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Predictive Marker of Disease Activity in Breast Cancer Patients with and without Metastasis

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A B S T R A C T

Introduction: Levels of the human epidermal growth factor receptor 2 (HER2) gene are low in normal breast tissue, but half of the patients with breast cancer exhibit higher levels of this receptor. The differential expression of the HER2 gene in normal and malignant cells makes it an excellent biomarker for therapeutic purposes. In this study, we evaluated the degree of HER2 overexpression in patients and its relationship with age and the occurrence of metastases.

Materials & Methods: In this retrospective, registry-based, two-center cohort study, information on 1500 breast cancer patients recruited from Shahid Mostafa Khomeini Hospital in Ilam Province was collected from 2020 to 2023.

Results: The likelihood of metastasis in cancer patients with HER2 gene expression was three times higher (adjusted OR: 2.82; 95% CI: 1.79–3.29; P=0.001). Additionally, the involvement of lymph nodes (adjusted OR: 2.01; 95% CI: 0.87–3.79; P=0.03) was significantly associated with increased metastasis.

Conclusion: This study demonstrates that HER2 gene expression and the number of involved lymph nodes are significant prognostic factors that increase the risk of metastasis. Therefore, implementing comprehensive breast cancer screenings can play an important role in the treatment and prevention of metastasis in breast cancer patients.

Keywords: Breast Neoplasms, ERBB2 Protein, Humans, Neoplasm Metastasis

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Introduction

Breast cancer, the second leading cause of death after lung cancer, is one of the most common cancers among people aged 45 to 55 (1). Each year, more than 1.1 million women worldwide are affected by breast cancer (2-4). Several factors have been identified as risk factors for breast cancer, including breast trauma, history of radiation therapy, and menopause. Monitoring certain biomarkers, such as Ki67, HER2, progesterone receptor (PR), and estrogen receptor (ER), is useful in the diagnosis and treatment of breast cancer (5).

The nucleus-associated Ki-67 antigen is a protein marker associated with significant cellular proliferation, and its expression is correlated with invasion (6). The immunohistochemical investigation of HER2, along with ER and PR, has recently become a routine clinical practice integrated into the management of breast cancer patients in Ivory Coast, where data on HER2 are scarce (7). HER2 (human epidermal growth factor receptor 2) is a proto-oncogene located on chromosome 17q, coding for the tyrosine kinase receptor found on the surface membrane of breast epithelial cells (8, 9). It affects epidermal growth factor to regulate various cellular functions, including apoptosis, cell survival, and cell proliferation. Understanding the basic mechanisms of HER2 overexpression in breast cancer has led to the discovery of new anti-HER2 targeted therapies for better management of this disease (3, 10).

The HER2 gene plays a crucial role in the diagnosis and treatment of breast cancer patients, which is important both in Iran and globally (11). Therefore, this study was conducted to compare gene expression in cancer patients with and without metastases.

Materials and methods

Sampling

This retrospective cohort study was based on registry data of 150 breast cancer patients referred to oncology clinics in Ilam province from 2020 to 2023, focusing on the use of clinical registries. Inclusion criteria were confirmed breast cancer in the pathology report, availability of tissue samples, and absence of malignancy in other body organs. Exclusion criteria were patient dissatisfaction at any stage of the study and destroyed tissue samples. The Medical Ethics Committee of Ilam University of Medical Sciences approved this research (Ethics code: IR.MEDILAM.REC.1402.140).

Laboratory data

To conduct this study, all patients referred to oncology clinics in Ilam province were evaluated, and those whose diagnosis of breast cancer was confirmed by a pathologist based on histological samples were included. Initially, informed consent was obtained from the patients, who were also given the option to withdraw from the study at any stage. A detailed history of the disease was taken from the patients included in the study, and their demographic information was recorded on the patient information collection sheet. Subsequently, the presence of metastasis was assessed through history and clinical examination, supplemented by paraclinical measures as needed, based on the patients' complaints and symptoms.

For instance, a chest x-ray (CXR) was taken for patients with complaints of shortness of breath or cough, and any effusion fluid was sent for cytology. In cases of bone pain, a bone scan was performed. Patients with hepatomegaly, right upper abdominal pain (RUQ), or liver enzyme disorders (LFT) underwent an abdominal CT scan. Additionally, patients with neurological symptoms were subjected to brain MRI to check for brain metastases.

After collecting the necessary information and assessing metastasis, the patients' tissue samples were re-evaluated by a trusted pathologist using immunohistochemical (IHC) staining for HER2 receptor analysis.

After obtaining the necessary information from the patients and examining for metastasis, the patients' tissue samples were re-evaluated by a trusted

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pathologist using immunohistochemistry (IHC) staining for the HER2 receptor. Four-micron thick sections were prepared from paraffin blocks of the patients' samples. The slides were then stained for HER2 using the streptavidin-biotin immunohistochemistry method with the DAKO kit. After staining, the stained tumor cells were evaluated by a pathologist, and the HER2 grade was determined on a scale from 0 to 3.

- Grade 0: No membrane staining or staining in less than 10% of cells.
- Grade 1: Focal membrane staining in more than 10% of cells.
- Grade 2: Complete but weak to moderate membrane staining in more than 10% of cells.
- Grade 3: Complete and strong membrane staining.

Cases graded as 0 and 1 were considered negative, while cases graded as 3 were considered positive. Cases graded as 2, being borderline, were further examined using the Fluorescence in Situ Hybridization (FISH) method to minimize false negatives. Grade 3 cases in IHC evaluation and positive cases in FISH were considered HER2 positive.

Tumor grade was analyzed based on histopathological diagnosis, age, tumor size, and lymph node involvement. Agreement tables and the odds ratio coefficient were calculated. Multiple logistic regression tests were also used for prediction. The software used for data analysis was STATA version 12 (STATA Corporation, College Station, TX).

Results

The mean age of participants in the metastasis group was 49.9 ± 7.6 years, and in the non-metastasis group, it was 51.34 ± 6.4 years. Most of the studied breast cancer patients had undergraduate or postgraduate education (33.9%), the majority were married (93.5%), and most were housewives (82.3%). None of the patients in the study reported a history of smoking, alcohol, or drug use.

The expression level of the HER2 gene was inversely correlated with the age of patients in the study, indicating that higher HER2 positivity was associated with younger age (P value = 0.04). The results of this study showed that 48% of the breast cancer patients were positive for HER2 gene expression.

Table 1. Comparison of Mean Variables and Tumor Size in Individuals with and without Metastasis.

Characteristics	Metastasis=732(%)	Non-Metastasis=768(%)	P-Value
Age	49.9± 7.6	51.34 ± 6.4	0.35
The size of the tumor (cm)	4.32 ± 2.3	3.12 ±1.01	0.164
Multiple lymph nodes are involved	7.45± 5.8	4.23 ± 4.8	0.044
The number of lymph nodes is removed	13.07±4.23	10.78 ± 4.76	0.88

Table 2. Univariate Regression Models for Factors Associated with Metastasis

Risk factors	Crude odds ratio (95% CI)	P-value
Age	1.09 (0.89-2.14)	0.23
Tumor size (cm)	0.96 (0.79-1.16)	0.69
0-20mm	1**	

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20-50mm	1.47 (1.27 – 1.65)	0.06
>50	1.62 (1.00 – 3.15)	<0.001*
Multiple lymph nodes involved		
0	1**	
<3	1.47 (1.27 – 1.65)	0.013
4-9	1.93 (0.87 – 3.79)	<0.001*
>9	1.37 (0.86– 2.69)	0.028
The number of lymph nodes removed	0.93 (0.78 – 1.11)	0.83
ER	1.57 (1.11-2.23)	0.٠39
PR	0.98 (0.93-1.02)	0.41
Grade	1.09 (0.89-1.35)	0.23
Her2	2.76 (1.43 – 3.29)	<0.001*

**Reference category
*Significant

Univariate analysis using the logarithmic distribution confirmed a significant association between HER2 level and metastasis (TR: 2.76; 95% CI: 1.43 – 3.29; $p < 0.001$), indicating an increased risk of metastasis with higher HER2 levels (Table 2).

Table 3. Comparison of Mean Variables and Tumor Size in Individuals with and without Metastasis.

Risk factors	Adjusted Odds Ratio (95% CI)	P-value
Multiple lymph nodes are involved		
0	1**	
<3	2.14 (1.27 – 1.65)	0.010
4-9	2.01 (0.87– 3.79)	<0.001*
>9	2.51 (0.86– 2.69)	0.061
Her2	2.82 (1.79 – 3.29)	<0.001*

**Reference category
*Significant

In the multivariate model adjusted for other variables, the adjusted TR for HER2 level was 2.82 (95% CI: 1.79 – 3.29; $p < 0.001$). For lymph node involvement (4-9 nodes), the adjusted TR was 2.01 (95% CI: 0.87 – 3.79; $p = 0.03$) (Table 3).

Discussion

According to the results of this study, increased HER2 gene expression in breast cancer patients in the Ilam region was associated with the development of metastases, which negatively impacts the patient's condition. The average age of the women referred to the clinics was 49.9 years, which aligns with other domestic studies (12). This average age is approximately a decade lower than in some other countries (13).

This study found that 48% of breast cancer patients were positive for HER2 gene expression, consistent with results from a study conducted in Tehran by Kadivar et al. (14). In contrast, studies in other regions have reported different percentages: 23.3% in Isfahan (15), 32% in Gilan (16), 13.6% in Arak (17), and 30.8% (18).

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Variations in HER2 overexpression reports can be attributed to differences in operator techniques, which may explain some of the discrepancies between studies. Additional evidence from Turkey (32.1%, 19), Palestine (16.1%, 20), Saudi Arabia (32.3%, 21), Ivory Coast (15.6%, 22), Japan (34.2%, 23), Kenya (17.6%, 24), Hong Kong (21%, 25), Puerto Rico (20.9%, 26), and Jordan (17.5%, 27) indicates diverse percentages of HER2 gene positivity.

This study demonstrated a positive association between HER2 gene expression and the presence of metastases. Specifically, HER2 gene expression was 2.82 times higher (OR = 2.82) in the metastasis group compared to patients without metastasis. These findings are consistent with those of Khudabakhshi et al. (28), who reported a direct relationship between HER2 overexpression and the occurrence of metastasis in breast cancer patients. Najaf et al. found that the probability of metastasis or death was 2.64 times higher in HER2-positive patients compared to HER2-negative patients, with more metastases observed in HER2-positive cases. Similarly, Mirzaei et al. found that HER2-positive patients had more advanced tumors. Although these studies support our findings, Naini et al. did not find a correlation between HER2 gene hypermethylation and tumor size or grade according to disease stage.

Estrogen receptor status has been identified as a prognostic factor in some studies (29). However, in the present study, its significance was not observed, a finding that is consistent with another research (30, 31).

In our sample, an increase in tumor size was associated with a higher relative risk of metastasis. Nevertheless, this difference was not statistically significant, aligning with the findings of Lakoska's study (33). In contrast, other studies have identified tumor size as a significant prognostic factor (29).

Given that increased HER2 gene expression is commonly observed in breast cancer patients and is associated with the development of metastases, it is crucial to further investigate the effectiveness of drugs targeting this receptor.

Diagnosing HER2 gene overexpression through tissue sample examination is both invasive and costly. Despite its importance in diagnosing breast cancer, there is a need to explore alternative methods, such as measuring serum HER2 levels, and to compare the sensitivity and specificity of these methods with histological examination (34).

HER2 overexpression is strongly associated with a high risk of recurrence, reduced overall survival, and increased mortality in breast cancer patients.

Conclusion

The results of this research provide valuable insights into the state of breast cancer among women in Ilam, specifically regarding HER2 marker expression. These findings can be utilized to develop targeted prevention and treatment strategies, enhancing the management of breast cancer in the region. The study faced limitations due to the cost of measuring HER2 serum levels and the availability of this biomarker in a limited number of patients. Consequently, the statistical population of this research was restricted. To mitigate this limitation, sampling was conducted with precision to minimize the loss of samples.

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Conflict of interest

The authors declare that there are no conflicts of interest.

Authors' contributions

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ESH developed and designed the study, collected the clinical data (NM), and drafted the manuscript. HN and ESH participated in the study conception and design, supervised the study, and critically revised the manuscript for important intellectual content. NH and AN critically revised the manuscript for important intellectual content. All authors have read and approved the final manuscript.

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