

EVALUATION OF IMMUNOHISTOCHEMICAL BIOMARKERS (KI-67, P53, AND HER2) IN BREAST CARCINOMA: CORRELATION WITH TUMOR GRADE AND PROGNOSTIC OUTCOMES

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ABSTRACT:

Background: Breast carcinoma had remained one of the most common malignancies affecting women, and accurate assessment of tumor biology had been essential for predicting disease behavior and guiding treatment. Immunohistochemical biomarkers such as Ki-67, p53, and HER2 had provided valuable information regarding cellular proliferation, tumor suppressor abnormalities, and receptor status. Their association with histopathological tumor grade had been clinically relevant for prognostic assessment.

Aim: The study aimed to evaluate the expression patterns of Ki-67, p53, and HER2 in breast carcinoma and to determine their correlation with histopathological tumor grade and prognostic outcomes.

Methods: This hospital-based observational study was conducted at Mayo Hospital, Lahore, from August 2025 to January 2026. A total of 80 patients with histopathologically confirmed breast carcinoma were included. Tumor specimens were evaluated for histological grade, while immunohistochemical staining was performed for Ki-67, p53, and HER2. Ki-67 expression was assessed according to the percentage of positively stained tumor cells, whereas p53 expression was evaluated based on staining intensity and proportion of positive cells. HER2 expression was interpreted according to established immunohistochemical scoring criteria. Associations between biomarker expression, tumor grade, and recorded prognostic parameters were analyzed statistically.

Results: Among the 80 breast carcinoma cases, higher tumor grades were associated with increased Ki-67 proliferative activity and greater p53 expression. HER2 positivity was also observed more frequently among higher-grade tumors. Tumors demonstrating high Ki-67 expression showed a greater proportion of aggressive histopathological features, while p53 overexpression was associated with poorer tumor differentiation. HER2-positive tumors demonstrated higher-grade pathological characteristics compared with HER2-negative tumors. The combined assessment of Ki-67, p53, and HER2 provided additional information regarding tumor biological aggressiveness and prognostic characteristics.

Conclusion: The study demonstrated that Ki-67, p53, and HER2 immunohistochemical biomarkers had shown meaningful associations with tumor grade and prognostic characteristics in breast carcinoma. Their combined evaluation alongside conventional histopathological grading had provided useful information for assessing tumor aggressiveness and supporting prognostic stratification.

KEYWORDS: Breast carcinoma; Ki-67; p53; HER2; Immunohistochemistry; Tumor grade; Prognosis; Breast cancer biomarkers.

INTRODUCTION:

Breast carcinoma had remained one of the most frequently diagnosed malignancies among women and had represented a major cause of cancer-related morbidity and mortality worldwide. The disease had demonstrated considerable biological and clinical heterogeneity, with tumors differing substantially in their morphological characteristics, molecular profiles, rates of proliferation, responses to treatment, and clinical outcomes [1]. Histopathological evaluation had remained the fundamental approach for establishing the diagnosis and determining tumor grade; however, conventional morphological assessment alone had not always provided sufficient information regarding tumor behavior and prognosis. Consequently, immunohistochemical (IHC) biomarkers had increasingly been incorporated into the pathological assessment of breast carcinoma to provide additional information about tumor biology and therapeutic implications.

Tumor grade had served as an important prognostic parameter because it had reflected the degree of cellular differentiation, nuclear abnormalities, and mitotic activity within breast carcinoma [2]. Higher-grade tumors had generally demonstrated more aggressive biological behavior and had been associated with an increased risk of recurrence and poorer clinical outcomes. Nevertheless, tumors with similar histological grades had sometimes

shown different clinical courses, indicating that additional molecular and proliferative markers had been required for more comprehensive prognostic assessment [3]. IHC evaluation had therefore provided a practical method for characterizing specific molecular alterations and proliferative patterns within breast tumor tissues.

Ki-67 had been recognized as an important marker of cellular proliferation and had been expressed during active phases of the cell cycle but not during the resting phase. The proportion of tumor cells showing Ki-67 positivity had been used as an indicator of proliferative activity. Increased Ki-67 expression had frequently been associated with higher tumor grade, increased aggressiveness, and less favorable outcomes in breast carcinoma [4]. Assessment of Ki-67 had therefore provided valuable information regarding the biological activity of a tumor and had potentially complemented conventional histopathological grading.

The tumor protein p53 had also played an important role in the biological behavior of breast carcinoma. The TP53 gene had encoded a tumor-suppressor protein that had contributed to the regulation of cell-cycle progression, DNA repair, and apoptosis. Alterations in TP53 had impaired these protective mechanisms and had promoted uncontrolled cellular proliferation and malignant progression [5]. Abnormal p53 protein accumulation detected through IHC had been considered a surrogate indicator of underlying TP53 abnormalities in many breast carcinomas. Increased or aberrant p53 expression had been associated in several studies with higher tumor grade, aggressive pathological characteristics, and unfavorable prognosis, although the strength of these associations had varied according to tumor subtype and assessment criteria [6].

Human epidermal growth factor receptor 2 (HER2) had represented another clinically important biomarker in breast carcinoma. HER2 had been a transmembrane receptor involved in cellular growth and proliferation, and amplification or overexpression of HER2 had characterized a clinically significant subgroup of breast cancers. HER2-positive tumors had historically been associated with more aggressive disease characteristics and poorer outcomes in the absence of targeted treatment [7]. However, identification of HER2 status had become particularly important because HER2-targeted therapies had substantially improved treatment outcomes for appropriately selected patients.

The combined evaluation of Ki-67, p53, and HER2 had therefore provided a broader assessment of proliferative activity, tumor-suppressor abnormalities, and receptor-related biological behavior. Examining the expression of these biomarkers in relation to histological tumor grade had potentially improved understanding of the biological heterogeneity of breast carcinoma. Such an assessment had also been particularly relevant in hospital-based populations where pathological and immunohistochemical findings had guided prognostic assessment and treatment planning [8].

The present study had therefore been conducted to evaluate the immunohistochemical expression of Ki-67, p53, and HER2 in breast carcinoma and to determine their relationship with tumor grade and prognostic outcomes. The study had aimed to assess whether variations in these biomarkers had corresponded with differences in pathological characteristics and had provided additional prognostic information beyond conventional histopathological evaluation [9].

MATERIALS AND METHODS:

Study Design and Setting

A hospital-based observational study was conducted at the Department of Pathology, Mayo Hospital, Lahore. The study was carried out over a period of six months, from August 2025 to January 2026. The study was designed to evaluate the immunohistochemical expression of Ki-67, p53, and HER2 in breast carcinoma and to determine their relationship with histopathological tumor grade and selected prognostic characteristics.

Study Population and Sample Size

The study population consisted of 80 patients who had histopathologically confirmed breast carcinoma and underwent diagnostic or therapeutic breast tissue sampling at Mayo Hospital, Lahore, during the study period. A consecutive sampling technique was used to include eligible cases. Patients of different age groups and histological grades were included to allow assessment of biomarker expression across the spectrum of tumor differentiation.

Inclusion and Exclusion Criteria

Patients were included if they had a confirmed diagnosis of primary breast carcinoma on histopathological examination and had adequate formalin-fixed, paraffin-embedded tissue available for immunohistochemical analysis. Cases with sufficient clinical and histopathological information for tumor grading were also included. Patients were excluded if tissue samples were inadequate, poorly preserved, or unsuitable for immunohistochemical staining. Cases with recurrent breast carcinoma, metastatic tumors to the breast from another primary site, or incomplete pathological records were also excluded.

Histopathological Examination

Representative tissue specimens were fixed in 10% neutral buffered formalin, processed routinely, and embedded in paraffin wax. Sections of approximately 3–4 μm thickness were prepared and stained with hematoxylin and eosin. Histopathological examination was performed to confirm breast carcinoma and determine the histological type and tumor grade. Tumor grading was assessed according to the Nottingham histological grading system, which incorporated tubule formation, nuclear pleomorphism, and mitotic activity.

Immunohistochemical Assessment

Immunohistochemical staining was performed on selected paraffin-embedded tissue sections for Ki-67, p53, and HER2 biomarkers using standardized laboratory protocols. Appropriate positive and negative controls were included with each staining batch to ensure staining quality and reliability.

Ki-67 expression was assessed by determining the percentage of tumor cell nuclei showing positive staining. Cases were categorized according to the proportion of positively stained tumor cells into low- and high-proliferative groups using a predefined cutoff. p53 expression was evaluated according to the percentage and intensity of nuclear staining and was categorized into low and high expression groups according to the predefined study criteria. HER2 expression was assessed by evaluating membranous staining intensity and completeness in invasive tumor cells. HER2 results were classified according to established immunohistochemical scoring criteria, with equivocal cases identified separately where applicable.

Data Collection

Relevant demographic and clinicopathological information was obtained from patient records and pathology reports. Variables included age, histological type, tumor grade, and immunohistochemical expression of Ki-67, p53, and HER2. Where available, selected prognostic characteristics were also recorded. All collected information was entered into a structured data collection form before statistical analysis.

Statistical Analysis

The collected data were entered and analyzed using SPSS version 26.0. Categorical variables were presented as frequencies and percentages, while continuous variables were summarized using mean and standard deviation. Associations between Ki-67, p53, and HER2 expression and tumor grade were assessed using the chi-square test or Fisher's exact test where appropriate. Associations between biomarker expression and selected clinicopathological characteristics were also evaluated. A p-value of <0.05 was considered statistically significant.

Ethical Considerations

The study was conducted after obtaining approval from the relevant institutional ethical/research committee. Patient confidentiality was maintained throughout the study, and identifying information was not included in the research database or final analysis. The study procedures were performed in accordance with accepted ethical principles for research involving human tissue and clinical records.

RESULTS:

A total of 80 patients with histopathologically confirmed breast carcinoma who were evaluated at Mayo Hospital, Lahore, from August 2025 to January 2026 were included in the study. Immunohistochemical staining for Ki-67, p53, and HER2 was performed and the findings were correlated with histological tumor grade and selected prognostic characteristics. The patients had a mean age of 51.8 ± 10.6 years, with most cases occurring between 41 and 60 years of age. Invasive ductal carcinoma constituted the predominant histological type.

Table 1. Distribution of patients according to tumor grade and immunohistochemical biomarker expression (n=80):

Variable	Category	n	%
Tumor grade	Grade I	18	22.5
	Grade II	38	47.5
	Grade III	24	30.0
Ki-67 expression	Low (<20%)	25	31.3
	Intermediate (20–40%)	27	33.8
	High (>40%)	28	35.0
p53 expression	Low/negative (<10%)	29	36.3
	Intermediate (10–50%)	24	30.0
	High (>50%)	27	33.8
HER2 expression	Negative (0/1+)	49	61.3
	Equivocal (2+)	8	10.0
	Positive (3+)	23	28.8

Table 1 showed that Grade II tumors were the most frequently observed histological category, accounting for 38 (47.5%) cases, followed by Grade III tumors in 24 (30.0%) and Grade I tumors in 18 (22.5%) cases. Ki-67 demonstrated variable proliferative activity. Low expression was observed in 25 (31.3%) cases, intermediate expression in 27 (33.8%), and high expression in 28 (35.0%) cases. Thus, a relatively greater proportion of tumors demonstrated high proliferative activity.

p53 expression was low or negative in 29 (36.3%) cases, intermediate in 24 (30.0%), and high in 27 (33.8%) cases. HER2 was negative in 49 (61.3%) patients, equivocal in 8 (10.0%), and positive in 23 (28.8%) patients. The distribution indicated that HER2-negative tumors predominated in the study population.

Table 2. Association of Ki-67, p53, and HER2 expression with tumor grade (n=80):

Biomarker	Expression	Grade I n (%)	Grade II n (%)	Grade III n (%)	Total
Ki-67	Low (<20%)	12 (48.0)	11 (44.0)	2 (8.0)	25
	Intermediate (20–40%)	6 (22.2)	17 (63.0)	4 (14.8)	27
	High (>40%)	0 (0.0)	10 (35.7)	18 (64.3)	28
p53	Low/negative (<10%)	13 (44.8)	13 (44.8)	3 (10.3)	29
	Intermediate (10–50%)	5 (20.8)	13 (54.2)	6 (25.0)	24
	High (>50%)	0 (0.0)	12 (44.4)	15 (55.6)	27
HER2	Negative (0/1+)	15 (30.6)	25 (51.0)	9 (18.4)	49
	Equivocal (2+)	2 (25.0)	4 (50.0)	2 (25.0)	8
	Positive (3+)	1 (4.3)	9 (39.1)	13 (56.5)	23

Table 2 demonstrated a progressive increase in Ki-67 expression with increasing tumor grade. Among the 25 tumors with low Ki-67 expression, 12 (48.0%) were Grade I, 11 (44.0%) were Grade II, and only 2 (8.0%) were Grade III. In contrast, among 28 tumors with high Ki-67 expression, 18 (64.3%) were Grade III and none were Grade I. This distribution suggested that increased cellular proliferative activity was associated with higher histological grade.

A similar pattern was observed for p53. High p53 expression was not observed among Grade I tumors, whereas 12 (44.4%) of the high-expression cases were Grade II and 15 (55.6%) were Grade III. Low or negative p53 expression was predominantly observed in Grade I and Grade II tumors, with only 3 (10.3%) cases occurring in Grade III. These findings indicated an increasing frequency of strong p53 expression among higher-grade carcinomas.

HER2 expression also demonstrated a relationship with tumor grade. Of the 23 HER2-positive tumors, 13 (56.5%) were Grade III, 9 (39.1%) were Grade II, and only 1 (4.3%) was Grade I. Conversely, HER2-negative tumors were more frequently distributed among Grade I and Grade II categories. The findings therefore showed that HER2 positivity was more commonly encountered in higher-grade tumors.

Overall, the results demonstrated that high Ki-67, high p53, and HER2 positivity were more frequently observed in Grade III breast carcinomas, whereas low biomarker expression was more commonly associated with lower-grade tumors. The combined immunohistochemical assessment provided complementary information regarding tumor proliferative activity and biological aggressiveness. These findings supported the use of Ki-67, p53, and HER2 assessment alongside conventional histopathological grading for characterization of breast carcinoma.

DISCUSSION:

The present study evaluated the immunohistochemical expression of Ki-67, p53, and HER2 in breast carcinoma and examined their relationship with tumor grade and prognostic characteristics. The findings demonstrated that these biomarkers provided complementary information regarding the biological behavior of breast carcinoma. Their expression patterns were particularly relevant because conventional histopathological grading primarily reflected morphological features, whereas immunohistochemical biomarkers provided additional insight into cellular proliferation, genetic alterations, and receptor-related tumor biology [10].

Ki-67 expression was observed to increase with increasing tumor grade. Higher-grade tumors showed a greater proportion of Ki-67-positive cells, indicating increased proliferative activity. This finding was consistent with the established biological role of Ki-67 as a marker of actively proliferating cells. Tumors with high proliferative indices were generally associated with more aggressive pathological characteristics. Therefore, Ki-67 assessment appeared to have provided useful supplementary information for distinguishing tumors with relatively lower and higher proliferative potential [11]. The observed association between Ki-67 and tumor grade also supported its potential value in prognostic assessment when interpreted together with conventional histopathological findings.

The study also demonstrated an association between p53 expression and tumor grade. Increased or abnormal p53 immunoreactivity was more frequently observed in higher-grade carcinomas. This pattern was biologically plausible because alterations involving the TP53 pathway had been associated with genomic instability, uncontrolled cellular proliferation, and aggressive tumor behavior. Abnormal p53 staining therefore appeared to have reflected underlying molecular abnormalities in a proportion of breast carcinomas [12]. However, p53 immunohistochemistry had not always represented a direct equivalent of TP53 mutation status, and its interpretation required consideration of staining intensity and distribution. Consequently, p53 findings were considered most informative when integrated with tumor morphology and other immunohistochemical markers.

HER2 expression also showed important clinical and pathological relevance. HER2-positive tumors had demonstrated distinct biological characteristics and were associated with an increased likelihood of aggressive tumor behavior in comparison with tumors lacking HER2 overexpression. The identification of HER2 expression had been particularly important because HER2 status had implications not only for prognosis but

also for therapeutic decision-making. Immunohistochemical assessment had therefore provided clinically relevant information that could have complemented routine histopathological examination. In cases with equivocal immunohistochemical findings, confirmatory testing using an appropriate method would have remained important [13].

The combined interpretation of Ki-67, p53, and HER2 appeared to have provided a broader assessment of breast carcinoma biology than evaluation of any single marker alone. Ki-67 primarily reflected proliferative activity, p53 provided information related to abnormalities in tumor-suppressor pathways, and HER2 characterized receptor-related tumor biology. Their combined assessment could therefore have assisted in identifying tumors with potentially more aggressive characteristics [14]. The relationship between these biomarkers and tumor grade further suggested that immunohistochemistry could have strengthened pathological risk stratification.

Despite these findings, several limitations were recognized. The study population was relatively limited, and the investigation was conducted at a single institution. Furthermore, prognostic outcomes could have been influenced by treatment modality, tumor stage, lymph-node status, hormone-receptor status, and follow-up duration. The interpretation of immunohistochemical staining could also have been affected by differences in tissue preparation, antibody selection, scoring systems, and laboratory procedures. Larger multicenter studies with longer follow-up and standardized scoring methods would therefore have been valuable [15].

Overall, the findings indicated that Ki-67, p53, and HER2 expression had been associated with important pathological characteristics of breast carcinoma, particularly tumor grade. Their combined evaluation appeared to have enhanced the pathological characterization of breast tumors and could have contributed to more comprehensive prognostic assessment and individualized clinical management.

CONCLUSION:

Immunohistochemical evaluation was performed on 80 breast carcinoma specimens to assess the expression of Ki-67, p53, and HER2 and their association with tumor grade. Ki-67 proliferation index showed a progressive increase with higher tumor grade, with low expression predominantly observed in Grade I tumors and high expression more frequently detected in Grade III tumors. p53 positivity was also more common among higher-grade carcinomas, indicating an association with unfavorable histopathological features. HER2 overexpression was identified in a subset of cases and was observed more frequently in Grade II and Grade III tumors than in Grade I tumors. Cases demonstrating high Ki-67 and p53 expression and HER2 positivity were associated with more aggressive tumor characteristics. Overall, the findings showed that increased Ki-67 and p53 expression and HER2 overexpression were correlated with higher tumor grade and adverse prognostic features.

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