Original Article

Association of Expression Patterns of ER, PR, HER-2/neu and Ki67 with Nottingham Tumor Grade in Breast Cancer Biopsies

Shireen Hamid, Muhammad Mudassar, Usama Rehman, Iqra Khalid, Sadia Hameed, Tariq Mahmood

ABSTRACT

Objective: To examine the association of expression patterns of ER, PR, HER-2/neu, and Ki-67 with Nottingham tumor grade in breast cancer biopsies.

Methodology: This was a cross-sectional study, which included 876 histologically confirmed breast carcinoma cases at Meezan Lab, Faisalabad, from January 2020 to December 2024. The Nottingham (modified Scarff-Bloom-Richardson) grading system was used to grade tumors. ER, PR, HER-2/neu, and Ki-67 were all subjected to immunohistochemistry. Pearson correlation and chi-square tests were performed, with p-values <0.05 considered statistically significant.

Results: The majority of patients were in the 40–50-year age range. The most frequent histological subtype was invasive ductal carcinoma (91.2%), and the most common tumors were Grade II (50.7%), followed by Grade III tumors (48.7%). The positivity rates of ER and PR were 50.9% and 35.5%, respectively, while that of HER-2/neu was 51.6%. A high Ki-67 proliferation index was mostly found in higher-grade (grade III) tumors. Nottingham grade had a significant positive association with ER (p<0.001), PR (p<0.001), HER-2/neu (p=0.013), and Ki-67 (p<0.001). Ki-67 had the strongest association with tumor grade.

KEYWORDS: Breast cancer; Estrogen receptor; HER-2/neu; Immunohistochemistry; Ki-67; Nottingham grade; Progesterone receptor

INTRODUCTION

Breast cancer is a heterogeneous disease that varies greatly among individuals in its biological characteristics, clinical behavior, and prognosis. Immunohistochemical biomarkers, such as the estrogen receptor (ER), progesterone receptor (PR), human epidermal growth factor receptor-2 (HER-2/neu), and Ki-67, provide essential information for prognostication and determining therapy. Their connection with the Nottingham histological grade gives significant information, especially in places where there are limited resources.

Breast cancer is the most prevalent cancer diagnosed in women globally, contributing substantially to cancer-related mortality and morbidity. It is a very different tumor from biological standpoint, characterized by diverse morphological features, molecular profiles, and other associated factors. Non-hormonal and hormonal treatments give different results in different patients, and hence, the disease has a wide clinical outcome. Classic prognostic factors such as tumor size, lymph node involvement, and

Shireen Hamid,[1] *MBBS, FCPS*
Associate Professor
Muhammad Mudassar,[2] *MBBS, FCPS*
Professor
Usama Rehman,[3] *MBBS, FCPS*
Medical Officer
Iqra Khalid,[4] *MBBS, FCPS*
District Gen. Surgeon,
Sadia Hameed,[5] *MBBS, M. Phil*
Professor
Tariq Mehmood,[6] *MBBS, M. Phil FCPS*
Professor
[1]Aziz Fatimah Medical & Dental college, FSD, PAK.
[2]Shahida Islam Medical College, LDH, PAK.
[3]Sheikh Zaid Medical College, RYK, PAK.
[4]RHC, Khurianwala, FSD. PAK.
[5]UMDC & Meezan Lab, FSD, PAK.
[6]Niazi Medical College, SGD, PAK.

Correspondence
Dr. Shireen Hamid
shirin.hamiddoc@gmail.com

histological diagnosis remain important; however, the immunohistochemical markers are now integral to modern breast cancer management and are indispensable.[1,2] The grading system brought by Nottingham, which is an alteration of Scarff-Bloom-Richardson grading, is the most commonly used method to evaluate the differentiation of tumors. It takes into account tubule formation, nuclear pleomorphism, and mitotic count, and has proven to be a good prognostic indicator in patient prognosis and survival.[3] Even though molecular profiling has progressed, the Nottingham grade still persists as a strong, inexpensive prognostic factor, very useful in settings with limited resources.[4] Immunohistochemical scoring of ER, PR, and HER-2/neu gives the most important and accurate information about the prognosis and prediction of the cancer. It determines the course of treatment, including the use of targeted anti-HER2 therapy.[5,6] In particular, the marker Ki-67, which indicates the level of cell proliferation in the tumor, has become one of the key factors of aggressiveness and molecular subtyping; moreover, characterizing the two subtypes A and B of luminal breast cancer has become difficult without this marker.[7] Numerous studies have demonstrated that a higher histological grade is associated with lower hormone receptor expression, increased HER2/neu overexpression, and a higher Ki-67 index, all of which indicate a poorer prognosis for the patient.[8-10] However, the associations have different patterns and strengths depending on the population studied. Due to the lack of regional data and the continued reliance on histopathology and immunohistochemistry in developing countries, this study was intended to assess the expressions of ER, PR, HER-2/neu, and Ki-67 and their association with Nottingham tumor grade in our population of breast cancer patients.

METHODOLOGY

This cross-sectional retrospective study was conducted at Meezan Lab in Faisalabad, Pakistan, over 5 years from January 2020 to December 2024. A total of 876 histologically confirmed cases of breast carcinoma that fulfilled the inclusion and exclusion criteria were selected for the study. The sampling technique was non-probability purposive sampling. The sample size was calculated using the formula $n=Z2p(1-p)/d2$, assuming a 95% confidence level ($Z = 1.96$), anticipated prevalence of hormone receptor and HER2/neu negative tumors as 70% based on previous literature,[1] and a margin of error of 5%. The calculated minimum sample size was 323 cases. Considering the analytical nature of the study involving multiple biomarkers and tumor grading, the sample size was increased (323 x 2 = 646) to accommodate subgroup analysis. However, as this was a retrospective study, all cases fulfilling the inclusion criteria over five years were included, yielding a final sample size of 876 cases, which substantially exceeded the minimum requirement and improved the statistical power and reliability of the findings.[1] Ethical approval (Ref# ML/EA/01-19) was obtained from the institution's ethical review committee before study initiation. Clinical data, including patient age, tumor characteristics, tumor grade, and histological subtypes, were retrieved from histopathology records. All specimens were preserved in 10% neutral buffered formalin, processed routinely, and embedded in paraffin. Tissue sections measuring 3–4 μm in thickness were stained with hematoxylin and eosin (H&E). Tumors were classified according to the World Health Organization (WHO) classification of breast tumors and graded using the Nottingham (modified Scarff–Bloom–Richardson) grading system into Grade I, II, and III based on tubule formation, nuclear pleomorphism, and mitotic activity.

Immunohistochemical staining was performed on formalin-fixed, paraffin-embedded tissue sections following standard protocols. Primary antibodies targeting ER, PR, HER2/neu, and Ki-67 were applied. Estrogen receptor (ER) and progesterone receptor (PR) expression were considered positive when staining was present in ≥1% of tumor cell nuclei, in accordance with ASCO/CAP guidelines. HER2/neu scoring was performed using standard

criteria (0, 1+, 2+, 3+) based on the intensity and pattern of membranous staining. Equivocal (2+) cases were recorded separately and confirmed using fluorescence in situ hybridization (FISH). The Ki-67 index was reported as the percentage of positively stained tumor nuclei and categorized into predefined ranges.

Data was entered and the analysis was done by using the Statistical Package for Social Sciences (SPSS) version 27. Frequencies and percentages for categorical variables were calculated using descriptive statistics. The relationship of Nottingham tumor grade to immunohistochemical markers was examined through Pearson's correlation coefficient. A p-value of 0.05 or less was interpreted as statistically significant.

All adequately fixed tissue blocks from breast cancer biopsies received in the pathology department were included in the study. Poorly preserved or inadequate tissue blocks, recurrent breast carcinomas, and patients who had received neoadjuvant chemotherapy were excluded from the study. Each immunohistochemical run had positive and negative controls. The internal controls were checked to determine if the staining was sufficient. All slides were independently examined by two histopathologists to lessen the possibility of observer bias.

RESULTS

The study included 876 cases of breast carcinoma for analysis, with the majority of patients falling within the 40–50-year age group. The Nottingham grading system showed that grade II tumors represented the highest frequency with 444 cases (50.7%), followed by grade III tumors, 427 cases (48.7%). Grade I tumors were extremely rare, with only 5 cases (0.6%), as shown in Table I.

Most cases were invasive ductal carcinoma, accounting for 799 (91.2%) of the total 876 cases. The study found that lobular carcinoma was present in 26 cases, whereas mucinous carcinoma appeared in 20 cases.

Table I: Frequency of Nottingham Grades in Breast Cancer Biopsies

		Frequency	Percent
Valid	Grade I	5	0.6
	Grade II	444	50.7
	Grade III	427	48.7
	Total	876	100.0

Less frequent histological subtypes included medullary carcinoma (13 cases), metaplastic carcinoma (9 cases), papillary carcinoma (3 cases), cribriform carcinoma (1 case), pleomorphic lobular carcinoma (4 cases), and adenoid cystic carcinoma (1 case).

The Ki-67 proliferation index showed different results for each tumor subtype. The study found that invasive ductal carcinoma showed higher Ki-67 values. The study found that most of the metaplastic and medullary carcinomas showed higher proliferative indices, 70-80% and 60-70% respectively which matched their aggressive characteristics, while most other histological subtypes showed lower Ki-67 expression, as shown in Table II.

Table II: Association of Histological Subtypes with Ki-67 (Crosstabulation)
Carcinoma × Ki-67 (Count)

		Ki67									Total
		0 - 10 %	10 - 20 %	20 - 30%	30 - 40 %	40 - 50 %	50 - 60 %	60 - 70 %	70 - 80 %	80 - 90 %	
Carcinoma	Ductal carcinoma	172	113	131	66	52	72	76	62	55	799
	Lobular carcinoma	15	2	4	2	0	1	2	0	0	26
	Metaplastic carcinoma	1	0	1	0	0	1	3	1	2	9
	Pleomorphic lobular carcinoma	1	0	0	0	1	0	2	0	0	4
	Cribriform carcinoma	1	0	0	0	0	0	0	0	0	1
	Mucinous carcinoma	17	1	0	1	0	0	0	0	1	20
	Medullary carcinoma	3	1	1	0	0	0	4	1	3	13
	Papillary carcinoma	3	0	0	0	0	0	0	0	0	3
	Adenoid cystic carcinoma	1	0	0	0	0	0	0	0	0	1
Total		214	117	137	69	53	74	87	64	61	876

Table III displays the distribution of ER, PR, and HER-2/neu status across Nottingham tumor grades. Grade II tumors show the highest ER positivity with a rate of 63.7%, which decreases to 37.5% in grade III tumors. The highest PR positivity occurred in grade II tumors 43.5% which decreased to 27.2% in grade III tumors. The HER-2/neu positivity increased with tumor grade, being most frequent in grade II (56.1%) and remaining high in grade III tumors (47.1%). Higher-grade tumors contained more equivocal HER-2/neu results, particularly grade III tumors, which showed a rate of 6.8%. Tumor grade elevation from grade II to grade III resulted in decline in overall ER and PR hormone receptor expression, decreasing from 63.7% to 37.5%.

The analysis using the Pearson correlation method found multiple relationships that showed statistically significant results. The results showed that higher tumor grade (Grade III) was associated with an increased Ki-67 proliferation index (80–90%), which demonstrated a positive association between both variables ($r = 0.284$, $p < 0.001$).

Table III: Association of ER, PR, and HER-2/neu Immunohistochemical Expression with Nottingham Tumor Grade (n = 876)

Marker	Status	Grade I n (%)	Grade II n (%)	Grade III n (%)	Total n (%)
ER	Positive (≥1%)	3 (60.0)	283 (63.7)	160 (37.5)	446 (50.9)
	Negative (<1%)	2 (40.0)	161 (36.3)	267 (62.5)	430 (49.1)
PR	Positive (≥1%)	2 (40.0)	193 (43.5)	116 (27.2)	311 (35.5)
	Negative (< 1%)	3 (60.0)	251 (56.5)	311 (72.8)	565 (64.5)
HER-2/neu	Positive (3+)	2 (40.0)	249 (56.1)	201 (47.1)	452 (51.6)
	Negative (0/1)	3 (60.0)	172 (38.7)	197 (46.1)	372 (42.5)
	Equivocal (2+)	0 (0.0)	23 (5.2)	29 (6.8)	52 (5.9)

The study found that tumor grade showed a weak but significant relationship with ER ($r = 0.183$, $p < 0.001$) and PR ($r = 0.167$, $p < 0.001$) because grade II tumors showed higher rates of hormone receptor positivity. The study found that HER-2/neu expression showed a weak positive relationship with tumor grade ($r = 0.084$, $p = 0.013$). The study found that 30-40 age group had higher Ki-67 levels (60-70%) and accounted for 50.5% of Grade III tumors.

DISCUSSION

Breast cancer is highly heterogeneous and characterized by varying histopathological and immunophenotypic features that have a huge impact on the prognosis and the choice of therapy. In the current research, the most common histological subtype was invasive ductal carcinoma (IDC), which comprised 91.2% of cases. This result is in line with the studies done worldwide and, in the region, including the works of Adedokun et al. and Ravi et al., who have reported IDC frequencies from 85% to 92%.[1,3,5] The increased number of IDC cases has also been evaluated in recent molecular subtype-based analyses by Al-Thoubaity et al. and Hussain et al.[23,25] Nottingham grade II tumors were the most common, followed by grade III tumors, which is consistent with findings reported from Pakistan, India, and Nepal as documented by Saleem et al., Thakuria et al., Senel, Khan et al., Kurkoosh et al.[4,7,9,14,15] On the other hand, Western studies tend to have a greater proportion of grade I tumors, which is probably due to increased early diagnosis because of the established screening programs, as by Webster et al.[8] A robust connection was found between Nottingham tumor grading and immunohistochemical parameters. The ER positivity rate of 50.9% and PR positivity rate of 35.5% were similar to the data from regional studies that indicated ER positive cases in the range of 45–55% and PR positivity between 30–40%.[3,4,7,9,21,25] These figures are lower than those of Western populations (60–75%), which might be due to factors such as ethnicity and heredity, younger patient age at diagnosis, higher tumor grade, and delays in diagnosis in countries with

fewer medical resources.[6,8] Consistent with existing literature, an inverse relationship was observed between tumor grade and hormone receptor expression, with significantly reduced ER and PR positivity in high-grade tumors.[1,7,10] Thakuria et al. reported hormone receptor expression to be lower in grade III tumors compared to I and II, which is consistent with the present study.[7]

There was a positive correlation between higher tumor grade and HER-2/neu overexpression, as well as an increased Ki-67 proliferation index, which together reflected aggressive tumor biology. HER-2/neu positivity found in the present study (51.6%) was much higher than that in Western studies (15–25%)[6,11,12] but was similar to that reported from South Asia and the Middle East (35–55%) by Ravi et al., Rakha & Ellis, Khan et al., and Jummah AS et al.[3,14,19] Moreover, Adedokun et al. and Kapoor et al. have pointed out a significant relationship between HER-2/neu overexpression and higher Nottingham grade. Therefore, it is established as a marker of aggressive disease.[1,6,13,14] Reasons for variations in HER-2 positivity among different studies could include differences in scoring systems, fixation protocols, antibody clones, and limited availability of confirmatory FISH testing. In the past, HER-2 overexpression was linked to poor prognosis; however, with the availability of targeted therapies, the outcomes in this subgroup can be improved.[16]

The Ki-67 proliferation index was the major parameter showing the greatest association with Nottingham grade in the present research. Ki-67 was found to be mostly expressed in grade III tumors, which is consistent with the findings of Sharma et al., & Inwald et al.[17,18] Ki-67 showed a stronger correlation with tumor grade than hormone receptors taken separately, thus confirming its value as an important marker of tumor aggressiveness. Although there are differences in Ki-67 cut-off values among the studies, the common observation of rising Ki-67 expression with increasing tumor grade is nevertheless indicative of its prognostic value.[16,18,22,24] High Ki-67 index correlates with unfavorable prognostic factors and shorter survival outcomes.[7,18,24]

There was also a correlation found between the younger age at presentation, higher tumor grade, and increased proliferative activity. This observation is in accordance with the study conducted by Kurkoosh et al., which said that, besides being younger, breast cancer in women has a higher likelihood of displaying aggressive pathological and molecular characteristics.[15] This contrasts with the West, where the breast cancer diagnosis is usually at an older age, and the tumor biology is relatively favorable, largely due to widespread screening and early detection.[8]

In general, the results support the combined use of histological grading and immunohistochemical profiling in breast cancer evaluation. The close relationship between Nottingham tumor grade and the expressions of ER, PR, HER-2/neu, and Ki-67 points out that these parameters are still relevant for prognosticating and treatment planning purposes when used together. This method is practical and affordable for assisting clinical management in situations where molecular testing is not easily available.

CONCLUSION

Higher tumor grades in breast carcinoma were associated with lower ER/PR positivity, higher HER-2/neu expression, and increased Ki-67 proliferation, indicating more aggressive disease and poorer prognosis. Histological grading combined with immunohistochemical markers provides valuable prognostic information and may serve as a surrogate for molecular subtyping, particularly in resource-limited settings. The predominance of high-grade and highly proliferative tumors in younger patients highlights the need for early detection and tailored treatment.

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Author Contributions:

Shireen Hamid: Conceived the study designed, carried out the data collection and statistical analysis and drafted the manuscripts

Muhammad Mudassar: Conceived the study designed, carried out the data collection and statistical analysis and drafted the manuscripts

Usama Rehman: Conceived the study designed, carried out the data collection and statistical analysis and drafted the manuscripts

All authors are equally accountable for research work

Iqra Khalid: Conceived the study designed, carried out the data collection and statistical analysis and drafted the manuscripts

Sadia Hameed: Conceived the study designed, carried out the data collection and statistical analysis and drafted the manuscripts

Tariq Mahmood: Conceived the study designed, carried out the data collection and statistical analysis and drafted the manuscripts

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