Journal of Cardiovascular Disease Research

ISSN:0975 -3583,0976-2833 VOL14, ISSUE 10, 2023

Original research article

A study on evaluation of prognostic significance of Cyclin D1 in breast carcinoma

[1]Dr. Pranshu Saklani, [2]Dr. Karthik LR, [3]Dr. Chaitra N, [4]Dr. R. Naveen Shyam Sundar

[1]Senior Resident, Department of Pathology, Varun Arjun Medical College, Shahjahanpur, Uttar Pradesh, India

[2,3]Assistant Professor, Department of Pathology, Sri Siddhartha Medical College, Tumkur, Karnataka, India

[4]Senior Resident, Department of Community Medicine, Dr. Sushila Nayar School of Public Health, Mahatma Gandhi Institute of Medical Sciences, Sewagram, Wardha, Maharashtra, India

Corresponding Author:
Dr. Chaitra N

Abstract

Introduction: Breast cancer is a heterogeneous disease, comprising numerous distinct entities that have different biological features and clinical behaviour. There are many biomarkers that are related to the progression and therapeutic response of invasive breast cancer. One emerging biomarker is Cyclin D1 which is a key cell cycle regulatory protein, encoded by the gene, CCND1 or PRAD1, positioned on chromosome11q13. Thus, the present study is an attempt to analyse immunohistochemical expression of Cyclin D1 in breast carcinoma and correlate it with other known histopathological prognostic markers.

Materials and Methods: A study of forty breast carcinoma patients carried out in the Dept. of Pathology during 2019-2022. Immunohistochemical staining of Cyclin D1 was performed. The intensity of Cyclin D1 staining will be scored on a scale from 0 to 3 and results were tabulated. Data was entered in MS Excel and analyzed using Pearson's Chi- square test and fisher exact test. A P value <0.05 was considered as statistically significant.

Results: Statistically significant results were obtained with Cyclin D1 and grade of the tumor (p value 0.04). However, correlation of Cyclin D1 with size, lymphnode status and lymphovascular invasion was not statistically significant.

Conclusion: Our study confirms the strong association between Cyclin D1 and grade of the tumour which is one of the oldest and the strongest prognostic marker known in breast carcinoma. Cyclin D1 expression appears to be a good independent marker in breast cancer and can be included in the pool of prognostic markers like tumor size, nodal status, histopathological grade.

Keywords: Cyclin D1, Breast carcinoma, prognostic marker

Introduction

Breast cancer is a heterogeneous disease, comprising numerous distinct entities that have different biological features and clinical behaviour. The extent of the heterogeneity of breast cancer is highlighted by microarray-based studies which have identified multiple molecular subgroups [1]. The expression of estrogen receptor (ER), progesterone receptor (PR) and HER2neu, and the identification of molecular subtypes (Luminal A, Luminal B, HER2 enriched and Basal like) have important prognostic and predictive roles in the clinical management of breast cancer. However, there are many other biomarkers that are related to the progression and therapeutic response of invasive breast cancer, but a lack of consistent results in different studies has limited their use in clinical practice [2].

One such emerging biomarker is Cyclin D1 which is a key cell cycle regulatory protein, encoded by the gene, CCND1 or PRAD1, positioned on chromosome11q13. Cyclin D1 is necessary for the normal lobulo-alveolar development of the breast. Cyclin D1 is also one of the key regulatory molecules in cell cycle and plays a crucial role in cell cycle progression from G1 to S phase by regulating the activity of cyclin-dependent kinases (CDKs) [3].

Amplification and overexpression of the gene encoding cyclin D1 has been frequently observed in breast cancers. Cyclin D1 binds directly to the estrogen receptors (ERs) and thereby propagates the downstream effects of estrogen in a CDK independent and Rb independent fashion. Although it has shown its prognostic value in several studies, use of cyclin D 1 as a routine prognostic tool is still under debate [4].

Higher expression of Cyclin D1 is known to be positively associated with ER and PR positive tumors and negatively with Her 2 neu positive cases [5, 6, 7].

Breast cancer is dependent on various histopathological prognostic factors including tumor size, histopathological grade, lymph node metastasis, hormonal receptor status and Her2neu expression [4].

Thus, the present study is an attempt to analyse immunohistochemical expression of Cyclin D1 in breast carcinoma and correlate it with other known histopathological prognostic markers like tumor size,

ISSN:0975 -3583,0976-2833 VOL14, ISSUE 10, 2023

histological grade and lymph node status.

Materials and Methods: A study of forty breast carcinoma patients carried out in the Dept. of Pathology during 2019-2022.

Inclusion criteria: All cases biopsied and diagnosed as breast carcinoma.

- All types of biopsies, ranging from needle core to Mastectomy specimens were studied.

Exclusion criteria: Biopsy samples of recurrent cases.

- Inadequate biopsies.
- Poorly processed biopsies
- History of any hormonal or chemotherapy prior to biopsy
- Specimen was fixed in 10% formalin followed by paraffin embedding and staining with haematoxylin and eosin. Histopathological examination of the tissue with breast carcinoma was done. The Hematoxylin and Eosin-stained slides were examined for adequacy and as a rule of thumb we took a minimum of 60-70% well-preserved tumor area and tissues with less than 10% of necrotic area for Cyclin D1 IHC staining. This was done to prevent staining artefacts and for obtaining better results.
- Corresponding blocks were retrieved and four thin (4 to 5 microns) sections were taken to perform immunohistochemical study on Cyclin D1.
- Immunostaining for Cyclin D1 was considered as positive when at least 10% or more of the tumor cells show nuclear expression of the marker with a moderate to strong intensity of staining.
- The intensity of Cyclin D1 staining will be scored on a scale from 0 to 3 where,
- 0 = Negative staining.
- 1 = Weak staining.
- 2 = Moderate staining.
- 3 = Strong staining.

Statistical analysis

Data was entered in MS Excel. Tables and charts were generated using MS Excel. Quantitative data was presented as mean $\pm$ SD. Qualitative data was presented with the help of frequency and percentage table. Relationship between the cyclin D1 expression and known prognostic markers were analyzed using Pearson's Chi- square test and fisher exact test. A P value <0.05 was considered as statistically significant.

Results

The present study comprised of forty patients, who were diagnosed as carcinoma of the breast in our institute during the study period from January 2019-September 2022. The age of the patients ranged from 29 to 90 years with a mean age of 44 years. Majority of the cases (59.3%) were found to be ≥50years. Out of 40 cases, 39(98%) cases were females and 01(2%) case male patient.

Cyclin D1 expression in the study

Cyclin D1 expression was determined for all the 40 cases by immunohistochemistry. All cases showing >10% of tumor cells with moderate to strong nuclear staining were considered as positive.

Out of the total 40 cases studied, 30 (75%) cases were found to be Cyclin D1 positive. (Table 1). The range of Cyclin D1 expression in the study was 0-90%. The Mean Cyclin D1 was 38% while median was 40% in IDC NOS type. In our study, 10 (25%) cases showed Cyclin D1 negativity.

Table 1: Cyclin D1 expression in the study

Cyclin D1 Status	Number of cases
Cyclin D1 Positive	30 (75%)
Cyclin D1 Negative	10 (25%)
Total	40 (100%)

Correlation between Cyclin d1 and size of the tumor

As seen in table below, out of the 30 MRM cases, maximum cases belonged to T3 and T4 category (50%). 50% of the T1 tumors, 84.6% of T2 and 53.3% of T3&T4 cases showed positivity to cyclin D1. (Table 2) This was not statistically significant (p value was 0.188).

Table 2: Correlation cyclin D1 with size

Size of tumor	Cyclin Positive D1	Cyclin Negative D1	Total	
T1	1 (50%)	1 (50%)	2 (100%)	P value = 0.188
T2	11 (84.6%)	2 (15.3%)	13 (100%)	
T3 & T4	8 (53.3%)	7 (46.6%)	15 (100%)	

ISSN:0975 -3583,0976-2833 VOL14, ISSUE 10, 2023

Total	20(66.6%)	10 (33.3%)	30(100%)	

Correlation between Cyclin D1 and grade of the tumor

Out of the 40 cases studied, 35 cases belonged to Infiltrating ductal carcinoma (NOS) type which were graded according to Bloom Richardson grading system. All the 9(100%) cases of grade I (Figure 1), 15 (71.4%) cases of grade II (Figure 2) and 2 (40%) cases of grade III (Figure 3) tumors were positive for cyclin D1 (Table 3). A statistically significant correlation (p value of 0.04) was noted between grade of the tumor and Cyclin D 1 expression.

Table 3: Correlation between Cyclin D1 and grade of the tumor

Grades	Cyclin Positive D1	Cyclin Negative D1	Total	
Grade I	9 (100%)	0(0%)	9 (100%)	
Grade II	15 (71.4%)	6 (28.6%)	21 (100%)	P value = 0.04
Grade III	2 (40%)	3 (60%%)	5 (100%)	
Total(n=35)	26 (72.5%)	9 (27.5%)	35 (100%)	

Correlation between Cyclin D1 expression and lymph node status

Lymph node status was assessed in the 35 MRM cases studied. Out of the 26-lymph node positive cases, 10(55.5%) showed Cyclin D positivity while 08(44.5%) showed negativity. 76.5% of node negative cases showed Cyclin D1 positivity (Table 4). No statistical correlation was noted between lymph node status and Cyclin D1 expression in the study.

Table 4: Correlation between Cyclin D1 expression and lymph node status

Lymph node	Cyclin D1 Positive	Cyclin D1 Negative	Total	
Lymph node positive	10 (55.5%)	8 (44.5%)	18 (100%)	
Lymph node negative	13 (76.5%)	4 (23.5%)	17 (100%)	P value = 0.192
Total	26 (72.5%)	9 (27.5%)	35 (100%)	

Correlation of cyclin d1 with lymphovascular invasion

Lymphovascular invasion was seen in 19 cases in which 11 (57.9%) cases showed positivity for Cyclin D1 expression (Table 5). The p value is 0.370 which was not statistically significant.

Table 5: Correlation of Cyclin D1 with Lymphovascular Invasion

Lymphovascular Invasion	Cyclin Positive D1	Cyclin Negative D1	Total	
Present	11 (57.9%)	8 (42.1%)	19 (100%)	P Value = 0.370
Absent	15 (71.4%)	6 (28.6%)	21 (100%)	
Total	26 (72.5%)	9 (27.5%)	35 (100%)	

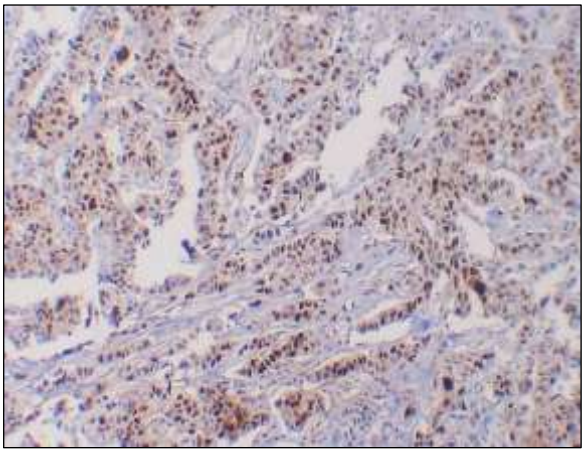

Fig 1: Grade 1 Invasive carcinoma, NOS showing strong nuclear positivity to Cyclin D1 immunostaining. IHC; 400x

ISSN:0975 -3583,0976-2833 VOL14, ISSUE 10, 2023

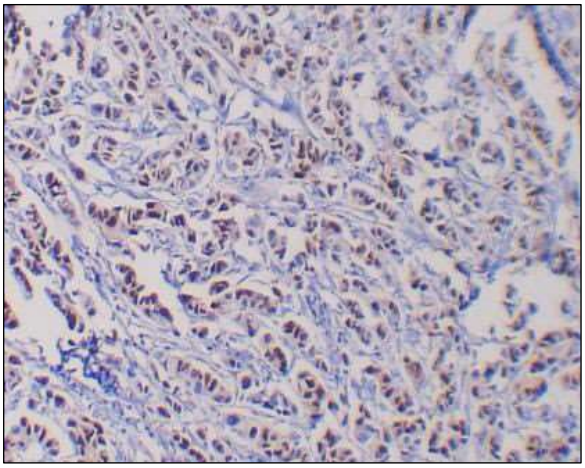

Fig 2: Grade 2 Invasive carcinoma, NOS showing strong nuclear positivity to Cyclin D1. IHC; 400x

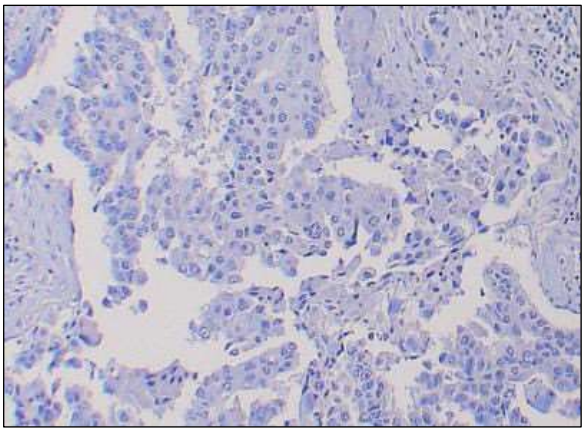

Fig 3: Grade 3 Invasive carcinoma, NOS showing negative Cyclin D1 immunostaining. IHC; 400x

Discussion

It is a well-known fact that breast carcinoma is quite heterogenous in origin with multiple genetic alterations leading to its pathogenesis. Most genetic alterations inherently contribute in the development of the tumor while few help in the progression of the disease. Amplification of Cyclin D1 resulting in its overexpression is one such genetic alteration playing crucial role in breast carcinogenesis [4].

In the present study, 26 (72.5%) cases showed positivity to Cyclin D1. Similar findings have been observed in the past by various authors where Cyclin D1 expression has a wide frequency ranging from 43 to 70.9% expression of the cases studied. Singh *et al*., (57.14%) and Hafez *et al*., (35.2%) reported a slightly lesser number of positive cases in their study. This wide variation has been largely attributed to the use of different antibodies, techniques and scoring system being adopted while interpreting cyclin D1 [8, 9]. Various studies have shown that the gross size of tumor is one of the most significant prognostic factors in breast carcinoma and there is increased incidence of axillary lymph node metastasis and decreased survival with increasing size of the tumor [8, 10].

Hence, we correlated the size of the tumor with Cyclin D1 expression to find an association between the two. Tumor size (T) was assessed in the 30 MRM cases received. Among them, most of the tumors belonged to size >2cm (93.3%). Expression of Cyclin D1 and size did not show any statistical correlation (p value =0.188). Only two cases in the study belonged to T1 category (<2 cm) of which one of them showed negativity to Cyclin D1. This was a case of IDC-grade II tumour. Our findings are similar to studies done by Dalal M Nemenqani, [11] Mohammedi *et al*., [12] Ravikumar *et al*., and Lengare *et al*., [4, 13] who also did not find any statistical correlation between size and expression of Cyclin D1. Conflicting to this, Hafez *et al*., has observed a statistically significant correlation between the two parameters [9].

Out of the 26 Cyclin D1 positive Infiltrating ductal carcinoma, NOS cases, 9 cases belonged to grade I, 15 (71.5%) cases belonged to grade II and 2 (40%) cases were of grade III. Cyclin D 1 positivity was noticed in 26(72.5%) out of 40 cases of IDC-NOS type. This finding is comparable to studies done by Gayathri *et al*., [4] and Sarkar *et al*., [14] Among the grade I tumours, Cyclin D1 showed positivity in all the 100% of the cases. This finding is similar to studies done by Sarkar *et al*., [14] and Gayathri *et al*., [4] unlike in study done by Mohammedi *et al*., where Cyclin D 1 was negative in both the grade I tumours studied. This could be due to the lower incidence of grade I tumours in their study. Among grade II tumours, our study showed 71.5% positivity which is in concordance to the studies done Gayathri *et al*., [4] and Sarkar *et al*., [14].

ISSN:0975 -3583,0976-2833 VOL14, ISSUE 10, 2023

Among grade III tumours, only 40% showed Cyclin D1 positivity which was lower in comparison to grade I and II tumours in the study.

Hence, histological grade of the tumor was seen to have a statistically significant (p= 0.04) negative correlation with Cyclin D 1 expression. This is consistent with majority of studies done in the past where higher expression of Cyclin D 1 positivity been noticed in low and moderate tumor grade. This suggests that higher expression of Cyclin D1 may directly or indirectly result in maturation of the tumor cells [15]. However, Singh *et al*., and Ravikumar *et al*., did not find any statistical correlation between grade and Cyclin D1 expression in their studies [4, 8]. This may be attributed to the smaller sample size studied.

Lymph node involvement is a valuable indicator of short-term survival. Node-positive patients have about four to eight times higher mortality than those without nodal involvement. Prognosis for patients with 10 or more involved axillary nodes showed 70% more deaths at 10 years than for those with 1-3 involved nodes [16]. Lymph node assessment was done in 30 cases of MRM cases and correlated with the Cyclin D 1 positivity. The finding of our study was comparable to most other studies in the literature who did not find any positive correlation between the two [17].

Conclusion

Our study confirms the strong association between Cyclin D1 and grade of the tumour which is one of the oldest and the strongest prognostic marker known in breast carcinoma. A high rate of expression of Cyclin D1 in the study highlights the importance of estimation of Cyclin D1 with other prognostic factors. Since studies pertaining to Cyclin D1 expression and disease- free survival are limited, larger population-based studies with better research designs and standardized measurements of Cyclin D 1 will throw more light on the exact mechanism of Cyclin D1 in Breast carcinoma. Our study concludes that Cyclin D 1 expression appears to be a good independent marker in breast cancer and can be included in the pool of prognostic markers like tumor size, nodal status, histopathological grade.

References

1. Feng Y, Spezia M, Huang S, Yuan C, Zeng Z, Zhang L, *et al*. Breast cancer development and progression: Risk factors, cancer stem cells, signaling pathways, genomics and molecular pathogenesis. Genes & diseases. 2018 Jun;5(2):77-106.
2. Ortiz AB, Garcia D, Vicente Y, Palka M, Bellas C, Martin P. Prognostic significance of cyclin D1 protein expression and gene amplification in invasive breast carcinoma. PloS one. 2017 Nov;12(11):e018-8068.
3. Sherr CJ, Roberts JM. CDK inhibitors: positive and negative regulators of G1-phase progression. Genes & development. 1999 Jun;13(12):1501-12.
4. Ravikumar G, Ananthamurthy A. Cyclin D1 expression in ductal carcinoma of the breast and its correlation with other prognostic parameters. J Can Res Ther. 2014;10:671-5.
5. Parvin T, Das C, Choudhury M, Chattopadhyay B, Mukhopadhyay M. Prognostic Utility of Cyclin D1 in Invasive Breast Carcinoma. IAOSO, 2018, 167-173.
6. Mohammadizadeh F, Hani M, Ranaee M, Bagheri M. Role of cyclin D1 in breast carcinoma. J Res Med Sci. 2013;18:1021-1025.
7. Lengare PV, Sinai Khandeparkar SG, Joshi AR, Gogate BP, Solanke SG, Gore SH. Immunohistochemical expression of cyclin D1 in invasive breast carcinoma and its correlation with clinicopathological parameters. Indian J Pathol. Microbiol. 2020;63:376-8.
8. Singh S, Mabood A, Dwivedi N, Alam MQ, Gangwar A. Correlation of cyclin D1 between hormones receptors and other clinical parameters in invasive breast carcinoma. International Journal of Research in Medical Sciences. 2020 Dec;8(12):4436.
9. El-Hafez AA, Shawky AE, Hasan B. Cyclin D1 overexpression associates with favourable prognostic factors in invasive breast carcinoma. Cancer Biomarkers. 2013 Jan;12(4-5):149-54.
10. Sen A, Sunita BS. CD44positive/CD24negative breast carcinoma cells as marker of tumor aggression, medical journal armed forces India. 2017Jan:73(1):29-35.
11. Dalal M. Nemenqani, Expression of cyclin D1 in breast carcinoma and its relation to other prognostic markers in Saudi female patients International Journal of Advanced Research. 2015;3(9):990-996.
12. Mohammedi L, Doula FD, Mesli F, Senhadji R. Cyclin D1 overexpression in Algerian breast cancer women: correlation with CCND1 amplification and clinicopathological parameters. African Health Sciences. 2019 Aug;19(2):2140-6.
13. Lengare PV, Sinai Khandeparkar SG, Joshi AR, Gogate BP, Solanke SG, Gore SH. Immunohistochemical expression of cyclin D1 in invasive breast carcinoma and its correlation with clinicopathological parameters. Indian J Pathol. Microbiol. 2020;63:376-8.
14. Daniel N, Goneppanavar M, Srinivasan S, Vaithy KA. Study of hormone receptor status and HER/2-neu expression in breast malignancies and its implication in molecular subtyping in a tertiary care hospital. Indian J Pathol. Oncol. 2020;7(4):537-542.

ISSN:0975 -3583,0976-2833 VOL14, ISSUE 10, 2023

15. Geethamala K, Murthy SV, Vani BR, Rao S. Histopathological Grade Versus Hormone Receptor Status In Breast Carcinoma-Treasure The Past. Int. J Biomed Res. 2015;6(7):466-71. Doi: 10.7439/ijbr.v6i7.2203.
16. Parvin T, Das C, Choudhury M, Chattopadhyay BK, Mukhopadhyay M. Prognostic utility of cyclin D1 in invasive breast carcinoma. Indian Journal of Surgical Oncology. 2019 Mar;10:167-73.
17. Dutta V, Chopra GS, Sahai K, Nema SK. Hormone Receptors, Her-2/Neu and chromosomal Aberrations in Breast Cancer. MJAFI. 2008;64:11-15.
18. Li CI, Uribe DJ, Daling JR. Clinical characteristics of different histologic types of breast cancer. British journal of cancer. 2005 Oct;93(9):1046-52.
19. Gogoi G, Borgohain M, Saikia P, Fazal SA. Profile of molecular subtypes of breast cancer with special reference to triple negative: A study from Northeast India. Clin Cancer Investig J. 2016 Sep;5(5):374-83.
20. Iqbal BM, Buch A. Hormone receptor (ER, PR, HER2/neu) status and proliferation index marker (Ki-67) in breast cancers: Their onco-pathological correlation, shortcomings and future trends. Med J DY Patil. Univ. 2016;9:674-9.