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Original Article

Pathology Section

CDK4 Expression in Invasive Ductal Carcinoma of the Breast and its Association with Prognostic Parameters: A Cross-sectional Study

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ABSTRACT

Introduction: Breast cancer is a growing public health concern in India, often diagnosed at advanced stages, leading to poor outcomes. Identifying effective biomarkers is crucial. Cyclin-Dependent Kinase (CDK)4, a key regulator of the cell cycle, is frequently overexpressed in breast cancer, promoting tumour growth and emerging as a potential therapeutic target.

Aim: To determine the expression of CDK4 marker in Invasive Ductal Carcinoma (IDC) of the breast and its association with prognostic parameters.

Materials and Methods: The present cross-sectional analytical study utilised retrospective data from Sri Devaraj Urs Medical College, Kolar, Karnataka, India, utilising data spanning from January 2018 to December 2021.The study period extended over 18 months, from April 2023 to October 2024. Ninety histologically confirmed IDC breast cases were analysed, excluding recurrent cases, chemotherapy/radiotherapy-treated cases, improperly fixed specimens, male breast carcinomas and secondary metastases. Immunohistochemical analysis of CDK4 was conducted, evaluating nuclear and cytoplasmic staining separately and its association with prognostic parameters as size, grade, stage of tumour, metastatic lymphnodes, lymphovascular invasion, tumour infiltrating lymphocytes and expression of ER, PR, Her2/Neu, Ki67 in IDC breast was done. All data were entered into Microsoft Excel and analysed using Statistical Package for Social Sciences (SPSS) 22 version software. Categorical variables were presented as percentages. Associations between CDK4 expression and clinicopathological factors were tested using Chi-square test. A p-value <0.05 was considered statistically significant.

Results: The study included 90 IDC cases, mean age was 52.5 years. CDK4 showed cytoplasmic positivity in 63.3% (n=57) and nuclear positivity in 47.8% (n=43). Nuclear CDK4 positivity showed significant association with HER2/Neu positivity (p=0.004), advanced stage (p<0.001), larger tumours (T3/T4) (p<0.001), presence of LVI (p=0.022), poor prognosis (p<0.001), and higher tumour grade (p<0.001). Cytoplasmic CDK4 expression showed no significant association with prognostic parameters.

Conclusion: As per the present study findings nuclear CDK4 expression significantly associated with aggressive tumour characteristics and worse prognosis in breast IDC, highlighting its potential as a prognostic biomarker and therapeutic target. Cytoplasmic CDK4 expression, though frequent, had limited prognostic utility. The findings emphasize the biological importance of subcellular localisation of CDK4 in breast cancer.

Keywords: Breast cancer, Cyclin dependent kinase 4, Prognosis

INTRODUCTION

Breast cancer remains a significant global public health concern, particularly among women. According to the World Health Organisation (WHO), breast cancer was the most frequently diagnosed cancer among females worldwide in 2020, with approximately 2.3 million new cases and 685,000 deaths. In India, the burden of breast cancer has notably increased, reflecting a 39.1% rise in incidence over the past 26 years [1]. As per GLOBOCAN 2020 data, breast cancer ranks as the most common cancer among Indian women, accounting for 13.5% of all malignancies and contributing to 10.6% of cancer-related mortalities [2]. In Karnataka, breast cancer presents a significant health issue with age-adjusted incidence rates reaching 27.8 cases per 100,000 women as reported by the National Cancer Registry Program of India in 2020 [3]. The frequent late stage diagnosis prevalent in India complicates treatment, consequently leading to higher mortality rates. In Kolar district, Karnataka, breast cancer constitutes a major public health issue, ranking third among cancers affecting females and representing 10.8% of all female cancers [4].

Addressing breast cancer effectively requires enhanced understanding of its biological and molecular markers, such as Cyclin-Dependent Kinase 4 (CDK4), which plays a pivotal role in driving G1-S cell cycle progression by phosphorylating RB1 in complex with cyclin D1, a process essential for proliferation in several cancers, especially $HR^+/HER2^-$ breast, prostate, and Ewing sarcoma cells. These tumours are often highly dependent on CDK4 but not CDK6, making CDK4 an attractive selective therapeutic target that can spare hematopoietic cells and reduce neutropenia risk. Selective inhibition of CDK4 allows higher drug exposure, achieving deeper tumour growth suppression compared with dual CDK4/6 inhibitors. Furthermore, CDK4 inhibition can be combined with endocrine therapy, CDK2 inhibitors, HER2-targeted agents, or immune checkpoint inhibitors to overcome resistance and broaden anti-tumour efficacy. Therapeutically, three relatively selective inhibitors of CDKs 4 and 6 have entered clinical development (palbociclib, ribociclib, and abemaciclib). Each of these is a potent inhibitor of CDK4 and 6 [5-7].

This study investigates the expression of CDK4 marker in Invasive Ductal Carcinoma of the breast (IDC) and association of CDK4 expression with established prognostic parameters such as size, grade, stage of tumour, metastatic lymphnodes, Lymphovascular Invasion (LVI), Tumour-Infiltrating Lymphocytes (TILs), and Estrogen Receptor (ER),Progesterone Receptor (PR) HER2/neu, Ki67expression in IDC of breast. Given that similar comprehensive studies evaluating CDK4 expression and its prognostic significance in IDC are scarce in the Indian literature, this research aims to bridge this gap.

MATERIALS AND METHODS

The present cross-sectional analytical study was conducted in the Department of Pathology at Sri Devaraj Urs Medical College,

Tamaka, Kolar, Karnataka, India, utilising retrospective data spanning January 2018 to December 2021. The study period extended over 18 months, from April 2023 to October 2024. Ethical approval was obtained prior to the commencement of this study. IEC Number DMC/KLR/IEC/9/2023-24.

Inclusion and Exclusion criteria: All histologically proven cases of IDC breast were included. Cases of male breast carcinoma, recurrent carcinoma, prior chemotherapy or radiotherapy, inadequately fixed specimens, or secondary metastasis to the breast were excluded.

Sample size selection: Based on previously published literature by Peurala E et al., [6], which reported a 70% positivity rate for CDK4 in breast carcinoma, the sample size was calculated to be 90 cases, considering an absolute error of 10% with 95% confidence interval. Retrospective data collection included histologically confirmed cases of IDC of the breast diagnosed between January 2018 and December 2021.

Study Procedure

Datacollection: Relevant demographic, clinical, and histopathological data, including tumour size, stage according to American Joint Committee on Cancer (AJCC) 8th Edition [8], histopathological grade assessed using Modified Bloom-Richardson's Histological Grading System [8], lymph node status, LVI, TILs, and hormonal biomarker status (ER, PR, HER2/neu, Ki67), were systematically retrieved from the departmental archives.

LVI was assessed in peritumoural regions on Haematoxylin and Eosin (H&E) stained sections of surgically excised breast tissue, with LVI defined as the presence of carcinoma cells within endothelial lined lymphatic vessels or as tumour emboli invading the vessel walls [9].

TILs were scored per 2014 International TILs Working Group guidelines on H&E sections (×200-400), estimating the percentage of invasive-tumour stroma filled by lymphocytes/plasma cells while excluding neutrophils, necrosis, DCIS, normal lobules, and biopsy artefacts. For descriptive analysis, TILs were categorised as low (<10%), intermediate (10-50%), and high (>50%) [10].

Immunohistochemistry and scoring: Formalin-fixed, paraffin-embedded tissue sections (3 µm) were subjected to IHC staining as per the routine lab protocol which utilised a primary antibody, CDK4, which is a rabbit monoclonal antibody. For secondary detection, the MACH™1 Detection Kit was employed.

Analysis of IHC: Immunohistochemical analysis was done based on a semiquantitative scoring as described [9]. The cytoplasmic and nuclear staining were evaluated separately. The scoring system was based on two parameters: staining intensity and the proportion of positively stained cells. Staining intensity was graded as follows: 0 for negative, 1 for weak, 2 for moderate, and 3 for high. The percentage of positive cells was scored as 0 for none, 1 for less than 25%, 2 for 25% to 50%, and 3 for more than 50% positive cells. The final score was obtained by summing both components (A + B), with a maximum possible score of 6. A total score greater than 3 was interpreted as positive expression [11]. CDK4 IHC staining was analysed for nuclear and cytoplasmic expression, and scored separately.

H&E Slides were reviewed, and tumour parameters including size, stage, grade, lymph node status, LVI, TILs, and ER/PR/HER2/Ki67 status were recorded. Association between CDK4 and prognostic markers (size, grade, stage of tumour, metastatic lymphnodes, LVI, low, intermediate and high TILs, molecular typing, expression of ER, PR, HER2/neu and Ki67) as well as Nottingham prognostic index was assessed.

STATISTICAL ANALYSIS

All data were entered into Microsoft Excel and analysed using SPSS 22 version software. Categorical variables such as tumour grade, stage, ER, PR, HER2/Neu status, LVI, and TILs were presented as percentages. Associations between CDK4 expression (nuclear vs. cytoplasmic) and clinicopathological factors such as tumour size, grade, stage, lymph-node status, hormone receptors, molecular subtype, Ki-67 index, and LVI were tested using Chi-square test. A p-value <0.05 was considered statistically significant.

RESULTS

Patient demographics and clinical characteristics: The study comprised 90 female patients with invasive ductal carcinoma of the breast. Patient ages ranged from 32 to 82 years, with a median age of 53 years. The highest proportion of cases occurred in the 50-59 year age group (26.1%; n=23), followed by 40-49 and 60-69 years (each 20.5%; n=18). Tumour sizes were predominantly classified as T2 (2-5 cm) in 56.7% (n=51) of cases, followed by T3 (>5 cm, 22.2%; n=20), T4 (14.4%; n=13), and T1 (≤2 cm, 6.7%; n=6). Lymph node positivity was seen in 48.9% (n=44) of cases. Grade 1 tumours were the most common (51.1%; n=46),followed by Grade 2 (31.1%; n=28) and Grade 3 (17.8%; n=16). LVI was documented in 62.2% (n=56) of tumours. TILs levels varied, with the majority showing low levels (45.6%; n=41), intermediate (23.3%; n=21), or high levels (31.1%; n=28) [Table/Fig-1].

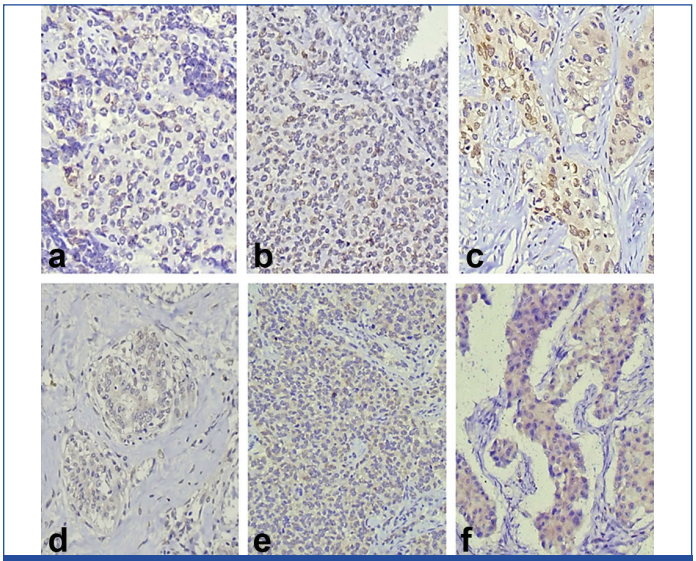
Parameters	Number of cases (%)
Tumour size	
T1 (≤2 cm)	6 (6.7%)
T2 (2-5 cm)	51 (56.7%)
T3 (>5 cm)	20 (22.2%)
T4	13 (14.4%)
Lymph node status	
Positive	44 (48.9%)
Negative	46 (51.1%)
Tumour grade	
Grade 1	46 (51.1%)
Grade 2	28 (31.1%)
Grade 3	16 (17.8%)
Tumour-Infiltrating Lymphocytes (TILs)	
Low (<10%)	41 (45.6%)
Intermediate (10-50%)	21 (23.3%)
High (>50%)	28 (31.1%)
Stage of disease	
Stage I	5 (5.5%)
Stage II	54 (60.0%)
Stage III	30 (33.3%)
Stage IV	1 (1.1%)
Hormone receptors and HER2/Neu	
ER-positive	52 (57.8%)
ER-negative	38 (42.2%)
PR-positive	48 (53.3%)
PR-negative	42 (46.7%)
HER2/Neu-positive	25 (27.8%)
HER2/Neu-negative	65 (72.2%)
Ki-67 proliferative index	
<14% (low)	45 (50.0%)
≥14% (high)	45 (50.0%)
Molecular subtype	
Luminal A	34 (37.8%)
Luminal B	23 (25.6%)
HER2/Neu-enriched	10 (11.1%)

Triple-negative (TNBC)	23 (25.6%)
Nottingham Prognostic Index (NPI)	
Excellent (I; <2.4)	4 (4.4%)
Good (II; 2.4-3.4)	28 (31.1%)
Moderate (III; 3.4-5.4)	32 (35.6%)
Poor (IV; >5.4)	26 (28.9%)
CDK4 expression	
Nuclear positive	43 (47.8%)
Nuclear negative	47 (52.2%)
Cytoplasmic positive	57 (63.3%)
Cytoplasmic negative	33 (36.7%)

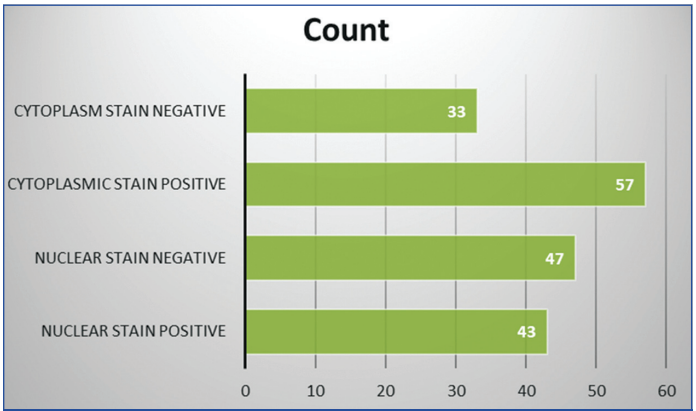
[Table/Fig-1]: Patient clinicopathological characteristics.

Hormonal biomarker profiles and molecular types: Hormone receptor status revealed ER positivity in 57.8% (n=52) and PR positivity in 53.3% (n=48) of tumours. HER2/Neu overexpression was observed in 27.8% (n=25). The Ki67 proliferative index demonstrated an equal distribution between high (≥14%) and low (<14%) proliferative groups (each 50%). Molecular subtyping identified Luminal A subtype as the most frequent (37.8%; n=34), followed by Luminal B (25.6%; n=23), triple-negative (25.6%; n=23), and HER2/Neu -enriched subtypes (11.1%; n=10) [Table/Fig-1].

CDK4 expression patterns: CDK4 expression was evaluated separately as nuclear and cytoplasmic expression [Table/Fig-2]. Nuclear positivity was noted in 47.8% (n=43) of cases, while cytoplasmic positivity was higher, observed in 63.3% (n=57) [Table/Fig-3].



[Table/Fig-2]: Microphotograph showing a) Weak nuclear expression; b) Moderate Nuclear expression; c) Strong nuclear expression; d) Weak cytoplasmic expression; e) Moderate Cytoplasmic expression; f) Strong Cytoplasmic Expression of CDK4 staining in Invasive ductal Carcinoma (Original magnification, x400).



[Table/Fig-3]: Distribution of cases based on CDK4 Nuclear expression and cytoplasmic expression.

Association of CDK4 expression with prognostic parameters: Nuclear CDK4 Expression: Statistically significant associations were found between nuclear CDK4 positivity and increased tumour size (p=0.00014), higher histopathological grade (p<0.00000000000531727), advanced clinical stages III and IV (p<0.0000002092), presence of LVI (p=0.022), and HER2/Neu-positive status (p=0.004). In addition, nuclear CDK4 positivity was significantly higher in cases with poor prognosis based on Nottingham Prognostic Index (NPI, p<0.00000000706). However, no significant association was found with ER status, PR status, Ki67 proliferation index, TIL levels, or molecular subtypes.

Cytoplasmic CDK4 expression: Significant associations were noted between cytoplasmic CDK4 expression and higher tumour grade (p=0.018) and presence of LVI (p=0.013). Cytoplasmic CDK4 expression did not significantly associate with tumour size, lymph node involvement, clinical stage, ER, PR, HER2/Neu status, Ki67 proliferation index, molecular subtypes, or TIL levels.

DISCUSSION

Breast cancer remains one of the leading causes of cancer-related mortality among women globally. It is a multifactorial disease driven by genetic, environmental, and lifestyle-related factors. Among the molecular markers routinely used in breast carcinoma like ER, PR, HER2/Neu and BRCA [12], Cyclin-Dependent Kinase 4 (CDK4) has emerged as an important factor in breast cancer pathogenesis, through both canonical (cell cycle regulation) and non-canonical mechanisms, including modulation of metabolism, senescence, and immune signaling and also its subcellular localisation as nuclear vs cytoplasmic may offer distinct insights into tumour behaviour [13]. In this study the authors investigated the expression of CDK4 marker in IDC breast and association of CDK4 expression with established prognostic parameters such as size, grade, stage of tumour, metastatic lymphnodes, LVI, TILs, ER, PR, HER2/neu, Ki67 expression in IDC breast [Table/Fig-4].

In the present study, the CDK4 expression was analyzed in both nucleus and cytoplasm of the tumour cells. 47.8% of the tumours showed nuclear expression of CDK4, while CDK4 expression in the cytoplasm was seen in 63.3% of the cases. In a study done by Peurala E et al., only nuclear expression of CDK4 was observed which was seen in 58.8% of the cases and no cytoplasmic expression was noted [6]. In a study done by An HX et al., combined score of nuclear and cytoplasmic expression of CDK4 was considered as over expression and it was seen in 18.9 % of the cases [11].

Association of CDK4 expression and tumour size: Comparative studies showed variable CDK4 positivity by tumour size. The current study found positivity increased with size. An HX et al., reported overall low positivity (16.6%), highest in T1 and T2 (6.3% each) and lowest in T3 (3.1%; p<0.05) [11]. Peurala E et al., noted higher overall positivity (58.8%), highest in T1 (38.2%, p>0.05) and decreasing markedly in larger tumours: T2 (13.8%), T3 (4.9%), T4 (1.9%) [6]. In this study, overall CDK4 positivity was 47.8%, significantly increasing with tumour size (p<0.001): T1 tumours had 33.3%, T3 tumours reached 75%, and T4 tumours 84.6%. This suggests CDK4 nuclear expression strongly correlates with tumour aggressiveness, possibly reflecting demographic and detection differences compared to earlier studies. CDK4 cytoplasmic expression also showed a progressive increase with size but lacked statistical significance (p=0.345), suggesting a weaker association.

Association of CDK4 expression and lymph node status: There was a significant inverse association between CDK4 nuclear expression and lymph node positivity (p<0.001). Surprisingly, CDK4 nuclear positivity was more frequent in node-negative tumours, a finding in contrast to previous studies which showed no significant association. Studies done by An et al., and Peurala E et al., found no statistically significant correlation between CDK4 Nuclear expression and lymph node status, with 9 out of 15 CDK4 positive

Variables	Category	CDK4 Nuclear Negative n (%)	CDK4 Nuclear Positive n (%)	p-value (Nuclear)	CDK4 Cytoplasmic Negative n (%)	CDK4 Cytoplasmic Positive n (%)	p-value (Cytoplasmic)
Tumour size	T1 (≤2 cm)	4 (66.7)	2 (33.3)	<0.001	3 (50.0)	3 (50.0)	0.345
	T2 (2-5 cm)	36 (70.6)	15 (29.4)		21 (41.2)	30 (58.8)	
	T3 (>5 cm)	5 (25.0)	15 (75.0)		4 (20.0)	16 (80.0)	
	T4	2 (15.4)	11 (84.6)		5 (38.5)	8 (61.5)	
Lymph node	Negative	14 (31.8)	30 (68.2)	<0.001	13 (29.5)	31 (70.5)	0.170
	Positive	33 (71.7)	13 (28.3)		20 (43.5)	26 (56.5)	
Tumour grade	I	41 (89.1)	5 (10.9)	<0.001	23 (50.0)	23 (50.0)	0.018
	II	5 (17.9)	23 (82.1)		5 (17.9)	23 (82.1)	
	III	1 (6.2)	15 (93.8)		5 (31.2)	11 (68.8)	
ER status	Negative	20 (52.6)	18 (47.4)	0.947	15 (39.5)	23 (60.5)	0.637
	Positive	27 (51.9)	25 (48.1)		18 (34.6)	34 (65.4)	
PR status	Negative	20 (47.6)	22 (52.4)	0.978	28 (66.7)	14 (33.3)	0.539
	Positive	23 (47.9)	25 (52.1)		29 (60.4)	19 (39.6)	
HER2/Neu Status	Negative	25 (38.5)	40 (61.5)	0.004	38 (58.5)	27 (41.5)	0.122
	Positive	18 (72.0)	7 (28.0)		19 (76.0)	6 (24.0)	
Ki-67 index	<14%	27 (60.0)	18 (40.0)	0.140	19 (42.2)	26 (57.8)	0.274
	≥14%	20 (44.4)	25 (55.6)		14 (31.1)	31 (68.9)	
Molecular Subtype	Luminal A	21 (61.8)	13 (38.2)	0.309	15 (44.1)	19 (55.9)	0.142
	Luminal B	9 (39.1)	14 (60.9)		5 (21.7)	18 (78.3)	
	HER2/Neu Enriched	4 (40.0)	6 (60.0)		2 (20.0)	8 (80.0)	
	TNBC	13 (56.5)	10 (43.5)		11 (47.8)	12 (52.2)	
Stage	Stage I & II	43 (72.9)	16 (27.1)	<0.001	25 (42.4)	34 (57.6)	0.121
	Stage III & IV	4 (12.9)	27 (87.1)		8 (25.8)	23 (74.2)	
TILs	Low	21 (51.2)	20 (48.8)	0.457	17 (41.5)	24 (58.5)	0.371
	Intermediate	9 (42.9)	12 (57.1)		5 (23.8)	16 (76.2)	
	High	17 (60.7)	11 (39.3)		11 (39.3)	17 (60.7)	
LVI	Absent	23 (67.6)	11 (32.4)	0.022	18 (52.9)	16 (47.1)	0.013
	Present	24 (42.9)	32 (57.1)		15 (26.8)	41 (73.2)	
NPI / Prognosis	Excellent	3 (75.0)	1 (25.0)	<0.001	2 (50.0)	2 (50.0)	0.317
	Good	25 (89.3)	3 (10.7)		13 (46.4)	15 (53.6)	
	Moderate	18 (56.2)	14 (43.8)		12 (37.5)	20 (62.5)	
	Poor	1 (3.8)	25 (96.2)		6 (23.1)	20 (76.9)	

[Table/Fig-4]: CDK4 expression in breast cancer in relation to clinicopathological variables.

cases in the former group being lymph node positive (p>0.05) and 26 out of 60 in the latter group (p=0.478) [6,11].

No significant association was found between cytoplasmic CDK4 and lymph node status (p=0.170), with no comparative literature available.

Association of CDK4 expression and tumour grade: In the present study, CDK4 nuclear positivity was observed most frequently in grade 2 tumours (n=23), followed by grade 3 (n=15) and least was observed in grade 1 (n=5). The association between CDK4 nuclear expression and tumour grade was statistically significant (p<0.01), which suggests a potential role of CDK4 in progression of tumour and its aggressiveness. This finding is similar to study done by Peurala E et al., where higher CDK4 nuclear positivity was seen in grade 2 (22 cases) and grade 3 (24 cases) tumours, while only 14 cases of grade 1 were positive. However, their analysis was not statistically significant (p=0.156) [6]. Similarly, An Hx et al., reported a low number of CDK4 positive grade 1 tumours (n=1), with slightly more in grade 2 (n=8) and grade 3 (n=6). Although higher grade tumours showed increased CDK4 amplification in this study, this too lacked statistical significance (p>0.05) [11]. In the study done by An Hx et al. gene amplification was used as the marker whereas in the present study and Peurala E et al. focused on protein expression by immunohistochemistry. This difference in detection method may account for some of the variation [6,11].

The higher CDK4 expression in grade 2 and 3 tumours may be explained by the role of CDK4 in promoting cell cycle progression from G1 to S phase, which is commonly upregulated in proliferative and poorly differentiated tumours similar to study done by An HX et al., [11]. This finding supports the role of CDK4 contribution in tumour aggressiveness and could serve as a marker of intermediate to high grade malignancy.

In this study, CDK4 cytoplasmic staining was separately associated with grade in contrast to other studies where only nuclear staining of CDK4 was considered. CDK4 cytoplasmic expression showed a statistically significant association with tumour grade (p=0.018), indicating that CDK4 cytoplasmic expression increases with tumour grade. CDK4 cytoplasmic positivity was observed in 50% of grade 1 tumours, whereas in grade 2 tumours this increased markedly to 82.1% and in grade 3 tumours, 68.8% were positive. The findings indicate an increase in the cytoplasmic expression of CDK4 as tumours exhibit higher grade. Association between cytoplasmic CDK4 expression and tumour grade remains unexplored in the current literature.

Association of CDK4 expression and LVI: The present study revealed a statistically significant association between LVI and nuclear expression of CDK4 (p=0.030), which further supports the role of CDK4 in tumour invasiveness and progression. Among LVI positive tumours, 57.1% demonstrated CDK4 nuclear expression compared to only 32.4% in LVI negative tumours. This finding

suggests that higher nuclear CDK4 expression is more prevalent in tumours with lymphovascular invasion which is a known adverse prognostic feature in breast cancer [14].

In the present study, a significant association was observed between LVI and cytoplasmic CDK4 expression (p=0.023), indicating a possible link between CDK4 localisation and tumour aggressiveness. Among the cases showing presence of LVI, 73.2% showed CDK4 cytoplasmic expression when compared to only 47.1% in tumours where LVI was absent. This finding suggests that tumours exhibiting lymphovascular invasion which is an established marker of metastatic potential are more likely to display aberrant cytoplasmic localisation of CDK4.

CDK4 which is a central regulator of the cell cycle exerts its biological activity primarily in the nucleus by phosphorylating the retinoblastoma protein (pRB), promoting G1-S phase progression. Its increased nuclear and cytoplasmic expression in LVI present tumours may indicate enhanced proliferative activity and aggressiveness of the tumour , which is consistent with previous studies linking elevated CDK4 activity with poor outcomes [13]. No comparative studies were available in the literature for association between CDK4 expression and LVI.

Association of CDK4 expression and TILs: Nuclear CDK4 showed no significant association with TILs (p=0.457), occurring in 39.3%, 57.1% and 48.8% of high, intermediate and low TIL tumours, respectively suggesting its nuclear localisation is independent of immune infiltration. Cytoplasmic CDK4 expression did not correlate significantly with TIL levels (p=0.371), occurring in 60.7% of high-TIL, 76.2% of intermediate-TIL, and 58.5% of low-TIL tumours. Although expression was marginally higher in the intermediate-TIL group, this difference was not significant. To date, no studies have directly examined the CDK4–TIL relationship, as previous research has focused on CDK4's proliferative role or TILs' prognostic value separately [6].

Association of CDK4 expression and stage: In this study the distribution of CDK4 nuclear expression across different tumour stages were assessed. It was found that there was a significant association between CDK4 expression and tumour progression. In early stage tumours (Stage I & II), only 16 out of 59 cases (27%) showed positive CDK4 nuclear expression while the majority (72.9%) were negative. Meanwhile, advanced stage tumours (Stage III & IV) showed a markedly higher expression, with 27 out of 31 cases (87.1%) showing positive expression and only 12.9% were negative. These findings suggest that there is a strong association between CDK4 nuclear expression and advanced tumour stages, emphasizing on its possible role in cancer progression.

Although cytoplasmic expression was more frequent in advanced stages (74.2%), it did not reach statistical significance (p=0.121), consistent with its less defined role in staging.

Association of CDK4 expression and hormonal biomarkers: The association of hormonal biomarker positivity and CDK4 nuclear expression was assessed and compared with other studies. In a study done by Peurala E et al. it was found that CDK4 nuclear positivity had no statistically significant association with ER, PR, HER2/Neu and Ki67 [6]. Nuclear CDK4 showed no significant link with ER, PR, or Ki-67, but correlated strongly with HER2/neu positivity (p=0.004). This mirrors Piezzo M et al.,'s finding that CDK4/6 drive proliferation in HER2-positive breast cancer, supporting CDK4-inhibitor use in this subtype [15]. In the present study, association of the CDK4 cytoplasmic expression with biomarkers revealed no statistically significant results.

Association of CDK4 expression and molecular subtypes: Although CDK4 nuclear positivity was highest in HER2/Neu enriched (60%) and Luminal B (60.9%) tumours, the association was not statistically significant (p=0.309), potentially due to sample size limitations. These subtypes are known for higher proliferation, correlating with increased CDK4 activity. Cytoplasmic CDK4 was also most frequent in HER2/Neu enriched and Luminal B subtypes (80% and 78.3%, respectively), again without statistical significance (p=0.142).

Association of CDK4 expression and NPI: There was a strong and statistically significant association between CDK4 nuclear expression and poor prognosis (p<0.001). Nearly all patients with poor NPI scores were CDK4 positive, while CDK4 negativity was more common among those with good or excellent prognosis. In contrast, although cytoplasmic expression of CDK4 was more frequently observed in poor prognosis cases (76.9%) in the present study, the association did not reach statistical significance (p=0.317). Collectively, these findings underscore CDK4's potential role as a prognostic biomarker and therapeutic target, particularly highlighting its relevance in aggressive tumour subtypes. Further multicenter studies are recommended to validate CDK4's clinical utility in breast cancer management.

Limitation(s)

The study's retrospective design have introduced selection bias, limiting generalisability. Being single-centre and region-specific (Southern India), findings may not be widely applicable. Survival analysis was not performed, preventing conclusions on long-term outcomes. Additionally, the lack of a standardised CDK4 assay and scoring system hinders reproducibility and clinical applicability.

CONCLUSION(S)

This study highlights the prognostic value of CDK4 expression in invasive ductal carcinoma, emphasising the importance of its subcellular localisation. Nuclear CDK4 correlates with aggressive features like larger tumour size, higher grade, LVI, advanced stage, HER2/Neu positivity and may serve as a prognostic biomarker and therapeutic target in aggressive subtypes. In contrast, cytoplasmic CDK4, though more prevalent, showed limited prognostic relevance. Further research with larger cohorts and long-term follow-up is needed to validate these findings and explore the functional role of CDK4 localisation in breast cancer.

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