ORIGINAL ARTICLE

Correlation between grade of the tumour and HER2NEU status in breast cancer.

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ABSTRACT… Objective: To evaluate the distribution of histological grades of breast cancer and its association with HER2/neu expression. **Study Design:** Cross-sectional study. **Period:** March 2024 to August 2024. **Setting:** Oncology, Jinnah Postgraduate Medical Center, Karachi, Pakistan. **Methods:** Patients diagnosed with breast carcinoma, with complete records for molecular subtype and hormone receptor status, and no history of recurrent, metastatic, or multiple cancers. Exclusion criteria were patients who refused participation, had benign lesions, or received neoadjuvant chemotherapy prior to presentation, were analyzed. Demographics, tumor site, histological grade, stage at diagnosis, and hormone profile of the patients were documented using a structured questionnaire. **Results:** In a total of 286 patients, 233 (81.5%) were over 40 years old, while 164 (57.3%) were overweight or obese. There were 257 (89.9%) patients who belonged rural areas of residence. The family history of breast cancer was noted among 42 (14.7%) women. A significant percentage of participants were HER2/neu positive, with expression varying across different tumor grades. Almost all patients with Grade I, Grade III, and Grade IV tumors were HER2/neu positive (100%, 99%, and 100%, respectively), while a slightly lower percentage was observed in Grade II tumors (95.9%). **Conclusion:** The nearly ubiquitous HER2/neu positivity observed across most tumor grades, with only a slight reduction in Grade II tumors, underscores the high prevalence of HER2/neu expression in this population. Routine HER2/neu testing in Pakistani breast cancer patients seems important, irrespective of tumor grade, for prognosis and treatment decisions.

Key words: Breast Cancer, HER2/neu Expression, Histology, Prognosis, Tumor.

INTRODUCTION

Breast cancer represents a diverse set of conditions that differ in clinical behavior and outcomes. Research highlights the significant role of hormone receptor status, particularly progesterone and estrogen receptors (PR, ER), along with HER-2/neu, in determining clinical prognosis.[1] These biomarkers serve as both predictive and prognostic tools in breast cancer, as they can help forecast how cancer cells might respond to hormonal treatments. Studies indicate that combining hormone receptor status with HER-2/neu expression is highly valuable in the diagnostic process for breast cancer.[2]

HER-2/neu, a proto-oncogene and part of the erbB-like oncogene family, is linked to the epidermal growth factor receptor. It is overexpressed in 15-20% of breast cancer cases and is associated with more aggressive disease progression.[3] Multiple studies have confirmed the overexpression of HER-2/neu in human breast cancer, establishing it as a key predictor of both overall survival and recurrence rates. In fact, HER-2/neu amplification is considered a more powerful prognostic marker than many traditional factors, including ER and PR status.[3] Research has demonstrated a strong correlation between patient age, tumor grade, and prognosis.[4-6] Younger women tend to have lower survival rates and higher chances of both local and distant relapse compared to older patients.[7,8] While the histological characteristics of breast cancer in younger women remain a topic of debate, age is widely recognized as a critical prognostic factor. Studies show that younger patients tend to have poorer outcomes than their older counterparts.[9,10]

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In Pakistan, 24-36% of breast cancer patients are under the age of 40, which is notably higher compared to the 12% seen in Western populations.[8] The majority of these tumors are high-grade, invasive ductal carcinoma, not otherwise specified (NOS), and in 56% of cases, they are larger than 5 cm at diagnosis.[7-10] Hormone receptor-negative tumors are more common in Pakistani women than in Western populations. One study from Pakistan analyzed 4,366 breast cancer cases, revealing that 24.6% of tumors exhibited HER-2/neu positivity.[10] Another study found an association between HER-2/neu and estrogen receptor status, as well as lymph node involvement, although no significant relationships were identified with other clinical parameters.[11]

The financial burden is increased by the scarcity of HER-2/neu diagnostic facilities, which are very expensive. An established prognostic marker called HER-2/neu may change how patients with relapsed or advanced metastatic breast cancer are treated. Thus the present study was undertaken to evaluate the distribution of histological grades of breast cancer and its association with HER-2/neu expression.

METHODS

This cross-sectional study was conducted at the department of oncology, Jinnah Postgraduate Medical Center (JPMC), Karachi, Pakistan, from March 2024 to August 2024. Inclusion Criteria were patients diagnosed with breast carcinoma who were managed and treated at the institute, those with no history of recurrent or metastatic breast cancer or multiple cancers, patients with complete records for molecular subtype of breast cancer (HER2/neu positive or negative), and patients with a complete hormone receptor status (ER, PR) profile. Exclusion Criteria included patients who refused to participate in the study, those with benign lesions, and those who received neoadjuvant chemotherapy prior to presentation. The sample size was determined to be 286 with a frequency of HER2 positive breast cancer in Pakistan being 24.6%[10], confidence level 95%, and margin of error 5%. A non-probability consecutive sampling technique was used for the study. After obtaining ethical approval from the "Institutional

Review Board (IRB)" of JPMC (No.F.2-81/2022-GENL/343/JPMC, dated: 30-12-2022), the study was commenced. Patients fulfilling the eligibility criteria were included in the study. Wemen were asked to sign a consent form for this study.

A predefined proforma and structured questionnaire were used to collect data from the participants. No personal identifiers were collected to ensure anonymity and privacy. All patients underwent physical examination and history taking by the principal investigator. The socio-demographic data, including the age of the patient, menopausal status, ethnicity, residence, as well as clinical characteristics of the patient, including stage, grade, and hormone receptor profile, were documented. "Body mass index (BMI)" was calculated and women were classified into categories: those with a BMI below 16.0 were considered severely underweight, 16.0 and 18.4 as underweight, 18.5 and 24.9 as normal, 25.0 and 29.9 as overweight, 30.0 or greater as obesity, 30.0 and 34.9 were classified as Obese Class I. For the analysis plan, data were analyzed using "IBM-SPSS Statistics, version 26.0". All continuous variables, including age and the number of children, were illustrated as mean and standard deviation. All categorical parameters, like gender, stage, and grade, were illustrated as proportions. To assess the relationship between HER2/neu status and grade of breast cancer, the chi-square test was applied taking p-value of $\leq$ .05 as statistically significant.

RESULTS

In a total of 286 patients, 233 (81.5%) were over 40 years old, while 164 (57.3%) were overweight or obese. There were 257 (89.9%) patients who belonged rural areas of residence. The family history of breast cancer was noted among 42 (14.7%) women. Table-I is showing details about the demographic characteristics of women studied.

The most frequency type of carcinoma were invasive ductal carcinoma, reported in 228 (79.7%) women. The most tumors were found at stages II, and III (41.6% and 43.4%, respectively), while the majority were grade III (72.7%). Table-II

is showing details of tumor related characteristics.

Demographic Variables		Frequency (%)
Age (years)	<25	11 (3.8%)
	25 to 40	42 (14.7%)
	>40	233 (81.5%)
Body Mass Index	Severely Underweight	24 (8.4%)
	Underweight	50 (17.5%)
	Normal Weight	48 (16.8%)
	Overweight	59 (20.6%)
	Obesity	32 (11.2%)
	Obese Class I	73 (25.5%)
Residential Status	Urban	29 (10.1%)
	Rural	257 (89.9%)
Ethnicity	Afghani	5 (1.7%)
	Balochi	11 (3.8%)
	Hindi	7 (2.4%)
	Punjabi	0
	Pushtoon	108 (37.8%)
	Sindhi	26 (9.1%)
	Urdu	44 (15.4%)
	Others	9 (3.1%)
Family History of Breast Cancer		42 (14.7%)
Marital Status	Married	159 (55.6%)
	Unmarried	41 (14.3%)
	Divorced/Separated	49 (17.1%)
	Widowed	37 (12.9%)
Menopausal Status	Premenopausal	233 (81.5%)
	Postmenopausal	53 (18.5%)

Table-I. Frequency distribution for demographic variables (N=286)

Tumor Related Characteristics		Frequency (%)
Type of Carcinoma	Invasive carcinoma with extensive intraductal component	30 (10.5%)
	Invasive ductal carcinoma	228 (79.7%)
	Invasive lobular carcinoma	20 (7.0%)
	Others	8 (2.8%)
Tumor Stage	I	4 (1.4%)
	II	119 (41.6%)
	III	124 (43.4%)
	IV	39 (13.6%)
Tumor Grade	I	1 (0.3%)
	II	74 (25.9%)
	III	208 (72.7%)
	IV	3 (1.0%)
Tumor size (cm), Mean±SD		5.42±2.62

Table-II. Tumor related characteristics (N=286)

Figure-1 shows that a very high percentage of participants (98.3%) were HER2Neu positive. Additionally, 56.6% were PR positive and 64.7% were ER positive, suggesting that a majority of the tumors were hormone receptor-positive. However, a significant portion of participants (39.9% PR negative, 31.8% ER negative) had tumors that were hormone receptor-negative.
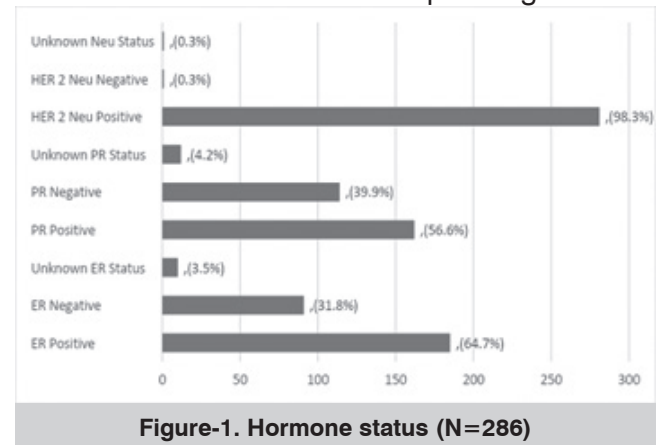

Figure-1. Hormone status (N=286)

Table-III reveals a high prevalence of HER2Neu positive status across all tumor grades: 100% in Grade I and IV, 95.9% in Grade II, and 99.0% in Grade III. Despite small variations, the P-value of 0.375 indicates that there is no statistically significant difference in HER2Neu positive status across different tumor grades. Hence, the data does not support a correlation between tumor grade and HER2Neu status in this population. This high prevalence of HER2Neu positivity across all grades suggests that HER2Neu status is not associated with the differentiation of the tumor cells (tumor grade) in this study population.

Tumor Grade	Her 2 Neu Positive		P-Value
	Yes	No	
Grade I	1 (100.0%)	-	
Grade II	71 (95.9%)	3 (4.1%)	0.375
Grade III	206 (99.0%)	2 (1.0%)	
Grade IV	3 (100.0%)	-	

Table-III. Comparison between tumor grade and Her 2 Neu positive status (N=286)
Applied Chi-Square test

DISCUSSION
The current study was designed to investigate the association between the histological grades of breast cancer and HER2/neu expression in a sample of Pakistani patients. A significant percentage of the participants were found to be HER2/neu positive, with the expression notably varying across different tumor grades.

Specifically, almost all of the patients with Grade I, Grade III, and Grade IV tumors were HER2/neu positive (100%, 99%, and 100%, respectively), while a slightly lower percentage was observed in Grade II tumors (95.9%). This highlights a high prevalence of HER2/neu positivity across all tumor grades, with only a marginal decrease in Grade II tumors. Breast cancer is a complex and multifactorial disease with varying biological characteristics and clinical behaviors. The studies mentioned provide essential insights into the molecular markers and their association with different clinicopathological parameters. Shokouh et al.[12] conducted a retrospective observational study of 566 patient records, identifying a significant correlation between age and tumor characteristics such as type, grade, and lymph node involvement. They found that tumors with a higher Ki67 index were associated with increased HER2/neu overexpression, larger tumor size, and greater lymph node involvement, suggesting a more aggressive cancer behavior. Younger patients with breast carcinoma exhibited higher rates of Ki67, HER2/neu overexpression, and p53 mutations, further indicating aggressive tumor behavior in this demographic.

Chand et al.[13] analyzed 100 cases of infiltrating ductal carcinoma and observed that most tumors were positive for estrogen and progesterone receptors (ER and PR) and negative for HER2/neu. They found significant correlations between ER and PR with patient age, tumor size, and grade, while HER2/neu expression was correlated only with tumor size. No association was noted between HER2/neu and axillary lymph node metastasis. ER and PR expression were positively correlated with each other, but neither showed a correlation with HER2/neu. Pokhrel et al.[14] investigated the diagnostic potential of serum HER2/neu testing using "chemiluminescent immunoassay (CLIA)" in 52 breast cancer patients and compared the results with "immunohistochemistry (IHC)" for HER2/neu expression. Their findings revealed a significant correlation between serum HER2 concentration and tissue HER2/neu expression, as well as histological tumor grade. Serum HER2 levels were negatively correlated with ER status but showed no association with PR status. Hussein et al.[15] conducted a clinicopathological study involving 83 paraffin blocks and histological slides of primary invasive ductal breast carcinomas. They evaluated the immunoexpression of the BRCA1 oncoprotein and its relationship with prognostic markers such as ER, PR, and HER2/neu, alongside other clinical parameters.

The study found that altered BRCA1 expression was significantly associated with advanced tumor grade and stage. Most cases with negative BRCA1 expression also demonstrated negative ER, PR, and HER2/neu expression. Gogia et al.[16] retrospectively analyzed 550 breast cancer patients and found that the majority were positive for ER/PR, with 29% of cases being HER2/neu positive. "Triple-negative breast cancer (TNBC)" accounted for 28% of the patients, many of whom were younger and presented with advanced-stage disease. Aliyu and Musa[17] studied 259 breast cancer cases in Nigeria, assessing the molecular expression patterns of ER, PR, and HER2/neu and their association with tumor variables. Though the complete results were not provided, the study aimed to understand the correlation between these biomarkers and various clinicopathological characteristics in the Nigerian population.

Routine assessment of molecular markers and clinicopathological parameters of breast cancer seems crucial for prognostic evaluation and tailoring appropriate treatment strategies for breast cancer patients. Being a single center study, our findings need further verification in large multicentric trials. Cross-sectional study designs limited the ability to follow up these women for prospective evaluation and outcomes.

CONCLUSION
In conclusion, the study demonstrates a strong association between the histological grades of breast cancer and HER2/neu expression in Pakistani patients. The nearly ubiquitous HER2/neu positivity observed across Grade I, Grade III, and Grade IV tumors, and only a slight reduction in Grade II tumors, underscores the high prevalence of HER2/neu expression in this population. Irrespective of the tumor grade, the molecular markers may have significant implications for

prognosis and treatment decisions.

CONFLICT OF INTEREST
The authors declare no conflict of interest.

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AUTHORSHIP AND CONTRIBUTION DECLARATION

No.	Author(s) Full Name	Contribution to the paper	Author(s) Signature
1	Faiza Mahar	Data collection, drafting, responsible for data's integrity, approved publication.	
2	Ghulam Haider	Study approval, Proof reading, critical revisions, approved publication.	
3	Priyanka	Data synthesis, Literature review, critical review, approved publication.	
4	Saima Zahoor	Data synthesis, Literature review, critical review, approved publication.	