Immunohistochemical Expression of p16 in Breast Carcinoma and its Correlation with Clinicopathological Parameters

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ABSTRACT
Background:
Breast carcinoma is the most prevalent malignancy among women globally. The tumour suppressor protein p16 (INK4a) plays a pivotal role in cell cycle. Aberrant p16 expression has been linked to distinct molecular subtypes of breast carcinoma. This study aimed to evaluate the immunohistochemical (IHC) expression of p16 in invasive ductal carcinoma (IDC) of the breast. **Materials and Methods:** A total of 50 cases of IDC, NOS, obtained from modified radical mastectomy specimens, were included from June 2020 to December 2025. **Results:** The mean age of presentation was in the fifth decade (46–65 years, 52%). p16 showed positive expression (moderate + strong) in 48% (n=24) of cases. p16 positivity was observed in TNBC (76.9%), and the lowest in HER2-enriched tumours (10.0%), with a near-significant trend (P=0.0526). **Conclusion:** p16 IHC expression demonstrates subtype-specific patterns in breast carcinoma, with predominant positivity in triple-negative tumours and consistent negativity in HER2-enriched tumours.

Keywords: p16, breast carcinoma, invasive ductal carcinoma, triple-negative breast carcinoma, (Estrogen Receptor) ER, (Progesterone Receptor) PR, HER2/neu.
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INTRODUCTION

Breast cancer remains to be a major cause of cancer-related death and is the most common disease diagnosed in women worldwide. Data from the Global Cancer Observatory indicates that 19.3 million new cases of cancer were reported worldwide in 2021, with India bearing the second-highest burden after China.[1] By 2040, there will be a predicted 2.08 million instances of cancer in India, a 57.5% rise from 2020 levels. About 70–80% of all breast cancers are invasive ductal carcinoma of no special type (IDC, NOS), which varies greatly in terms of shape, molecular characteristics, clinical behavior, and responsiveness to treatment.[2]

Based on the immunohistochemical evaluation of estrogen receptor (ER), progesterone receptor (PR), HER2/neu, and Ki-67, the molecular classification of breast carcinoma divides tumors into four subtypes: Luminal A, Luminal B, HER2-enriched, and triple-negative breast carcinoma (TNBC). Each subtype is distinguished by unique biological behavior, prognosis, and response to treatment. (3,30).

Cyclins, cyclin-dependent kinases (CDKs), and CDK inhibitors control the tightly controlled cell cycle. The retinoblastoma (Rb) pathway controls the G1–S phase transition, a crucial step in cellular growth. (4,9). When CDK4 and Cyclin D1 combine, the Rb protein is phosphorylated, which promotes cell cycle progression into the S phase. The tumor suppressor protein p16 (INK4a/CDKN2A), which suppresses CDK4 activity and preserves G1 arrest, adversely regulates this pathway. Unchecked CDK4 activity and unchecked cellular proliferation result from p16 loss or silence caused by genetic deletions, point mutations, or promoter hypermethylation. Paradoxically, p16 overexpression may occur in tumours with inactivated Rb protein, representing a feedback loop where the loss of Rb removes the negative signal that ordinarily suppresses p16 transcription. [5,18]

Aberrant p16 expression has been associated with higher tumour grade, increased proliferative index, and unfavourable clinical outcomes in several studies, though findings remain inconsistent across different populations. Data from Indian cohorts are limited, necessitating further investigation. The present study was undertaken to evaluate the immunohistochemical expression of p16 in Invasive ductal carcinoma of the breast and to correlate its expression with established clinicopathological parameters including tumour size, histological grade, tumour stage, lymph node status, hormone receptor status, HER2/neu, and molecular subtypes.

AIMS AND OBJECTIVES

To analyse the immunohistochemical expression of p16 in breast carcinoma and its

correlation with clinicopathological parameters including ER, PR, and HER2/neu status and to correlate p16 expression with clinicopathological parameters including age, tumour location, tumour size, histological grade, tumour stage, and lymph node status.

MATERIALS AND METHODS

This was a retrospective and prospective descriptive study conducted among the female patients aged 25–85 years who were histopathologically diagnosed with invasive ductal carcinoma and underwent modified radical mastectomy at Meenakshi Medical College Hospital & Research Institute (MMCH&RI), Kanchipuram, Tamil Nadu, India, over a period from May 2024 to December 2025. Tissue blocks from patients who received preoperative chemotherapy or radiotherapy; biopsies and inadequate tissue material; specimens without residual tumour; histological types other than invasive ductal carcinoma were excluded. Clinicopathological features including patient demographics, clinical presentation, tumour site and size, T-stage, and lymph node status were recorded. Haematoxylin and eosin (H&E) stained sections were evaluated for histopathological diagnosis and grading according to the Nottingham Histological Grading System (Elston–Ellis modification of the Scarff–Bloom–Richardson system).

Data Collection

Tumor appearance, location, size, depth of invasion, and margin status were all graphically examined in the prospective specimens of breast cancer cases. After examining the Department of Pathology's histopathological and cancer registers, gross information of retrospective cases were gathered. Formalin was used to fix all of the tissue samples for a full day. Grossing was carried out and representative tissue blocks were acquired in accordance with the CAP protocol. [6] After processing, these blocks were paraffin-embedded. The paraffin block of invasive ductal breast cancer cases was then cut into 4µm pieces, and H&E staining was performed. In accordance with CA P criteria, histopathological diagnosis, grading, and other pathological parameters were gathered. To get a conclusive histological diagnosis, these stained sections were examined by a trained pathologist. An immunohistochemistry analysis was performed on all 50 cases. The panel of IHC markers included ER, PR, HER2/neu and p16. The p16 expression was scored based on nuclear and/or cytoplasmic staining intensity as: negative (no staining or low diffuse staining), moderate (moderately intense staining), and strong (intense dark staining in tumour cells). Molecular subtyping was performed based on combined ER, PR, and HER2/neu expression: Luminal A (ER+ and/or PR+, HER2−), Luminal B (ER+ and/or PR+, HER2+), HER2-enriched (ER−, PR−, HER2+), and TNBC (ER−, PR−, HER2−).

Statistical Analysis

IBM SPSS Software Version 22 was used for statistical analysis after all the data was entered into an MS Excel spreadsheet. Pie charts, line charts, and tables were used to display all of the statistical data. A t-test with a target P value of less than 0.05 was used to correlate p16 expression with clinicopathological parameters including age, tumour location, tumour size, histological grade, tumour stage, and lymph node status.

RESULTS

The majority of the fifty cases (26, 52%) were found in the age group of 46–65 years old, followed by the age group of 25–45 years old (14, 28%) and the age group of 66–85 years old (10) representing 20% of the total. There were more cases of involvement in the right breast (26, 52%) than there were in the left breast (24, 48%). 16 percent of tumors were found in the upper outer quadrant, making it the most common location. Next in line was the lower inner quadrant (11) with 22%, followed by the central quadrant (6) with 12%, and finally the lower outer quadrant (5) with 10%. The upper inner quadrant (12) had 24% of the total. 31% of the tumors measured between 2 and 5 centimeters, 32% of the tumors measured more than 5 centimeters, and 6% of the tumors measured less than 2 centimeters. According to the TNM staging system, the most common stage was T2, which accounted for 28.6% of all cases. This was followed by T3 (11.2%), T4 (7.1%), and T1 (4.8%). In the Nottingham grading system, Grade III accounted for twenty percent of the total, Grade I for sixteen percent, and Grade II for fourteen percent. The presence of lymph node metastasis was found to be positive in (28) 56% of cases and negative in (22) 44% of cases. ER was positive in 24 (48%), PR was positive in 23 (46%), and HER2/neu was positive in 18 (36%). These findings pertain to the Receptor Status and Molecular Subtypes. The molecular subtype distribution was as follows: Luminal B at thirty percent, TNBC at twenty-six percent, Luminal A at twenty-four percent, and HER2-enriched 10 percent. In the process of determining the level of p16 expression in breast carcinoma, it was found that p16 exhibited positive immunoexpression (moderate to strong) in twenty-four. There were 22% of cases that showed strong expression, 26% that showed moderate expression, and 52% of cases that showed negative or weak expression (Table 1).

Table 1: Distribution of p16 IHC Expression in Breast Carcinoma

p16 Expression	No. of Cases (n=50)	Percentage
Negative/Weak	26	52.0%
Moderate	13	26.0%
Strong	11	22.0%

Table 2: Association of p16 Expression with Clinicopathological Parameters

Parameter	Total	p16 Negative	p16 Moderate	p16 Strong	P-value
Age 25–45 yrs	14	9 (64.3 %)	2 (14.3%)	3 (21.4 %)	**0.3512**
Age 46–65 yrs	26	14 (53.8 %)	6 (23.1%)	6 (23.1 %)	
Age 66–85 yrs	10	3 (30.0 %)	5 (50.0%)	2 (20.0 %)	
<2 cm	3	2 (66.7 %)	1 (33.3%)	0	**0.2347**
2–5 cm	31	17 (54.8 %)	5 (16.1%)	9 (29.0 %)	
>5 cm	16	7 (43.8 %)	7 (43.8%)	2 (12.5 %)	
Grade I	16	10 (62.5 %)	5 (31.2%)	1 (6.2 %)	**0.3248**
Grade II	14	5 (50.0 %)	2 (14.3%)	5 (35.7 %)	
Grade III	20	9 (45.0 %)	6 (30.0%)	5 (25.0 %)	
T1	4	3 (75.0 %)	1 (25.0%)	0	**0.411**
T2	28	14 (50.0 %)	5 (17.9%)	9 (32.1 %)	
T3	11	4 (36.4 %)	6 (54.5%)	1 (9.1 %)	
T4	7	5 (71.4 %)	1 (14.2%)	1 (14.2 %)	
Lymph node positive	28	13 (46.4 %)	8 (28.6%)	7 (25.0 %)	**0.669**
Lymph node negative	22	13 (59.1 %)	5 (22.7%)	4 (18.2 %)	

We found that there was no statistically significant association between p16 expression and age (P=0.3512), tumor location (P=0.4803), tumor size (P=0.2347), histological grade (P=0.3248), tumor stage (P=0.411), or lymph node status (P=0.669). This was determined by analyzing the Association of p16 Expression with Clinicopathological Parameters. Table 2 provides a comprehensive breakdown of the distribution. In the study that investigated the relationship between p16 expression and ER, PR, and HER2/neu status, it was discovered that there was no statistically significant association between p16 expression and either ER status (P=0.6510) or PR status (P=0.4030). On the other hand, a highly statistically significant inverse association was found to exist between p16 expression and HER2/neu positivity (P=0.0038). The p16 expression was found to be negative in 83.3% (15) of the 18 HER2-positive cases, while only 5.6% (1) of the cases showed strong p16 expression. On the other hand, 65.6% of HER2-negative cases (n=32) exhibited moderate or strong p16 expression, as shown in Table 3.

Table 3: Association of p16 Expression with ER, PR, and HER2/neu Status

Marker	Total	p16 Negative	p16 Moderate	p16 Strong	P-value
ER Positive	24	14 (58.3 %)	5 (20.8%)	5 (20.8 %)	0.6510
ER Negative	26	12 (46.2 %)	8 (30.8%)	6 (23.1 %)	
PR Positive	23	11 (47.8 %)	8 (34.8%)	4 (17.4 %)	0.4030
PR Negative	27	15 (55.6 %)	5 (18.5%)	7 (25.9 %)	
HER2 Positive	18	15 (83.3 %)	2 (11.1%)	1 (5.6 %)	0.0038*
HER2 Negative	32	11 (34.4 %)	11 (34.4%)	10 (31.2 %)	

*Statistically significant (P = < 0.05)

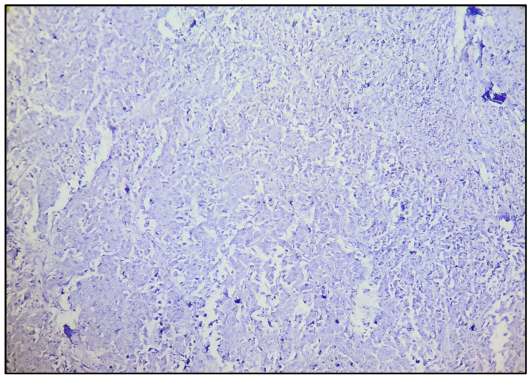

Figure 4.1: Photomicrograph of Invasive Ductal Carcinoma showing Negative p16 expression. (IHC, Magnification 10x).

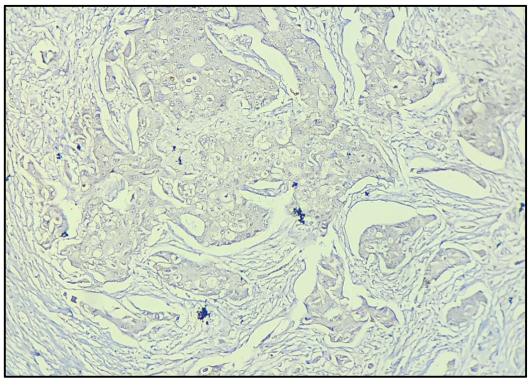

Figure 4.2: Photomicrograph of Invasive Ductal Carcinoma with Grade I showing low p16 expression. (IHC, Magnification 10x).

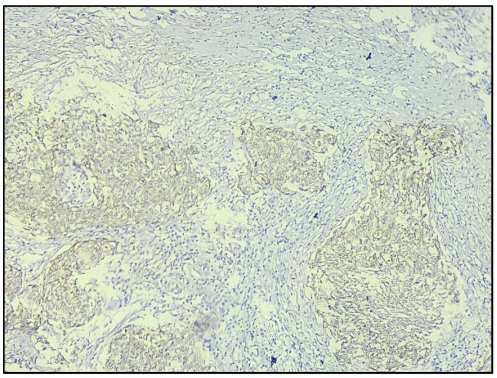

Figure 4.2: Photomicrograph of Invasive Ductal Carcinoma with Grade II showing Moderate p16 expression. (IHC, Magnification 10x).

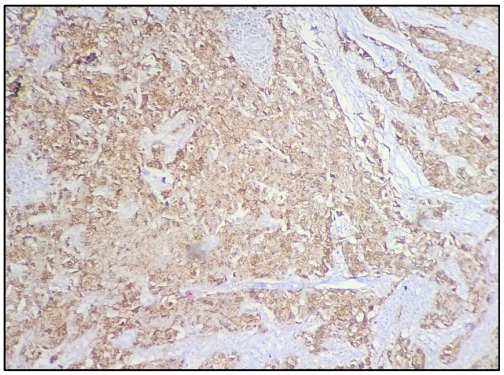

Figure 6.3: Photomicrograph of Invasive Ductal Carcinoma with Grade III showing Strong p16 expression. (IHC, Magnification 10x).

DISCUSSION

The present study assessed the immunohistochemical expression of p16 in 50 cases of invasive ductal carcinoma and evaluated their correlation with clinicopathological parameters, hormone receptor status, and molecular subtypes.

p16 Expression and Clinicopathological Parameters

In 48% of cases, p16 showed positive expression. This is similar to the 48% positivity rate reported by Malik et al. in their study of 50 cases of invasive breast carcinoma [7], and the study conducted by Victor et al. found p16 positivity in approximately 50–60% of their patients [8], which is similar to the present study. The lack of statistically significant correlations between p16 expression and traditional clinicopathological parameters like age (P=0.3512), tumor size (P=0.2347), histological grade (P=0.3248), tumor stage (P=0.411), and lymph node status (P=0.669) indicates that molecular subtype-specific mechanisms, rather than traditional indicators of tumor aggressiveness, are the main cause of p16 aberrations. Similarly, Malik et al. found no significant correlation between p16 expression and patient age [7]. However, a significant correlation between p16 positive and greater tumor grade (P=0.008) was observed by Victor et al. [8]. This finding was not repeated in the current investigation, perhaps as a result of the mixed grade distribution and relatively small sample size.

p16 and HER2/neu:

The most noteworthy discovery about p16 alone and its statistically significant inverse relationship with HER2/neu positive was a Significant Inverse Association (P=0.0038). 83.3% of HER2-positive tumors had negative p16 expression, whereas only 5.6% had robust expression. This discovery has significant biological ramifications. Instead of using the traditional CDK4–Rb–p16 route, HER2-enriched tumors

proliferate mostly through the HER2–MAPK/PI3K–AKT signaling cascade. As a result, in this subtype, p16 expression is either functionally unimportant or repressed. The results of the current investigation are directly supported by Peurela et al.'s specific discovery that p16 expression was strongly linked to HER2 negative and corresponded with better disease-free survival [9]. Although Malik et al.'s findings identified occasional p16 positivity in HER2-enriched patients, which contrasts with the continuously low positivity shown in the present study, they noted that p16 was mostly expressed in TNBC and HER2-enriched subtypes [7].

Expression of p16 in Different Molecular Subtypes

A biologically consistent pattern was found in the distribution of p16 expression among molecular subtypes. In line with the well-known occurrence of paradoxical p16 overexpression in basal-like tumors, TNBC showed the greatest positive (76.9%). The negative feedback loop that normally suppresses p16 transcription is disrupted in TNBC by the common inactivation of the Rb protein through mutations or functional loss, leading to p16 accumulation even in the absence of effective tumor suppression. Sugianto et al. discovered 75% p16 positive in high-grade TNBC [10], while Victor et al. showed that p16 expression was most commonly seen in TNBC (85.7%) [8]. The pathway-specific character of p16 expression is highlighted by the lowest p16 positivity in HER2-enriched tumors (10.0%). The correlation between p16 and molecular subtypes reached statistical significance (P=0.0115) on dichotomized analysis, supporting the marker's subtype-specific relevance.

LIMITATIONS

The sample size of 50 cases, while adequate for a single-institution study, limits statistical power for subgroup analyses. The mixed retrospective–prospective design may introduce selection bias. Exclusion of histological types other than IDC, NOS limits generalisability. Ki-67 was not assessed, preventing direct comparison with studies evaluating the p16–Ki-67 relationship. Follow-up data for survival analysis and molecular confirmation of Rb pathway status by FISH were not available. The intensity-based scoring system used may affect direct comparability with studies employing H-score methodology.

CONCLUSION

p16 immunoexpression in invasive ductal carcinoma of the breast exhibits subtype-specific patterns, with predominant positivity in triple-negative tumours and consistent negativity in HER2-enriched tumours. A statistically significant inverse association between p16 expression and HER2/neu positivity (P=0.0038) confirms that the p16–CDK4–Rb pathway is functionally suppressed in HER2-driven breast carcinoma.

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