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COMPARISON OF EXPRESSION OF HUMAN EPIDERMAL GROWTH FACTOR RECEPTOR 2 (HER-2) IN BREAST CANCER TISSUE AND AXILLARY LYMPH NODES, AND OTHER PROGNOSTIC FACTORS AND OUTCOMES IN THE NORTHWEST PART OF PAKISTAN

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ABSTRACT

Background: Breast cancer is a heterogeneous disease with rising incidence and mortality worldwide. It is influenced by environmental, genetic, reproductive, dietary, and lifestyle factors and presents with diverse molecular subtypes, clinical features, and treatment outcomes. Tumor size, grade, lymph node involvement, and hormone receptor status are key prognostic indicators. This study evaluated biomarkers, specifically HER-2, in primary breast tumors and corresponding axillary lymph nodes, and examined their association with other prognostic factors.

Methods: In this prospective study, HER-2 status in breast tumors and matched axillary lymph nodes was assessed using immunohistochemistry (IHC) according to American Society of Clinical Oncology (ASCO)/College of American Pathologists (CAP) guidelines. Scores 0 and 1+ were labeled as negative, scores 2+ as equivocal, and scores 3+ as positive. A total of 110 mastectomy specimens with axillary dissection were analyzed for HER-2 concordance between primary tumors and lymph nodes. Patients were followed for one year, until December 2021. Associations between HER-2 expression and prognostic factors were analyzed using SPSS version 27.

Results: The mean age at diagnosis was 48 years. Most women were married (89%) and multiparous, with equal distribution between pre- and postmenopausal status. A majority had breastfed their children. Seventy-nine percent received neoadjuvant chemotherapy, and 7.2% had a family history of breast cancer. The most common presenting complaints were a painless lump (27%) and a painful

lump (20%). Average tumor size was 3.1 cm, with 78% being invasive ductal carcinoma and 60% being grade 2 tumors. Fifty-six percent were metastasized to the axillary lymph node at the time of diagnosis. HER-2 (3+) overexpression was observed in 17% of primary tumors and 12.7% of axillary lymph nodes. Concordance in HER-2 (3+) expression between primary tumors and lymph nodes was observed in 70% of cases. One discordant case was noted ($r = 0.510$, $p = 0.01$). Among HER-2 (3+) cases, 82.3% had lymph node metastasis detected by H&E staining, and one additional case was identified via IHC ($r = 0.353$, $p = 0.001$). Among HER-2–positive cases, 58.8% were grade 2 tumors; no cases were grade 1 ($p = 0.01$, $r = 0.526$). HER-2 expression showed a weak positive correlation with tumor size ($r = 0.373$, $p = 0.01$). Sixty-six percent were diagnosed as early stage (I and II), and of all HER-2 positive cases, 53% were early-stage disease (r=.310, p = .01HER-2 expression was significantly correlated with age ($r = 0.441$, $p = 0.01$), with most HER-2 positive cases occurring in patients under 60 years. **Conclusion:** HER-2 overexpression was more common in younger patients and associated with greater metastatic potential. While most cases showed concordance in HER-2 expression between primary tumors and lymph nodes, a few discordant cases were observed. These may affect treatment planning and highlight the importance of assessing both sites for accurate diagnosis and therapy selection.

Keywords: Expression, human epidermal growth factor receptor-2 (HER-2), breast cancer, axillary lymph nodes, prognostic factors, outcomes, breast surgery, immunohistochemistry, anatomy, Pakistan.

INTRODUCTION

Breast cancer is the most frequently diagnosed malignancy among women and remains the leading cause of cancer-related mortality globally.(1) In Pakistan, approximately 1 in 9 women is estimated to develop breast cancer during her lifetime.(2, 3) Despite higher mortality rates in Western countries, the global incidence and mortality of breast cancer are rising across both developed and developing nations.(4) (5, 6) Among Asian countries, Pakistan has the highest reported incidence of breast cancer, nearly double that of its regional neighbors. (3, 7) Mostly, Breast cancer involves postmenopausal women in developed countries, while in developing countries, including Pakistan, half of breast cancer cases are diagnosed in premenopausal women. (8, 9)

Breast cancer is a biologically complex disease influenced by a range of reproductive, genetic, environmental, dietary, and lifestyle-related factors, many of which are linked to socioeconomic status. (10) Different modifiable and non-modifiable risk factors have been identified, (11, 12) With age and female sex being the most significant. In clinical practice, tumor size, histological grade, lymph node involvement, and hormone receptor status—including Estrogen Receptor (ER), Progesterone receptor (PR), and Human Epidermal Growth factor 2 (HER-2)—are key prognostic markers that guide diagnosis, treatment planning, and outcome prediction.

Advancements in breast cancer research have led to the identification of several biomarkers, including tumor grade, hormone receptors such as ER and PR, HER-2 status, and proliferation markers like Ki-67. Additionally, genomic assays such as Oncotype DX® and MammaPrint® provide further insights into disease prognosis and treatment response. These markers offer both prognostic and predictive value, helping to assess therapeutic benefit and potential resistance. As a result, evaluating hormone receptor status on core biopsy specimens has become standard practice in clinical management. (13)

Breast cancer is a hormone-dependent tumor. Estrogen, progesterone, and associated receptors have an established role in tumorigenesis, and inhibiting this pathway is the mainstay of treatment. (14) ER is involved in cellular proliferation and has a strong prognostic and predictive marker for response to endocrine therapies. (15) Endocrine treatment for ER-positive patients may reduce the chance of recurrence and reduce mortality. (16) About 50% of breast cancer patients with an ER+ expressing cancer will respond to endocrine therapy. The tissue distribution of ER is generally visualized by immunohistochemistry (IHC) using a monoclonal antibody-based biochemical method. PR expression relates to ER, resulting in different phenotypes and a different response to hormonal

therapy. PR shows a weak prognosis but a relatively strong predictive factor for hormone therapy. (13)

HER-2 plays a vital role in regulating key cellular processes, including differentiation, proliferation, migration, and survival. (17) While HER-2 is expressed in various tissues such as the breast, colon, stomach, brain, bladder, ovaries, and skin, its overexpression is most clinically significant in breast cancer. Approximately 15–30% of breast cancers exhibit HER-2 overexpression, which is associated with more aggressive tumor behavior and poor clinical outcomes.(18) The correlation between HER-2 status and resulting outcome is complex and variable. Survival time of patients with HER-2 positive breast cancer is considerably shorter than those with HER-2 negative tumors and is a negative predictor of overall survival.(19, 20)Studies show HER-2 positive breast cancer patients display 52% improvement in disease-free survival with a well-established therapeutic target (monoclonal antibody against HER2) like trastuzumab and adjuvant chemotherapy.(21)

METHODS

This was a prospective observational study conducted over two years, from October 2018 to September 2020. Patients were followed for one year after enrollment, with final follow-up completed by December 2021. A total of 110 female patients with histologically confirmed primary malignant breast cancer were recruited from tertiary care hospitals across Khyber Pakhtunkhwa, Pakistan. All patients had undergone mastectomy with axillary lymph node dissection at the time of diagnosis. Inclusion criteria include all female patients with primary malignant breast cancer with stage 2A (involving axillary lymph nodes). Exclusion criteria include any benign disease of breast cancer, or a patient with metastatic breast disease. After obtaining informed written consent, clinical histories—including demographic, reproductive, and risk factor information—were recorded. Details of preoperative investigations, surgical findings, and metastatic workup were reviewed. Postoperative follow-up included assessment during chemotherapy and radiotherapy sessions at the patients' respective hospitals. Surgical specimens were collected intraoperatively, immediately fixed in 10% neutral buffered formalin, and sent for histopathological evaluation at the Department of Histopathology, Khyber Medical University, Peshawar. Specimens were dissected, and tissue sections were processed using standard hematoxylin and eosin (H&E) staining techniques.

Tumors were classified according to the World Health Organization (WHO) Classification of Breast Tumors, and grading was performed using the Nottingham Grading System. Immunohistochemical (IHC) analysis was performed on formalin-fixed, paraffin-embedded (FFPE) tissue blocks to assess the expression of estrogen receptor (ER), progesterone receptor (PR), and human epidermal growth factor receptor 2 (HER-2). HER-2 scoring was conducted following the American Society of Clinical Oncology/College of American Pathologists (ASCO/CAP) 2018 guidelines: Scores of 0 and 1+ were considered negative, scores of 2+ were labeled as equivocal, score of 3+ was considered positive. Cases with a 2+ (equivocal) HER-2 score on IHC were further evaluated using fluorescence in situ hybridization (FISH) to confirm gene amplification status. Final HER-2 status was determined based on combined IHC and FISH results.

All patients were followed postoperatively through their chemotherapy and radiotherapy sessions at their respective treatment centers. Physiotherapy support was also provided as part of comprehensive care. Demographic, clinical, and pathological data—including patient age, histological subtype, tumor grade, lymph node status, and expression of ER, PR, and HER-2 in both primary tumors and corresponding axillary lymph nodes—were recorded using Microsoft Excel. The dataset was coded and analyzed using IBM SPSS Statistics version 27. Associations between HER-2 expression and clinicopathological variables were assessed using the Chi-square test for categorical data and the Spearman correlation coefficient for continuous or ordinal variables.

This study was reviewed and approved by the Advanced Study and Research Board (ASRB) and the Ethical Review Committee of Khyber Medical University, Peshawar (Ref No: DIR/KMU-AS&RB/EH/000903 and DIR/KMU-EB/EH/000650).

RESULTS

A total of 124 women diagnosed with primary breast cancer were initially enrolled in the study. Of these, 2 patients were lost to migration, 12 were excluded due to the absence of axillary lymph nodes in the surgical specimen, and another 12 were lost to follow-up after surgery. The final analysis included **110 patients** with complete clinical and pathological data.

Summary of demographic and clinical features

Age (years)	
Mean ± SD (Range)	48 ± SD 10.6 (26-80)
Menarche (years)	**percent (n)**
Normal (12-16)	99.1% (109)
Late onset (after 16)	0.9% (1)
Menstrual cycle	
Regular	77% (85)
Irregular	33% (25)
Menopausal status(years)	
Premenopausal	50% (55)
Postmenopausal	50% (55)
Average age ± SD (Range)	48± SD 3.8 (37-56)
Marital status	
Married	87% (96)
Unmarried	13% (14)
Parity n=96	
Nulliparous	2%
Multipara	98%
Children	
Mean (Range)	5(1-12)
Breastfeeding n=94	
Breastfed	95% (90)
Not breastfed	5% (4)
Neoadjuvant chemotherapy	
Received	79%
Complete response	18.3%

Presenting compliant	**% of patients (n)**
1) Lump(painless) Only	27.2 (30)
2) Breast pain only	3.6 (04)
3) Nipple Discharge Only	0.9 (01)
4) Lump, pain, and Changes in the Breast	6.3 (7)
5) Lump and Pain	20.9 (23)
6) Lump and Nipple Discharge	2.7 (3)
7) Lump, pain, and Discharge	8.1 (9)
8) Lump, Breast Pain, Weight Loss, and History of Malaise	17.27 (19)
9) Lump, pain, and history of weight loss	12.7 (14)

Forty-seven percent of cases were over 50 years of age. Tumor involvement was equally distributed between the right and left breasts (50% each), and only one patient presented with bilateral breast tumors at the same time. Quadrant involvement in right side breast tumor was: upper outer quadrant

45 %, upper inner quadrant 14.5%, lower outer quadrant 7%, lower inner quadrant 3.6 % and 11% were retro areolar, and 5.45 were involving more than one quadrant. Whereas quadrant involvement on the left side was: upper outer 22%, upper inner quadrant 13%, lower outer quadrant 25%, lower inner quadrant 9% and 18% were retro areolar, while 3.6% were involving more than one quadrant. Family history of breast cancer was positive for 7% (n=7), while 11% have a history of other cancers like ovarian, lung, gastric, skin, and blood cancer.

Histopathological features of breast tissue and axillary lymph nodes

Primary tumor	
Size (cm)	
Mean	3.1 cm± SD 0.48
Range	0.2-6.5cm
T1(<2)	19 %
T2 (2-5)	52 %
T3 (>5)	14 %
Histological type	**Percentage % (n)**
Invasive ductal carcinoma	78 (86)
Invasive lobular carcinoma	5 (6)
Pathological complete response (neoadjuvant)	13 (15)
Others*	4
Grade	
Well (I)	12% (12)
Moderately (II)	58% (55)
Poorly (III)	30% (28)
Lymph Node Metastasis	
Present	56% (57)
N1 (1-3)	68% (39)
N2 (4-9)	28% (16)
N3 (>9)	4% (2)
Absent	44% (51)

HER-2 expression of Primary Breast Tissue and axillary lymph nodes

According to ASCO guidelines, primary breast tissue was scored for HER-2 protein status. In this study, 64 cases were scored as 0, +1, and were labeled as negative, while in the axillary lymph node, 52 cases were labeled as negative. Eleven cases scored +2 and were labelled as equivocal in primary breast tissue, while three cases were labelled as equivocal in the axillary lymph node. Seventeen cases scored +3 and were labelled as positive in primary breast tissue, while fourteen cases were labeled as positive in the axillary lymph node.

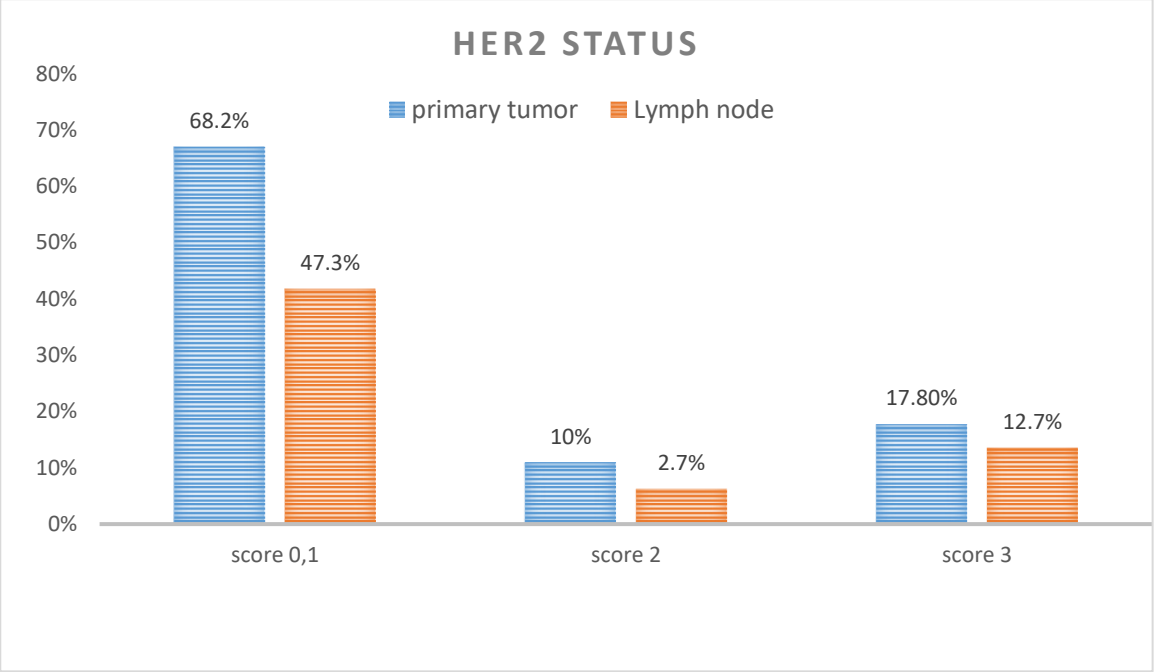

Figure: Percentages of HER-2 expression of primary breast tumor and ALN

HER-2 expression in primary breast tissue and corresponding Axillary Lymph node

The HER-2 status of primary breast tumors was compared with that of corresponding axillary lymph nodes (ALNs) to assess concordance and discordance patterns. Among patients with HER-2–negative primary tumors (score 0 or 1+, n = 64), 67.1% (n = 43) demonstrated concordant HER-2 negativity in ALNs. 28.1% (n = 18) had no metastatic involvement of the lymph nodes, while 4.7% (n = 3) exhibited discordance, with ALNs testing equivocal or positive despite negative primary tumor status. Notably, one HER-2–negative primary tumor showed HER-2 positivity in the corresponding metastatic ALN.

Among patients with HER-2–positive primary tumors (score 3+, n = 17), 70.2% (n = 12) showed concordant HER-2 positivity in ALNs. 23.5% (n = 4) had no nodal metastasis, and one case demonstrated discordance, with HER-2 positivity in the primary tumor but negativity in the ALN.

The distribution of discordant cases was statistically significant, indicating a non-random pattern of HER-2 status variation between primary and metastatic sites ($p = 0.01$, $r = 0.510$).

Relationship of HER2 status of primary breast tissue with clinicopathologic factors

HER-2 status of primary breast tissue has no significant association with age, menopausal status, histologic type, lymphovascular invasion, stage, or distant metastasis of the patient. However, a significant association is seen with grade, size, and nodal status as shown in the table.

Parameter	HER-2/neu positive	HER-2/neu Negative	HER-2/neu equivocal	p
Age years (%)				
<40(21%)	7	18	1	0.392
41-60(52.7%)	10	39	9	
≥60-80(9%)	0	9	1	
Menopausal status				
Premenopausal	9	33	6	0.940
Post menopausal	8	33	2	
Size of tumor(cm)*				
T1 <2(20.9%)	7	13	3	<0.001
T2 2-5(50.9%)	7	40	7	

T3 >5(14.5%)	3	12	1	
Grade of Tumor*				
G1	0	10	2	<0.001
G2	10	38	6	
G3	7	18	3	
Nodal Status				
N0(0)	3	4	24	
N1(1-3)	11	30	5	<0.001
N2(4-9)	1	11	2	
N3(>9)	2	1	0	
Histologic type*				
IDCa	13	53	7	
ILCa	2	2	1	0.529
Other	2	11	3	
Stage				
I (17%)	1	11	5	
II (46%)	10	30	5	0.324
III (19.5%)	3	15	0	
IV (13.3%)	3	10	1	
Lymphovascular invasion*				0.708
Present (17.7%)	2	14	2	
Absent (66.4%)	15	45	6	

*Sixteen cases (14.5%) show complete pathological response to neoadjuvant chemotherapy, IDCa Invasive Ductal Carcinoma, ILCa Invasive Lobular Carcinoma.

DISCUSSION

Breast cancer is a significant public health issue. Millions of cases are diagnosed every year, resulting in 400,000 deaths globally. (22) The risk of developing breast cancer increases with age. In this study majority of women were married with regular menstrual cycles, had breastfed their children. Most women presented with a painless lump mainly in the upper outer quadrant. A small number of patients had a positive family history of breast carcinoma. The morphology was invasive ductal carcinoma, mostly with grade 2, and more than half had already metastasized to ALNs.

According to study conducted in Iran,17% of women developing breast cancer were younger than 40 years of age, while in this study, it is estimated that 22% patients were younger than 40 year of age while another study conducted in united states estimated that 5-7% women presented with breast cancer at younger age which is low compared to our study. (1) This high percentage may be due to an increase in awareness in the young Pakistani population about breast cancer, and large young population, and a lack of screening programs, leading to an increased prevalence of this disease.

Our study shows that the percentage of HER-2 positive patients was less than 50 years of age when compared to another study, which also shows HER-2 expression in younger patients presented with breast cancer. (20) A study conducted by Nguiessanet et al shows no significant correlation of HER-2 expression with the age of the patient ($p = 0.568$). (23)

HER-2 overexpression and amplification result in the initiation, progression, and metastasis of tumor cells and are important prognostic factors. HER-2-positive tumors have a shorter survival than those that are negative for HER-2. (19) Analysis of hormone receptors in primary breast tissue is common in our set up but evaluation of receptors in ALN and metastatic site is not yet a routine practice, which may show different results and may change management of patient, In this study HER-2 status of both primary tumor and ALN were analyzed which shows that 18% of cases were positive for HER-2 expression in primary tumor as compared to another study conducted in north west area of Pakistan shows very high percentage of 49% which was very high as compared to our findings. (19)Similar

studies were conducted in Iran, which show 10% positivity for HER-2 in breast tissue and show more HER-2 positivity in the present study. (24) A study conducted in Switzerland for biomarker status at different intervals of time shows a lower frequency of positive HER-2 cases when compared to our study. Strict protocols and guidelines were used, and patients' reports were analyzed and compared using the FISH technique. (25) A study conducted by Aziun Nisa et al in Karachi shows HER-2 3+ of 24%; this high frequency of HER-2 expression was attributed to younger age at presentation. (20) Another study conducted by Mushood G. Nabi et al, in north India, shows similar results with a frequency of 15.8%. Our study suggests that overall, HER-2 positivity is increased in our population. The increase in HER-2 frequency as compared to other countries may be attributed to overrating of HER-2 expression.

Our study shows concordance in the majority of cases, while only one case showed discordance of primary tumor status, negative, which was positive for ALN. This case will not be considered for Herceptin treatment, which is a potentially useful therapy, as it only shows HER-2 expression in metastatic nodes. (24) Anna-Karin et al study shows only three such discordant cases, which indicated prognostic information. This discordance may show tumor progression; test artefacts were also proposed. Positive primary HER-2 status was linked to decreased survival (HR=2.6; P=0.03), while for HER-2 positive ALN (HR=3.0; P=0.04), suggesting ALN status can be a better predictor of early recurrence than HER-2 status of the primary tumor. (23, 26) Naoki et al study shows that discordance in HER-2 status has a bad prognosis compared to those who show concordance, and patients have improved prognosis with adjuvant chemotherapy. (27) Another study conducted to see concordance between primary breast tumor and ALN shows that the majority of cases show concordance between tissues, while a few cases show discordance. The discordance of HER-2 status can be principally explained by the biologic phenomenon of tumor heterogeneity that, even if quite rare (<5% of primary breast carcinomas), could determine the presence, in the primitive tumor, of a small neoplastic clone that not only may have a different HER-2 status, but also may acquire the genotypic and phenotypic capability to metastasize. This small neoplastic clone could be negligible in the tumor nodule and could not be detected in the immunohistochemical staining of the larger primary carcinoma; in this situation, the metastasis has a HER-2 status different from the primary carcinoma. (21) Breast cancer is a genetically and biologically heterogeneous disease, so discrepancies could be found between the expression of HER-2 in the primary tumor and metastatic ALN. (24)

In this study majority of cases were metastasized to synchronous ALN at the time of diagnosis, and HER-2 positive cases, more than eighty percent were metastatic to ALN. Studies have shown that the HER-2 status of the primary tumor is a more predictive response of the tumor than as a prognostic marker, as seen in this study. (28) According to another study conducted in Karachi, Pakistan shows the expression of HER-2 has been associated with spread to ALN. (20)

We compared our study with another study conducted in Pakistan in 2008, which also shows a strong association of HER-2 with high-grade tumors. In our study 34% HER-2 3+ cases were grade 2, and 24% were grade 3, while only 1 case was noted as grade 1when compared to another study, which shows that 41% of HER-2 3+ cases were grade 2, and 58% were grade 3, and no case for grade 1. (20) All study shows that 64% of HER-2 3+ cases were grade 3 and 34% were grade 2. (18) The present study showed that HER-2 overexpression is more related to intermediate and high-grade breast carcinoma. Nguiessan et al.'s study shows a correlation between HER-2 expression and the grade of the tumor ($p = 0.007$). This may suggest aggressive behavior of the HER-2-positive tumor. Ali Koyuncuer conducted a study in Turkey, which shows no association of HER-2 expression with grade, stage of tumor, and metastasis to lymph node. (29) Another study conducted by Shuling Zhou shows that HER-2 expression shows no significant correlation ($p > 0.05$) with size, grade, stage of tumor, and lymph node metastasis. (22) Ritu et al show HER-2/neu expression did not reveal any significant association with tumor grade ($p=0.94$). (30)

The menopausal status of our study was independent of HER-2 status, although premenopausal women overexpressed more HER-2 than postmenopausal women. Similar results were observed by another study conducted by Nguiessan et al. This lack of correlation is probably due to the relatively

short life expectancy of women in Sub-Saharan countries. Moreover, various studies have shown a lack of relationship between histological type and HER-2 status, which is in accordance with our result. (23) Another study, Nasrin et al., has found no significant correlation with the histological type of tumor and HER-2 status of the primary tumor. (30) No significant association of histological types was found with receptor expression of HER-2 primary tumor as studied by Ritun et al. (31). This study was limited by its single-region setting, modest sample size, and lack of genomic testing or longitudinal survival data. However, the prospective design and inclusion of matched lymph node analysis add strength to the findings.

The clinical relevance of HER-2 discordance lies in the potential undertreatment of patients if metastatic sites are not evaluated. In settings like Pakistan, where diagnostic and treatment resources are limited, routine assessment of HER-2 in both primary and nodal tissues may improve patient stratification and optimize use of targeted therapies.

CONCLUSION

In this study, HER-2 overexpression was observed in a subset of patients with breast cancer, particularly among younger women and those with higher-grade tumors and lymph node metastasis. While most cases showed concordant HER-2 expression between the primary tumor and axillary lymph nodes, discordance was identified in a small but clinically meaningful proportion. These findings highlight the importance of assessing HER-2 status not only in the primary tumor but also in metastatic lymph nodes, as discordant expression may impact therapeutic decisions—especially in selecting candidates for HER-2–targeted therapies such as trastuzumab. Given the high rate of late-stage presentation and limited access to screening in the region, improving diagnostic protocols—including routine receptor testing at multiple sites—may contribute to more personalized and effective breast cancer management in resource-limited settings.

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