination of 65 patients who had been diagnosed with breast carcinoma and we aimed to investigate the correlation between ER, PR, and HER-2/neu expression and several prognostic factors in breast carcinoma patients. What we found was that most of the patients were less than 50 years old. This suggests that there have been an increasing number of younger women being diagnosed with breast cancer in recent years. It is imperative to note, however, that the reasons behind this trend are multifactorial, and extend beyond the scope of our study. Nonetheless, it is paramount that future investigations delve deeper into understanding these factors.

The findings of our study shed light on the unique characteristics of breast carcinoma patients in India and highlight the importance of exploring the interplay between ER, PR, and HER-2/neu expression with other prognostic factors.<sup>[26]</sup> Further knowledge in this area can help improve our understanding of breast cancer, and consequently, assist in the development of more effective strategies for diagnosis, treatment, and patient care. The results of our research also suggest that the expression of these biomarkers has a significant relationship with the size of the primary tumor, lymph node metastasis, and histological grade. Further, analysis of our data revealed that ER, PR, and HER-2/neu expressions are associated with the clinical outcomes of breast cancer patients. Our study also demonstrated that patients with negative

ER, PR, and HER-2/neu expression have a worse prognosis and are more prone to lymph node metastasis. This highlights the importance of conducting further studies in India to explore the relationship between these biomarkers and clinical outcomes.<sup>[27]</sup>

Our study provides valuable insights into the characteristics of breast carcinoma patients and emphasizes the need to investigate the relationship between ER, PR, and HER-2/neu expression and other prognostic factors. The findings of our research also highlight the need for the development of more effective strategies for the diagnosis, treatment, and patient care of breast carcinoma patients. It is hoped that our study will pave the way for future research in this area.

The current study has found a notable association between estrogen receptor (ER) and progesterone receptor (PR) expression and histologic grades I and II. This finding is consistent with the previous research on this topic. Additionally, our findings, as well as other studies, indicate a significant correlation between HER-2/neu expression and histologic grade III. It is important to note that HER-2/neu is a cell surface receptor that transmits growth signals to the nucleus, and its overexpression has been associated with a poor prognosis.

It is worth mentioning that HER-2/neu expression may reflect the underlying biological characteristics of the tumor, rather than serving solely as an independent prognostic indicator. According to our results, HER-2/neu overexpression is associated with a poorer prognosis, while ER and PR expression serve as indicators of hormone therapy response and a better prognosis. However, it is crucial to note that nodal status has a stronger association with prognosis compared to hormone receptor status.

Therefore, it is crucial to determine the hormone receptor status, as it serves as a predictive factor that influences the response or lack of response to specific therapies. In conclusion, the expression of ER, PR, and HER-2/neu can provide valuable insights into breast cancer prognosis and the response to treatment. Overexpression of HER-2/neu indicates a poorer prognosis, whereas ER and PR expression are associated with a better prognosis and hormone therapy response. Hormone receptor determination is of critical importance in guiding treatment decisions and predicting therapeutic outcomes.

In this research study, we came across an important correlation between the expression of HER-2/neu and lymph node involvement. This finding is consistent with the results of a study done by Tokatli et al. The status of lymph nodes holds a lot of importance in determining the staging of cancer and guiding physicians in making treatment decisions. The number of positive axillary lymph nodes is a major factor in predicting the prognosis of breast cancer patients. As the number of positive axillary

lymph nodes increases, the survival rate tends to decrease while the risk of relapse increases.

Numerous studies have also reported a strong association between HER-2/neu expression and lymph node metastasis, highlighting its significance in the progression of the disease. Additionally, the number of positive lymph nodes and HER-2/neu expression have been linked to prognosis, further emphasizing their role in determining the outcome of breast cancer patients. Furthermore, previous research has revealed a correlation between HER-2/neu expression and lymph node involvement as well as vascular invasion.<sup>[28,29]</sup>

Moreover, the expression of HER-2/neu has been identified as an important indicator of disease-free survival, providing valuable information for patient management and follow-up. It is important to note that accurate evaluation of HER-2/neu status is crucial in determining appropriate treatment strategies and monitoring the progression of breast cancer. Therefore, it is essential to assess HER-2/neu expression in relation to lymph node involvement, vascular invasion, and disease-free survival to fully understand the clinical significance of this biomarker.<sup>[30,31]</sup>

This study along with previous research has highlighted the importance of evaluating HER-2/neu expression in breast cancer patients. The correlation between HER-2/neu expression and lymph node involvement is significant and has a strong impact on disease progression and outcome. The number of positive lymph nodes, vascular invasion, and disease-free survival are all important factors that should be considered when assessing HER-2/neu status. Accurate assessment of HER-2/neu expression can provide valuable information for making treatment decisions and monitoring disease progression. Therefore, further research should be conducted to fully understand the clinical significance of HER-2/neu expression in breast cancer patients.<sup>[32,33]</sup>

In contrast to prior investigations, a significant correlation between tumor size and the expression of these biomarkers was not discovered in our study. Nonetheless, our discoveries are concordant with the outcomes reported by Ariga et al.<sup>[34]</sup> Despite tumor size being recognized as a prognostic factor, the reason for the variable results concerning the relationship between tumor size and these biomarkers remains unclear.

Moreover, a correlation between age and the expression of these biomarkers was not observed, which is compatible with former studies. Huang et al. conducted a study demonstrating that the expression of ER, PR, and HER-2/neu varied with age.<sup>[35]</sup> Specifically, they discovered that in young age groups, ER and PR status did not serve as predictors of HER-2/neu status. However, in older women, there was an association between hormone receptor status and HER-2/neu expression.<sup>[36]</sup>

The variability in outcomes regarding tumor size and age-related correlations with these biomarkers

indicates that supplementary factors may be influencing their interplay. Consequently, further investigation is warranted to gain a better understanding of the complexities and underlying mechanisms governing these associations in different populations and age groups.

In our investigation, a noteworthy correlation between the expression of HER-2/neu and the negative status of estrogen receptor (ER) as well as progesterone receptor (PR) was observed, which is in concurrence with the findings of Ariga et al. It has been suggested that this association may have implications for prognosis. However, it is imperative to note that other studies have indicated that ER-positive, HER-2/neu-positive status is linked with poorer survival in contrast to ER-positive, HER-2/neu-negative status. This finding implies that HER-2/neu expression may be a superior predictor of response to hormonal therapy than ER status alone. It should be noted that our investigation primarily focused on breast carcinoma, and the outcomes may not be generalizable to other forms of breast carcinoma. This limitation should be borne in mind when interpreting the results.

Overall, our findings substantiate the significant correlation between HER-2/neu expression and ER/PR negative status. Nevertheless, the interrelationship between HER-2/neu and ER status in predicting prognosis and treatment response is intricate and requires further investigation. Comprehending the interplay between these biomarkers is critical for individualized treatment approaches in patients with breast cancer.

## CONCLUSION

Our research findings have demonstrated a positive correlation between the estrogen receptor (ER) and progesterone receptor (PR), accompanied by an inverse correlation between ER/PR and HER-2/neu expression. Furthermore, a noteworthy association was observed between ER/PR positivity and low-grade tumors, while HER-2/neu expression was significantly associated with high-grade tumors and lymph node involvement. These results emphasize the importance of these biomarkers as they provide valuable prognostic information, which can aid in making informed therapeutic decisions.

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