fortune Journals

Research Article

JOURNAL OF CANCER SCIENCE AND CLINICAL THERAPEUTICS

ISSN: 2637-5079

Significance of Cyclin D1 Immunoexpression in Breast Carcinoma

Dr. Mst Nazneen Nahar Rina[1*], Prof. Dr. Naba Kumar Saha[2], Dr. Mostafa Kamal[3]

Abstract

Background: Cyclin D1 plays a critical role in tumorigenesis and the regulation of the G1/S transition in the cell cycle. Overexpression of cyclin D1 in breast carcinoma is associated with poor prognosis and resistance to conventional hormone therapy.

Objectives: To assess the immunoexpression of cyclin D1 in breast carcinoma and to study the association between cyclin D1 immunoexpression with clinicopathological parameters such as age, tumor size, histomorphological types and grades, lymphovascular invasion and ER status.

Materials and Methods: The present study was a cross-sectional observational study conducted at the Department of Pathology, Sylhet MAG Osmani Medical College, Sylhet, during the period from March 2019 to February 2021. Twenty-three female patients were selected who underwent surgery, and forty-nine paraffin blocks from the growth of the breast were collected. The resected specimens were processed, and paraffin blocks were made. Then, sections from all blocks were stained with routine H&E stain to see the histologic type and grade of the tumor. The immunostaining of cyclin D1 and ER was done from paraffin blocks.

Results: In this study, 72 cases of invasive breast carcinoma were taken. The mean age of the patients was 50.06 (SD ± 10.25) years. The mean tumor size was 4.18 (SD ± 1.80) cm. Thirty-three (45.8%) cases belonged to grade III, 25 (34.7%) cases to grade II, and 14 (19.4%) cases to grade I. Lymphovascular invasion was found in 43 (59.7%) cases. Cyclin D1 was found to be strong positive in 7 (9.7%) cases, moderate positive in 24 (33.3%) cases, weak positive in 14 (19.4%) cases and negative in 27 (37.5%) cases. Estrogen receptor (ER) was positive in 33 (45.8%) cases and negative in 39 (54.2%) cases. There was a statistically significant association between tumor grade and cyclin D1 (p=0.046). There was a statistically significant association between tumor grade and cyclin D1 scoring (p=0.017). The association between cyclin D1 and estrogen receptor was also statistically significant (P=0.009).

Conclusion: The association of cyclin D1 immunoexpression with tumor grade and ER expression was statistically significant. Thus, cyclin D1 can be used as a prognostic marker and marker for resistance to hormone therapy, thereby dictating appropriate treatment modalities in patients with breast carcinoma.

Keywords: Invasive ductal carcinoma; Cyclin D1; ER; Immunohistochemical expression.

Affiliation:

[1]Assistant Professor, Department of Pathology, Khulna Medical College, Khulna, Bangladesh.

[2]Ex. Professor, Department of Pathology, Sylhet MAG Osmani Medical College, Sylhet, Bangladesh.

[3]Assistant Professor, Department of Cardiology, Khulna Medical College, Khulna, Bangladesh.

***Corresponding author:**
Dr. Mst Nazneen Nahar Rina, Assistant Professor, Department of Pathology, Khulna Medical College, Khulna, Bangladesh.

Citation: Dr. Mst Nazneen Nahar Rina, Prof. Dr. Naba Kumar Saha, Dr. Mostafa Kamal. Significance of Cyclin D1 Immunoexpression in Breast Carcinoma. Journal of Cancer Science and Clinical Therapeutics. 8 (2024): 321-326.

Received: October 26, 2024
Accepted: November 05 2024
Published: November 07, 2024

Dr. Mst Nazneen Nahar Rina, et al., J Cancer Sci Clin Ther 2024
DOI:10.26502/jcsct.5079255

Introduction

The breast is composed of glandular, ductal, and connective tissue, consisting primarily of two key structures: ducts and lobules. These structures are lined by two types of epithelial cells, luminal and myoepithelial cells, and are supported by interlobular and intralobular stroma. The adult breast contains 6–10 major ductal systems, which branch into smaller ducts, eventually forming the terminal duct lobular units (TDLUs)-the functional units where most breast cancers originate [1]. Breast cancer, the most prevalent malignancy among women worldwide, typically arises from the cells lining the ducts and lobules. Tumors arising in ducts are classified as ductal carcinomas, while those in lobules are termed lobular carcinomas. Globally, breast cancer incidence rates are higher in developed regions like Australia, New Zealand, Europe, and North America, while developing countries have shown a rising trend in recent years [2]. According to the 2018 GLOBOCAN report (a project of IARC, WHO), 2,088,849 new breast cancer cases were diagnosed globally, accounting for 24.2% of all cancers in women, with 626,679 deaths (6.6%). In Asia alone, 911,014 (22.3%) new cases and 310,577 (5.7%) deaths were reported. In Bangladesh, breast cancer accounted for 19% of all cancers in 2018, with 12,769 new cases and 6,846 deaths [3]. Breast cancer is a clinically and pathologically heterogeneous disease with several well-known risk factors, including early menarche, late pregnancy, late menopause, family history, atypical hyperplasia, and radiation exposure [4]. Traditional prognostic markers include age, tumor size, histological grade and type, lymph node metastasis, lymphovascular invasion, and the expression status of estrogen receptor (ER), progesterone receptor (PR), and human epidermal growth factor receptor 2 (HER2). Cyclin D1, an important cell cycle regulatory protein, has emerged as a significant but underutilized biomarker in breast cancer. Cyclins are a family of proteins that regulate the cell cycle, with Cyclins D, E, A, and B sequentially appearing to activate different cyclin-dependent kinases (CDKs). Cyclin D1, encoded by the CCND1 gene on chromosome 11q13.3, plays a key role in cell proliferation by binding to CDK4/6 and inducing phosphorylation of the retinoblastoma (Rb) protein, thereby promoting cell cycle progression from the G1 phase to the S phase [5]. Dysregulation of this pathway has been implicated in breast cancer development [6]. Parvin et al. (2019) reported that cyclin D1 is overexpressed in up to 90% of breast cancers and plays a dual role in cell proliferation by regulating both the cell cycle and the activity of ER, independently of Rb [7]. This interaction is crucial, as tamoxifen- standard therapy for ER-positive cancers often fails due to endocrine resistance linked to dysregulation of the Cyclin D1-CDK4/6-Rb pathway [8]. Emerging therapies, such as CDK4/6 inhibitors (e.g., Palbociclib), offer promising treatment options for overcoming such resistance [9]. Given its pivotal role in tumor progression and endocrine resistance, Cyclin D1 immunoexpression could serve as a valuable prognostic marker alongside ER and PR. Additionally, targeting this pathway offers new opportunities for therapeutic interventions in breast cancer [10]. This study aims to explore the significance of Cyclin D1 immunoexpression in breast carcinoma, emphasizing its potential as both a prognostic tool and a therapeutic target.

Methodology & Materials

This was a cross-sectional observational study carried out at the Department of Pathology in Sylhet MAG Osmani Medical College (SOMCH). From March 2019 to February 2021, 72 patients who were diagnosed with breast carcinoma by histopathological examination were enrolled in the study. After a full explanation of the study's details, informed written consent was obtained from the patients or attendants. Prior to the commencement of the study, ethical clearance was obtained from the Institutional Ethical Committee of SOMCH.

Inclusion criteria:

Histopathologically diagnosed cases of breast carcinoma of all ages.

Exclusion criteria:

Breast carcinoma patients who were treated by preoperative hormone therapy, chemotherapy or radiation therapy.

Data collection:

Data were collected using a pre-designed data collection sheet, meticulously prepared through a comprehensive review of relevant literature and consultation with supervisors and experts. The primary variables included histomorphological types and grades of breast carcinoma, lymphovascular invasion, and immunohistochemical expressions of Cyclin D1 and ER in breast carcinoma. The secondary variables recorded were age and tumor size. This structured approach ensured the systematic and accurate capture of essential data for analysis.

Specimen Collection

After admission, a diagnostic process was made from history, clinical examination and necessary investigations. Diagnosis of breast cancer was made by clinical examination by a surgeon and FNAC of breast lump by a pathologist. Mastectomy was done in the Department of Surgery by a competent surgeon. The mastectomy specimen was preserved in 10% formalin and carried by the investigator to the pathology department, SOMCH, for histopathological examination. Twenty-three female patients were selected following the enrolment criteria who underwent Surgery at the Department of Surgery, Sylhet MAG Osmani Medical College Hospital, Sylhet and forty-nine paraffin blocks

Dr. Mst Nazneen Nahar Rina, et al., J Cancer Sci Clin Ther 2024
DOI:10.26502/jcsct.5079255

from the growth of breast were collected from the National Institute of Cancer Research and Hospital, Dhaka. The resected specimens were processed, and paraffin blocks were made. Then, sections from all blocks were stained with routine H&E stain to see the histologic type and grade of the tumor. The paraffin blocks were then carried to the Armed Forces Institute of Pathology for immunostaining of cyclin D1 and ER.

Statistical analysis:

All the collected data were compiled and analyzed using the SPSS (Statistical Package for Social Science) version 27. Quantitative data were expressed as mean and standard deviation. Qualitative data were expressed as frequency and percentage. Chi-square test and Fisher-exact test were done to see the association between cyclin D1 immunoexpression and age, tumor size, tumor grade, lymphovascular invasion and ER status in breast carcinoma. A probability value (p) of <0.05 was considered statistically significant.

Results

The patient cohort had a mean age of 50.06 years (SD±10.25), with the majority aged 41-50 years (n=26, 36.11%), followed by 51-60 years (n=20, 27.78%). Smaller proportions were aged 31-40 years (n=15, 20.83%), 61-70 years (n=9, 12.50%), and 71-82 years (n=2, 2.78%). The mean tumor size was 4.18 cm (SD±1.80), with most tumors measuring 2-5 cm (n=51, 70.83%), while 20.83% (n=15) were larger than 5 cm and 8.33% (n=6) were smaller than 2 cm. Invasive ductal carcinoma was the predominant histologic type (n=71, 98.61%), with only one patients (1.39%) showing ductal carcinoma with lobular features. Regarding histologic grade, 45.83% of tumors (n=33) were classified as Grade III, followed by 34.72% (n=25) as Grade II, and 19.44% (n=14) as Grade I. Lymphovascular invasion was detected in 59.72% (n=43) of patients, in comparison,40.28% (n=29) were negative. Estrogen receptor (ER) immunoexpression was negative in 54.17% (n=39) of tumors, and 45.83% (n=33) were ER-positive. Figure 1 illustrates the distribution of cyclin D1 immunoexpression in invasive ductal carcinoma. The largest proportion of cases (37.50%) exhibited negative cyclin D1 expression, while 33.33% showed moderate positivity. Weak positive expression was observed in 19.44% of cases, and the least frequent category was strong positivity, accounting for 9.72% of patients. This distribution suggests that while a notable number of tumors express cyclin D1 at varying levels, a substantial portion remains negative, indicating variability in cyclin D1 expression across the cohort. The association of cyclin D1 expression with age, tumor size, and histopathological grade is summarized in Table 3. Cyclin D1 positivity was more frequent in patients aged 51-60 years (70.00%), followed by those aged 41-50 years (65.38%) and 31-40 years (53.33%). In the 61-70 and 71-80 age groups, the positivity rates were 55.56% and 50.00%, respectively, but the difference across age groups was not statistically significant (P=0.809). Regarding tumor size, cyclin D1 positivity was observed in 66.67% of patients with tumors <2 cm and 2-5 cm, but only 46.67% of those with tumors >5 cm, though the association was not significant (P=0.401). For histopathological grade, cyclin D1 positivity increased with higher grades, being most common in Grade III tumors (75.76%), followed by Grade I (64.29%) and Grade II (44.00%), with a statistically significant association (P=0.046). Table 4 explores the relationship between cyclin D1 expression, lymphovascular invasion, and estrogen receptor (ER) status. Among patients with lymphovascular invasion, 69.77% (n=30) were cyclin D1 positive, compared to 51.72% (n=15) of those without it. However, this difference was not statistically significant (P=0.121). A significant association was observed with ER status (P=0.009), as 78.79% (n=26) of ER-positive patients were cyclin D1 positive, whereas only 48.72% (n=19) of ER-negative patients expressed cyclin D1. These findings suggest that cyclin D1 expression is significantly linked to ER status but not to lymphovascular invasion.

Table 1: Demographic and clinical characteristics of patients

Variables	Frequency (N)	Percentage (%)
Age (years)		
31-40	15	20.83
41-50	26	36.11
51-60	20	27.78
61-70	9	12.5
71-82	2	2.78
Mean±SD	50.06 ± 10.25	
Size of tumor (cm)		
<2	6	8.33
05-Feb	51	70.83
>5	15	20.83
Mean±SD	4.18 ± 1.80	
Histologic type		
Invasive ductal carcinoma	71	98.61
Invasive ductal carcinoma with lobular feature	1	1.39
Histologic grade		
Grade I	14	19.44
Grade II	25	34.72
Grade III	33	45.83

Citation: Dr. Mst Nazneen Nahar Rina, Prof. Dr. Naba Kumar Saha, Dr. Mostafa Kamal. Significance of Cyclin D1 Immunoexpression in Breast Carcinoma. Journal of Cancer Science and Clinical Therapeutics. 8 (2024): 321-326.

Dr. Mst Nazneen Nahar Rina, et al., J Cancer Sci Clin Ther 2024
DOI:10.26502/jcsct.5079255

Table 2: Lymphovascular invasion and estrogen receptor immunoexpression distribution in study participants

Variables	Frequency (N)	Percentage (%)
Lymphovascular invasion		
Positive	43	59.72
Negative	29	40.28
Estrogen receptor immunoexpression		
Positive	33	45.83
Negative	39	54.17

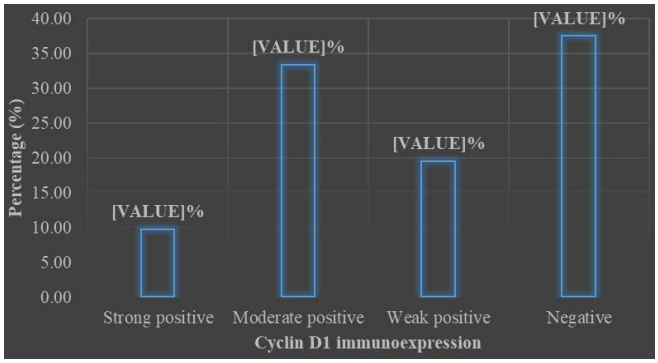

Figure 1: Distribution of cyclin D1 immunoexpression in invasive ductal carcinoma.

Table 3: Association of cyclin D1 expression with age, tumor size, and histopathological grade in patients

Cyclin D1 expression	Positive (N=45)		Negative (N=27)		P-value
	N	%	N	%	
Age					
31-40 years	8	53.33	7	46.67	0.809
41-50 years	17	65.38	9	34.62	
51-60 years	14	70	6	30	
61-70 years	5	55.56	4	44.44	
71-80 years	1	50	1	50	
Tumor size					
<2 cm	4	66.67	2	33.33	0.401
2-5 cm	34	66.67	17	33.33	
>5 cm	7	46.67	8	53.33	
Histopathological grade					
Grade-I	9	64.29	5	35.71	0.046
Grade-II	11	44	14	56	
Grade-III	25	75.76	8	24.24	

Discussion

Invasive breast cancer is a leading cause of mortality among women worldwide, with significant efforts dedicated to identifying prognostic biomarkers. This study evaluated cyclin D1 expression and its correlation with various clinicopathological parameters, including age, tumor size, grade, lymphovascular invasion, and ER status, in 72 cases of invasive ductal carcinoma. The findings provide insights into the role of cyclin D1 in breast cancer progression and align with prior studies, though some variations exist. Previous research presents mixed findings regarding the prognostic impact of cyclin D1 expression in breast cancer. Some studies associate cyclin D1 overexpression with a poorer prognosis [11, 12], while others report that cyclin D1 expression correlates with ER positivity and favorable outcomes [13, 14]. Moreover, higher levels of cyclin D1 have been linked to reduced overall survival and higher relapse rates [15, 16]. Interestingly, the majority of cyclin D1-overexpressing tumors are ER-positive, yet patients with these tumors experience worse outcomes compared to those with ER-positive tumors lacking cyclin D1 overexpression [12]. In this study, the mean age of the patients was 50.06 ± 10.25 years, consistent with reported data from similar studies [17-19]. The tumor size ranged from 2 to 5 cm, with a mean size of 4.18±1.80 cm. Notably, 91.7% of tumors exceeded 2 cm in diameter, which reflects delayed diagnosis, likely due to limited screening programs and social factors in the region. Tumor size did not show a significant association with cyclin D1 expression (p=0.401), aligning with previous studies [5, 7]. Histological grading revealed that 45.83% of tumors were grade III, 34.72% grade II, and 19.44% grade I. Cyclin D1 expression was more frequent in high-grade tumors, with a statistically significant correlation between cyclin D1 expression and histological grade (p=0.046). Among grade-III tumors, 75.76% showed positive cyclin D1 expression, while only 64.29% of grade-I tumors were positive. This suggests that cyclin D1 overexpression may contribute to increased tumor aggressiveness, consistent with findings by Mohammadizadeh et al and Parvin et al [5, 7]. Lymphovascular invasion (LVI) was observed in 59.72% of cases, but no significant association was found between LVI and cyclin D1 expression (p=0.121). This result is consistent with studies that also reported no significant correlation between these parameters [18]. ER status was positive in 45.8% of cases, similar to findings by Sarkar et al [20]. Among ER-positive tumors, 78.8% showed positive cyclin D1 expression, suggesting a significant association between cyclin D1 and ER status (p=0.009). This may reflect cyclin D1's role in regulating ER activity, as previously noted by Mohammadizadeh et al and Lengare et al [5, 18]. The study observed varying levels of cyclin D1 expression: 9.7% of cases showed strong positivity, 33.3% moderate, 19.4% weak, and 37.5% were negative. These findings align with Sarkar et al., who reported similar distribution patterns [20]. Although cyclin D1 expression did not significantly correlate with patient age (p=0.809), its higher expression in aggressive tumors suggests that cyclin D1 plays a role in tumor progression. In conclusion, the current study highlights the association between cyclin D1 expression and key

Dr. Mst Nazneen Nahar Rina, et al., J Cancer Sci Clin Ther 2024
DOI:10.26502/jcsct.5079255

pathological factors, particularly tumor grade and ER status. The lack of significant correlation with patient age, tumor size, or lymphovascular invasion suggests that cyclin D1's role may be more complex and context-dependent. Given the association with aggressive, ER-positive tumors, cyclin D1 may serve as a useful prognostic biomarker and potential therapeutic target in breast cancer. Further studies with larger sample sizes are recommended to validate these findings and explore the therapeutic potential of targeting cyclin D1 in ER-positive breast cancers.

Limitations of the study

Due to the COVID-19 pandemic, only twenty-three simple mastectomy specimens were available. Additionally, forty-nine paraffin blocks from breast carcinoma were collected from NICRH, Dhaka. Lymph node status could not be assessed due to the lack of radical mastectomy specimens. Tamoxifen was not administered, and patient follow-up was not conducted.

Conclusion and Recommendations

Invasive ductal carcinoma is the most common type of breast carcinoma and the leading cause of cancer mortality and morbidity in women. Conventional therapy like surgery, radioablation and chemoablation is not always effective to combat mammary carcinoma. Immunoexpression of ER, PR, and HER-2 is explored to give hormonal and immunotherapy. Cyclin D1 expression in breast carcinoma is associated with tumor progression and resistance to tamoxifen. So, overexpression of cyclin D1 with ER expression in breast carcinoma may give way for targeted therapy by palbociclib along with tamoxifen.

Funding

No funding sources

Conflict of interest

None declared

Ethical approval

The study was approved by the Institutional Ethics Committee.

References

1. Ellenson LH, Lester SC. Female genital system and breast. Robbins Basic Pathology, 10th ed, Elsevier, Amsterdam (2018): 759-794.
2. Hossain MS, Ferdous S, Karim-Kos HE. Breast cancer in South Asia: a Bangladeshi perspective. Cancer epidemiology 38 (2014): 465-470.
3. Bray F, Ferlay J, Soerjomataram I, et al. Global cancer statistics 2018: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. CA: a cancer journal for clinicians 68 (2018): 394-424.
4. Hussain SM, Hussein AA, Khashma BM. Immunohistochemical Expression of Cyclin D1 in Human Breast Carcinoma. J Integr Oncol S 1 (2016): 2.
5. Mohammadizadeh F, Hani M, Ranaee M, et al. Role of cyclin D1 in breast carcinoma. Journal of research in medical sciences: the official journal of Isfahan University of Medical Sciences 18 (2013): 1021.
6. Ortiz AB, Garcia D, Vicente Y, et al. Prognostic significance of cyclin D1 protein expression and gene amplification in invasive breast carcinoma. PloS one 12 (2017): e0188068.
7. Parvin T, Das C, Choudhury M, et al. Prognostic utility of cyclin D1 in invasive breast carcinoma. Indian Journal of Surgical Oncology 10 (2019): 167-173.
8. Gao A, Sun T, Ma G, et al. LEM4 confers tamoxifen resistance to breast cancer cells by activating cyclin D-CDK4/6-Rb and ERα pathway. Nature communications 9 (2018): 4180.
9. Akin S, Babacan T, Sarici F, et al. A novel targeted therapy in breast cancer: cyclin dependent kinase inhibitors. J buon 19 (2014): 42-46.
10. Assem M, Youssef EA, Rashad RM, et al. Immunohistochemical expression of cyclin d1 in invasive ductal carcinoma of human breast. Oncomedicine 2 (2017): 80-87.
11. McIntosh GG, Anderson JJ, Milton I, et al. Determination of the prognostic value of cyclin D1 overexpression in breast cancer. Oncogene 11 (1995): 885-891.
12. Kenny FS, Hui R, Musgrove EA, et al. Overexpression of cyclin D1 messenger RNA predicts for poor prognosis in estrogen receptor-positive breast cancer. Clinical cancer research 5 (1999): 2069-2076.
13. Gillett C, Smith P, Gregory W, et al. Cyclin D1 and prognosis in human breast cancer. International Journal of Cancer 69 (1996): 92-99.
14. Roy PG, Thompson AM. Cyclin D1 and breast cancer. The Breast 15 (2006): 718-727.
15. Umekita Y, Ohi Y, Sagara Y, et al. Overexpression of cyclinD1 predicts for poor prognosis in estrogen receptor-negative breast cancer patients. International journal of cancer 98 (2002): 415-418.
16. Rudas M, Lehnert M, Huynh A, et al. Cyclin D1 expression in breast cancer patients receiving adjuvant tamoxifen-based therapy. Clinical Cancer Research 14 (2008): 1767-1774.

Dr. Mst Nazneen Nahar Rina, et al., J Cancer Sci Clin Ther 2024
DOI:10.26502/jcsct.5079255

17. Khokher S, Qureshi MU, Riaz M, et al. Clinicopathologic profile of breast cancer patients in Pakistan: ten years data of a local cancer hospital. Asian Pacific Journal of Cancer Prevention 13 (2012): 693-698.

18. Lengare PV, Khandeparkar SG, Joshi AR, et al. Immunohistochemical expression of cyclin D1 in invasive breast carcinoma and its correlation with clinicopathological parameters. Indian Journal of Pathology and Microbiology 63 (2020): 376-381.

19. Huang HJ, Neven P, Drijkoningen M, et al. Association between tumour characteristics and HER-2/neu by immunohistochemistry in 1362 women with primary operable breast cancer. Journal of clinical pathology 58 (2005): 611-616.

20. Sarkar S, Kanoi A, Bain J, et al. Correlation between cyclin D1 expression and standard clinicopathological variables in invasive breast cancer in Eastern India. South Asian journal of cancer 4 (2015): 155-159.