RESEARCH PAPER

Significance of p16 immunohistochemical expression in invasive ductal carcinoma of breast and its association with molecular type, grade and stage of the tumor- A Cross sectional study.

Dr. Dheeraj kumar, Dr. Kalyani R*. , Dr. Gopinath K.S.

) Dr. Dheeraj kumar, Post Graduate Year 3, Sri Devaraj Urs Academy of Higher Eduction, kolar.

2) Dr. Kalyani Raju, MD, Phd, Professor and Former Head, Department of Pathology,Director of Research & Development, SDUAHER.

3) Dr. Kodaganur S. Gopinath, MS, FAMS, FRCS (Padmashree Awardee).

Corresponding author: Kalyani Raju
Email ID: drkalyanir@rediffmail.com

ABSTRACT

Introduction: Breast cancer is one of the leading causes of cancer deaths in women. Immunohistochemistry is commonly used to define subtypes by analysing the expression of ER, PR and HER2 proteins. p16 protein, is a negative cell regulator causing G1 arrest by preventing G1 to S transfer.Deletions and mutations of p16 in human cancer cell lines indicate its essential significance in carcinogenesis.Its connection to breast cancer is still debatable, though. The purpose of this study is to examine the expression of p16 in invasive ductal carcinoma of breast cancer while taking into account tumor stage, grade and molecular type.

Materials &methods: This is a cross-sectional analytical study utilizing formalin fixed paraffin embedded tissue blocks of diagnosed invasive ductal carcinoma of breast cases in females.A total of 60 subjects were considered of different age groups. The grading was given as per ASCO guidelines2024 , staging was given from Stage I to IV and molecular classification according to WHO criteria for tumor classification of breast 2024. p16 staining was done with the obtained paraffin blocks and scoring was given as per CAP protocol as p16 positive (≥70% nuclear and cytoplasmicstaining) and p16 negative (<70%, nuclear and cytoplasmicstaining).The staining intensity given as weak, moderate and strong.

Results: In this study mean age group of patients were 53±12.5 years with 58.3% cases of grade I, 56.7% cases of stage II and Luminal A molecular subtypes representing 33.3% cases followed by 26.7% cases of triple negative molecular subtype, p16 was strongly expressed in 96.7% of IDC cases. Stage showed a significant association p=0.0025 with no association with grade (p=0.72) and stage (p=0.67).

Conclusion: p16 showed high expression in IDC, with a significant association with advanced tumor stage but no correlation with grade or molecular subtype.

Keywords: Significance, p16, immunohistochemical expression, invasive ductal carcinoma, breast, association , molecular type,grade ,tumor

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Introduction

Breast cancer is one of the leading causes of cancer deaths in women. The World Health Organization (WHO) reported around 2.3 million new cases and 685,000 deaths from breast cancer globally in 2020.[1] By 2050, an estimated 3.2 million new instances of breast cancer would be identified each year.[1]The prevalence of Breast Cancer in Kolar reported as 6.4 % of total female cancers.[2]Breast cancer is a complex disease with hereditary factors influencing prognosis and treatment options. This cancer can originate from the epithelium of the glandular ducts (ductal carcinoma) or lobules (lobular carcinoma) and spread to other organs through the lymphatic system or blood circulation. Despite breakthroughs in cancer screening and therapy, around 30% of women diagnosed with invasive breast cancer develop metastases.[3] The high mortality rate of these patients emphasizes the need of early identification. However, because cancer cell activity differs greatly from patient to patient, predicting the prognosis of breast cancer is difficult. Currently, prognostic indicators include HER2, ER, PR, and Ki67.

Breast subtypes include luminal A, luminal B, HER2-overexpressing, and triple negative, each with unique gene expression, prognosis, and response to

chemotherapy.[4]Immunohistochemistry is commonly used to define subtypes by analysing the expression of ER, PR and HER2 proteins.[4]

The CDKN2A gene on chromosome 9p21 encodes the p16 protein, which regulates the cell cycle and suppresses tumours. P16 protein, produced by the gene, is a negative cell cycle regulator, causing G1 arrest by preventing G1 to S transfer.[4] P16 inhibits cyclin-dependent kinases 4/6 (CDKs 4/6). CDKs 4/6 phosphorylate pRb, which is thus inactivated during cell cycle progression.[5] Deletions and mutations of p16 in human cancer cell lines indicate its essential significance in carcinogenesis.[5]

It is commonly known that p16 protein expression plays a significant role in a variety of human cancer types. Its connection to breast cancer is still debatable, though.[6] The purpose of this study is to examine the expression of p16 in invasive ductal carcinoma of breast cancer while taking into accounttumor stage, grade and molecular type.

Materials & Methods:

The present study is a cross-sectional analytical study conducted in a tertiary health care center attached to the Department of Pathology at Sri Devaraj Urs Medical College, Tamaka, Kolar, Karnataka utilizing case files of invasive ductal carcinoma. Ethical clearance was obtained from the central ethics committee (SDUAHER/R&D/CEC/SDUMC-PG/09/NF/2025-26). Patient's data were obtained from the case files.Datafrom January 2022 to December 2023 was collected and MS excel sheet was prepared containing all the required data.

A total of 60 cases were consideredfor the study with different age groups,all females.

Sample size was calculated based on the intensity of p16 calculated on the invasive breast CA (96.2%) from the study done by Abdulhadi et al. [7]

Anonymity of the patient was considered. Clinicopathological features was obtained from the patient's medical records. Sociodemographic data, clinical features, molecular type, grade and stage was taken into consideration.

The pathological grade and stage of the disease were noted. The grading was given asperASCO 2024 guidelines, staging was given according to AJCC guideline 8th edition from Stage I to IV and molecular classification according to WHO criteria for tumor classification of breast 2024.

Inclusion Criteria: Clinicallydiagnosed cases of breast carcinoma, confirmed by histopathology as IDC.

Exclusion criteria: Post Chemotherapy, Post Radiotherapy cases, Post surgery cases, recurrent cases or any other cancer in the patient.

For immunohistochemical staining of p16,p16 primary antibody (M/s Biocare Medicals United states of America) was done as per the manufacturer's protocol. p16 scoring was taken into consideration according to CAP protocol.

p16 IHC results include:

• p16 IHC positive (≥70%, nuclear and cytoplasmic moderate to strong staining)

• p16 IHC negative (< 70%, nuclear and cytoplasmic moderate to strong staining)

ER, PR and HER2neu Scoring was done using ASCO/CAP guidelines (Allred scoring system).

Intensity score was done asstrong,moderate, weak (fig. 1,2,3).

All the data was entered in Microsoft excel sheet and analysed statistically.

Statistical analysis:

Data like ER, PR, HER2 and p16 IHC marker expression was entered in Microsoft excel sheet. The characteristics of the prognostic markers was shown as both medians and means. P16 IHC was correlated with molecular subtype, grade and stage of disease and its significance as prognostic biomarkers in breast carcinoma. Student's t-test was used to compare continuous characteristics between the groups. Pearson's test was used to analyse the correlation between two continuous variables. A minimum level of statistical significance was considered at p level of < 0.05.

Results:

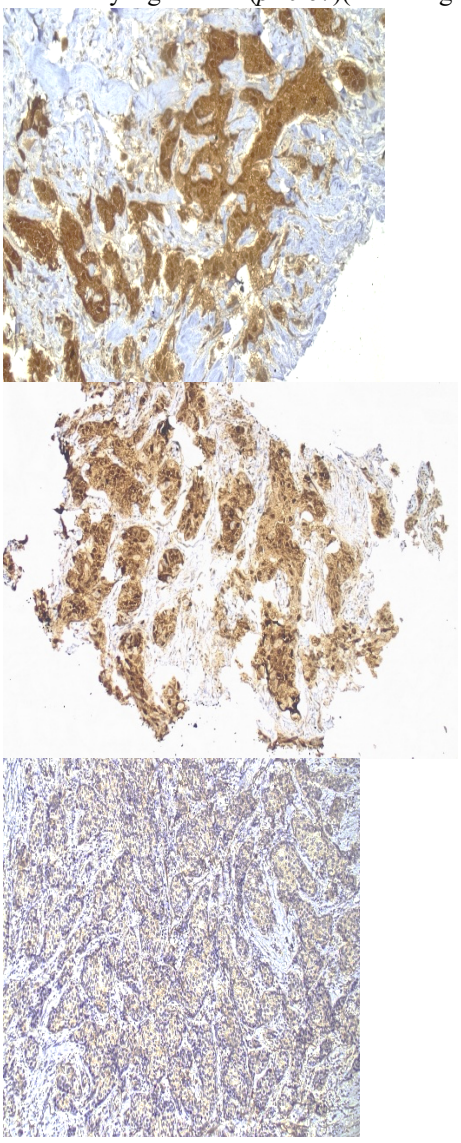
A total of 60 study subjects were enrolled in the study.

The cases included were from the age group of 30 years to 80 years, with a mean age group of 53.6 ± 12.5 years. Most patients belonged to the 5th to 7th decades of life, indicating that IDC commonly affects middle-aged and post-menopausal women.The highest incidence was observed in the 51–60 years group.The majority oftumors in this cohort were well (58.3%) to moderately differentiated (28.3%), with Grade I (35 cases) being the most common.Stage II tumors (56.7%) were the most frequently observed. A substantial proportion (36.7%) presented at Stage III, indicating late diagnosis in many patients.Luminal A was the most common subtype (33.3%), while TNBC accounted for 26.7%, representing a substantial proportion associated with more aggressive clinical behaviour.The majority of cases demonstrated high proliferative activity (63.3%), correlating with more aggressive tumor biology (Table/Fig.2).

p16 expression was highly prevalent (96.7%) across the cohort with no significance with the intensity of cytoplasm and nuclear staining showing p16 positive and p16 negative ($p > 0.05$). (Table/Fig3).

p16 showed high levels of expression across all grades, with Grade III tumors showing 100% positivity. However, the difference in p16 biomarker expression between Grade I, II and III was not statistically significant (p=0.72). (Table/Fig.4).

A statistically significant association was observed between p16 expression and tumor stage (p=0.0025). p16 expression was markedly higher in Stage II–IV tumors (Stage II-97.1%, Stage III-100% and stage IV- 100%), while Stage I (50%) showed lower and variable expression(Table/Fig.4). Although p16 positivity appeared highest in TNBC (100%) and HER2 positive (100%)tumors and slightly lower in Luminal A (95%) and Liminal B(93.3%) subtypes, the comparison was not statistically significant (p=0.67)(Table/Fig.4).



Table/Fig. 1 (a,b,c).p16 IHC stain in Invasive ductal carcinoma**a)** strong intensity, **b)** moderate intensity and **c)** weak intensity (Both nuclear and cytoplasmic) with p16 positivity (>70% stain). (p16 x400)

Table/Fig. 2 Clinicopathological parameters of the cases

Parameter	Category	Cases (n)	Percentage (%)
Age Group (years)	31–40	13	21.7
	41–50	12	20
	51–60	15	25
	61–70	14	23.3
	>70	6	10
Histological Grade	Grade I	35	58.3
	Grade II	17	28.3
	Grade III	8	13.3
Stage Group	Stage I	2	3.3
	Stage II	34	56.7
	Stage III	22	36.7
	Stage IV	2	3.3
Molecular Subtype	Luminal A	20	33.3
	Luminal B	15	25
	HER2-enriched	9	15
	Triple Negative (TNBC)	16	26.7
Ki-67 Expression	High (>14%)	38	63.3
	Low (<14%)	22	36.7

Table/Fig 3 : p16 Status

p16 Status	Cases (n=60)	%	Intensity Category	Cytoplasmic Intensity (%)	Nuclear Intensity (%)	p value
p16 Positive	58	96.7	Nil	0 (0%)	4 (6.89%)	0.159
			Weak	4 (6.89%	6 (10.3	

				)	4%)	
			Moderate	36 (62.06 %)	29 (58.0 %)	
			Strong	18 (31.03 %)	19 (32.75%)	
p16 Negative	2	3.3	Nil	0 (0%)	1 (50%)	1.0
			Weak	0 (0%)	0 (0%)	
			Moderate	1 (50%)	0 (0%)	
			Strong	0 (0%)	0 (0%)	

Table/fig. 4 : Association of p16 with grade, stage and molecular types.

Clinicopathological Parameter	**Category**	**Total Cases (n=60)**	**p16 Positive**	**p16 Negative**	**Positivity %**	**p value**
Tumor Grade	Grade I	35	34	1	97.10%	**0.72**
	Grade II	17	16	1	94.10%	
	Grade III	8	8	0	100 %	
Tumor Stage	Stage I	2	1	1	50.00%	**0.0025**
	Stage II	34	33	1	97.10%	
	Stage III	22	22	0	100 %	
	Stage IV	2	2	0	100 %	
Molecular Subtype	Luminal A	20	19	1	95.00%	**0.67**
	Luminal B	15	14	1	93.30%	
	HER2-enriched	9	9	0	100 %	
	TNBC	16	16	0	100 %	

Discussion:

In the present study, 60 histologically confirmed cases of Invasive Ductal Carcinoma (IDC) of the breast were evaluated to analyze the clinicopathological profile and the immunohistochemical expression of p16 and its association with tumor grade, stage, and molecular subtype. The mean age of patients was 53.6 ± 12.5 years, with the majority falling in the 5th to 7th decades of life. This age distribution aligns with global epidemiological data indicating that IDC predominantly affects peri- and post-menopausal women, particularly between 45 and 70 years of age.[8] The highest incidence in the 51–60 years age group in this study is consistent with existing literature from both Western and Asian populations.[9]

In this study, the majority of cases were Grade I (58.3%), followed by Grade II (28.3%) and Grade III (13.3%). Although IDC is frequently reported as moderately to poorly differentiated in several cohorts [10], a predominance of Grade I tumors has also been reported in screening-supported populations and tertiary care settings.[11] Most tumors presented at Stage II (56.7%), with 36.7% in Stage III, reflecting a substantial proportion of patients presenting with locally advanced disease. This trend is commonly observed in developing regions, where late clinical presentation and limited breast cancer screening contribute to delayed diagnosis.[12]This indicates that p16 expression is independent of tumor differentiation and does not correlate with histological gradeandalsosuggesting widespread activation of the p16 pathway in IDC.

Luminal A and Luminal B subtypes comprised 58.3% of cases collectively, reflecting the typically hormone-receptor-predominant biology of IDC.

TNBC constituted 26.7% of cases in this study, similar to the reported prevalence of 15–30% in South Asian populations.[13] TNBC is well recognized for its aggressive clinical behavior, early recurrence, and poor survival. In our study more TNBC and HER2 positive cases showed p16 positivity in all the cases but no significant correlation was obtained.

A high Ki-67 index (>14%) was seen in 63.3% of cases, indicating a high proliferative profile. This is consistent with reports that high Ki-67 is associated with higher stage, increased relapse risk, and poorer prognosis.[14]

p16 is a cyclin-dependent kinase inhibitor encoded by CDKN2A, classically associated with cell cycle arrest via inhibition of the CDK4/6-RB pathway.[15] Overexpression of p16 in breast cancer is often considered a marker of dysfunctional RB pathway, particularly in high-grade or aggressive tumors.[16,17] However, due to the small number of Stage I and IV cases, the finding should be interpreted cautiously.The most significant finding in this study was the high prevalence of p16 positivity, observed in 96.7% of cases.

Although Grade III tumors showed 100% p16 positivity, no statistically significant correlation between p16 expression and histological grade was observed ($p > 0.05$). Similar findings have been reported by Kim et al. and Besson et al., suggesting that while p16 may be more frequently overexpressed in high-grade tumors, its expression is not strictly grade-dependent.[18,19] Thus, p16 biomarker expression cannot be reliably used as a surrogate marker of differentiation.

A statistically significant correlation was observed between p16 expression and tumor stage ($p = 0.0025$), with higher positivity in Stage II–IV tumors and lower, variable expression in Stage I tumors. This suggests a possible association between p16 overexpression and disease progression. Previous studies have also demonstrated increasing p16 expression with tumor invasiveness and advanced pathological stage.[20,21]Thisindicate higher p16 expression in more advanced disease. However, due to the small number of Stage I and IV cases, the finding should be interpreted cautiously.Although significance was achieved, this result is influenced by small sample size in Stage I, therefore the association should be interpreted with caution.

Although p16 was most strongly expressed in TNBC and HER2-enriched subtypes, the association did not reach statistical significance ($p > 0.05$). This trend is consistent with other studies indicating higher p16 expression in basal-like and HER2-driven tumors, which are more likely to exhibit RB pathway abnormalities.[22,23] However, as shown in this study and others, p16 expression alone cannot reliably differentiate molecular subtypes.This indicates that p16 expression does not vary significantly among molecular subtypes and cannot be used to differentiate intrinsic breast cancer categories.

A prospective cross-sectional study of 50 patients with invasive breast carcinoma found that p16 positivity was strongly associated with proliferative activity. The mean Ki-67 index was significantly higher in p16-positive tumors than in p16-negative tumors (56.59% vs. 15.94%; **$p<0.001$**). p16 expression was also significantly associated with higher tumor grade (**$p=0.008$**) and was particularly frequent in triple-negative breast cancer (TNBC), with **85.7% of TNBC cases showing p16 positivity**. There was a trend toward larger tumor size and greater lymph-node involvement in p16-positive tumors, although these associations were not statistically significant. No significant association was observed between p16 expression and lymphovascular space invasion (**$p=0.746$**). The authors concluded that p16 overexpression may indicate aggressive tumor biology, particularly in TNBC [24].

Duran et al. evaluated **302 breast cancer patients** using immunohistochemical expression of PCNA, p16 and Ki-67. p16 expression showed a significant positive correlation with PCNA expression and with the Ki-67 index. However, p16 was not significantly associated with pathological stage, T-stage or lymph-node involvement and did not demonstrate independent prognostic significance for overall survival. In contrast, a high Ki-67 index (>25%) was associated with more advanced pathological stage, lower ER and PR expression, and shorter survival. The authors concluded that, although p16 reflects proliferative activity, **Ki-67 was more informative for prognostic stratification**, while the prognostic value of p16 requires further investigation.[25]

This study has certain limitation as single centre study and small number of sample.Further studies with larger sample sizes to be done to extrapolate the results to general population and survival analysis are recommended to validate the prognostic significance of p16 in IDC. However, the findings suggest that p16 may have potential value as a prognostic marker, particularly in identifying tumors with more advanced disease, but it is not useful for determining tumor grade or molecular subtype.

Conclusion: The expression of p16 biomarker shows statistical significant association with stage of the disease. However, the association with grade and molecular types was not statistically significant.

CONFLICT OF INTEREST

No conflicts of interest related to this study. No financial support or sponsorship was received from any commercial or non-commercial organization for conducting this research.

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