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Immunohistochemical Expression of Cyclin D1 in Invasine Breast Carcinoma and its Correlation with Other Prognostic Parameters

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ABSTRACT

Breast carcinoma is one the most common neoplasm in women ranking second among overall cancers in the world. Age, tumour size, histological grade, histological type, lymphovascular invasion, axillary lymph node metastasis and hormone receptor markers are regarded as prognostic markers in patients with breast carcinoma. Cyclin D1 is emerging as a significant biomarker in invasive breast cancer, not still used as a routine prognostic tool in breast cancer, although it has shown its prognostic value in several studies. The recent knowledge in molecular mechanisms of this cancer and consequent targeted treatments have attempted to improve its outcome. Aims of the present study are to assess Cyclin D1 expression in invasive breast carcinoma and to correlate Cyclin D1 expression with other prognostic parameters for invasive breast carcinoma. Patients fulfilling the inclusion criteria and who had undergone mastectomy were selected including both prospective and retrospective cases after obtaining waiver-off consent and clearance from The Institutional Research Committee and Institutional Ethics Committee. The study included 47 cases of Modified radical mastectomies done for various grades and stages of breast carcinoma. Tumour tissues were stained immunohistochemically with Cyclin D1. Semiquantitative scoring was done using Allred score method and its relationship with prognostic parameters was then determined. Cyclin D1 was strong, intermediate, weak/ negative expression in 31.9%, 42.6% and 25.5%.

***Key words*:** Breast carcinoma, Cyclin D1, prognostic parameters, mastectomy, breast, immunohistochemistry.

INTRODUCTION:

Breast cancer is the most common cancer among women worldwide[1]. In India, breast cancer has the highest incidence and death rate. Age, tumour size, histological grade, histological type, lymphovascular invasion, axillary lymph node metastasis and hormone receptor markers are regarded as prognostic markers in patients with breast carcinoma[2,3].

Cyclin D1 is emerging as a significant biomarker in invasive breast cancer,though not still used as a routine prognostic tool; it has shown its prognostic value in several studies. Recent knowledge in molecular mechanisms and consequent targeted treatments has attempted to improve its outcome. Cyclin D1, a regulatory protein in cell cycle plays an important role in regulating the progress of cell during the G1 phase of the cell cycle. Cyclin D1 binds to CDK4 and CDK6 and induces hyperphosphorylation of Rb gene, thus promoting cellular proliferation. CCND1 gene is amplified in approximately 20% of mammary carcinomas and the protein is over-expressed in approximately 50% of cases[4,5] . The present study aims to find the frequency of Cyclin D1 expression and relationship

between Cyclin D1 expressions with prognostic parameters in invasive breast carcinomas.

MATERIALS AND METHODS:

The present study was done in the Department of Pathology and Surgery at Sri Manakula Vinayagar Medical College and Hospital, Kalitheerthalkuppam, Puducherry. It was a hospital based analytical cross-sectional study. Patients fulfilling the inclusion criteria during the study period of one and a half years including both prospective and retrospective cases were included in the study with an inclusion criteria of patients diagnosed with breast carcinoma in mastectomy specimens were studied. Retrospective cases were included after obtaining waiver-off consent and clearance from The Institutional Research Committee and Institutional Ethics Committee. Patients with inadequate biopsy, biopsy samples of recurrent carcinoma, benign breast lesions, stromal tumors and history of any hormonal/ chemotherapy/radiotherapy prior to biopsy were excluded from the study. The sample size was calculated based on previous study by Reis-Filho J. et al.[6] After taking the absolute precision value of 13.5% and confidence interval of 95%, the sample size was calculated to be 47 using software Open epi version 3. The specimens sent to the Department of Pathology were examined.

Clinical data was obtained from requisition forms and hospital records. Following fixation, gross examination and tissue sectioning, the tissues were stained with Haematoxylin and Eosin, histologic grade, type and staging was done.

Immunohistochemical staining with Cyclin D1 was done using Polymer kit(DAKO) using standard protocol and tonsillar tissue was taken as positive control. The sections were evaluated by 2 Pathologists and slides were assessed under low power and 400x. Cyclin D1 intensity and proportion were semi-quantitatively scored using Allred score method.

Intensity was interpreted as 0 - negative (no staining of any nuclei even at high magnification); weak (only visible at high magnification); moderate (readily visible at low magnification) and strong (strikingly positive even at low power magnification). The intensity of Cyclin D1 was scored on a scale from 0 to 3[3].

Proportion of tumour nuclei showing positive staining was also recorded as either: 0 -None; 1 - 0, 2 -1% to 10%, 3 -11% to 33%, 4 - 34% to 66% and 5 - 67%. The proportion and intensity scores were then added to obtain a total score, which ranged from 0 to 8[3].

Tumors were then categorized into four groups: Negative/Weak expression (total scores 0-2), Intermediate expression (total scores 3-5) and Strong expression (total scores 6-8)[3]. Only nuclear staining was considered specific.

The results where then statistically analysed by using SPSS (Statistical Package for the Social Sciences) Statistics software 27.0.1 version.

ETHICAL CONSIDERATION:

The ethical approval for the study was obtained from the Institute ethics committee of Sri Manakula Vinayagar Medical College and Hospital, Puducherry with IEC study number EC/52/2022. All the ethical principles were adhered in the study. All the participants were briefed about the study in the language they could follow and their willingness to participate in the study was obtained through an informed consent form. All 47 patients in this study were very cooperative while giving their detailed history during the sample collection.

RESULTS :

The various clinicopathological features of breast carcinoma are presented in Table 1. The age of patients ranged from 31 to 79 years, with a mean value of 54.6 years. Highest cases were seen in the age group of 50-60 years (34%). Most common histologic type observed was Invasive Ductal carcinoma of no special type as shown in Graph 1.

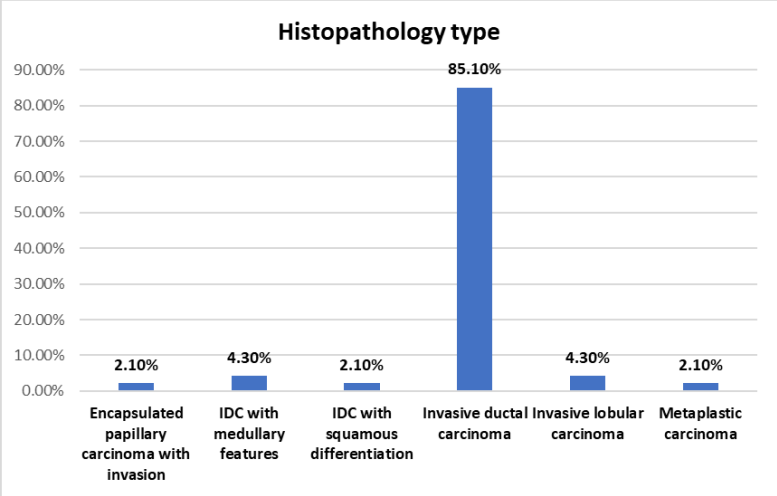
TABLE 1: Distribution of overall Clinicopathological parameters

	Parameters	**Frequency**	**Percentage (%)**
Age	30 to 40 years	3	6.4
	40 to 50 years	13	27.7

	50 to 60 years	16	34
	60 to 70 years	12	25.5
	70 to 80 years	3	6.4
Duration	Less than 6 months	26	55.3
	6 months to 1 year	21	44.7
Family history	Absent	44	93.6
	Present	3	6.4
Menstrual history	irregular	17	36.2
	Regular	30	63.8
Consumption of OCP.	consumed	34	72.3
	not consumed	13	27.7
Side of the tumor	Right	22	46.8
	Left	25	53.2
Tumor size	less than 2cm	3	6.4
	2 to 5cm	32	68.1
	more than 5cm	12	25.5
Quadrant involved	LIQ	7	14.9
	LOQ	7	14.9
	UIQ	4	8.5
	UOQ	18	38.3
	Central	11	23.4
Adjacent area changes.	Fibrosis	46	97.9
	Micro-calcification	1	2.1
Glandular (Acinar)/tubular Differentiation	Score 1 (>75% of tumor area forming glandular/tubular structures)	6	12.8
	Score 2 (10% to 75% of tumor area forming glandular/tubular structures)	25	53.2
	Score 3 (< 10% of tumor area forming glandular/tubular structures)	16	34
Nuclear Pleomorphism	Score 1	4	8.5
	Score 2	22	46.8
	Score 3	21	44.7
Mitotic Rate	Score 1 (≤3 mitoses per mm2)	14	29.8
	Score 2 (4-7 mitoses per mm2)	14	29.8

	Score 3 (≥8 mitoses per mm2)	19	40.4
Focality of the tumor	multifocal	2	4.3
	unifocal	45	95.7
Lymphovascular invasion	Absent	17	36.2
	present	30	63.8
Perineural invasion	Absent	42	89.4
	present	5	10.6
Lymph node metastasis	Absent	21	44.7
	Present	26	55.3
Histopathology type	Encapsulated papillary carcinoma with invasion	1	2.1
	IDC with medullary features	2	4.3
	IDC with squamous differentiation	1	2.1
	Invasive ductal ca	40	85.1
	Invasive lobular carcinoma	2	4.3
	Metaplastic ca	1	2.1
Histopathology grade	HP Grade 1	11	23.4
	HP Grade 2	19	40.4
	HP Grade 3	17	36.2
DCIS/ LCIS status	Absent	29	61.7
	present	18	38.3
Intensity of Cyclin D1 Staining	Negative	1	2.1
	Weak	19	40.4
	Moderate	19	40.4
	Strong	8	17
Proportion of Cyclin D 1	None	1	2.1
	<1%	11	23.4
	1% to 10 %	6	12.8
	11 % to 33%	16	34
	34% to 66%	9	19.1
	>=67%	4	8.5
Cyclin D 1 scoring	Negative / weak expression	12	25.5
	Intermediate expression	20	42.6
	Strong expression	15	31.9

Cyclin D1 scoring was done for 47 cases of breast carcinoma. Majority of cases showed intermediate expression comprising 20 cases (42.6%) followed by 15 cases (31.9%) of strong expression and 12 cases (25.5%) showed negative/ weak staining.



Graph 1: Distribution of Histopathology type of tumor

Histopathological grading was done according to Modified Bloom-Richardsons grading. Majority of cases 19 (40.4%) were classified as grade 2 followed by 17 cases of grade 3(36.2%) and 11 cases of grade 1(23.4%).

Table 2: Association between Cyclin D1 status and histopathological grade

		Histopathological Grade					
		1		2		3	
		Count	% of Total	Count	% of Total	Count	% of Total
Cyclin D 1 Status	Negative	1	9.09%	7	36.84%	4	23.52%
	Positive	10	90.9%	12	63.15%	13	76.47%
Total		11		19		17	
p value	0.237						

Association between Cyclin D1 and histopathological grade showed 11 cases with grade 1 tumour in which 10 cases (90.9%) exhibited Cyclin D1 overexpression, 19 cases of grade 2 tumors in which 12 cases (63.15%) showed overexpression of Cyclin D1 and 17 cases of grade 3 tumors in which 13 cases (76.47%) showed Cyclin D1 overexpression.

Table 3: **Association of Cyclin D1 with Clinicopathological parameters**

			Cyclin D 1 Status		**Total**	**p value**
			Negative	**Positive**		
Tumor Stage	T1	Count	2	1	3	

		% of Total	4.30%	2.10%	6.40%	0.235
	T2	Count	7	25	32	
		% of Total	14.90%	53.20%	68.10%	
	T3	Count	3	9	12	
		% of Total	6.40%	19.10%	25.50%	
Quadrant	LIQ	Count	5	2	7	0.04
		% of Total	10.60%	4.30%	14.90%	
	Central	Count	2	9	11	
		% of Total	4.30%	19.10%	23.40%	
	LOQ	Count	1	6	7	
		% of Total	2.10%	12.80%	14.90%	
	UIQ	Count	0	4	4	
		% of Total	0.00%	8.50%	8.50%	
	UOQ	Count	4	14	18	
		% of Total	8.50%	29.80%	38.30%	
Lymphovascular invasion	Absent	Count	5	12	17	0.646
		% of Total	10.60%	25.50%	36.20%	
	Present	Count	7	23	30	
		% of Total	14.90%	48.90%	63.80%	
Perineural invasion	Absent	Count	9	33	42	0.6
		% of Total	19.10%	70.20%	89.40%	
	Present	Count	3	2	5	
		% of Total	6.40%	4.30%	10.60%	
Lymph node	Absent	Count	6	15	21	0.668
		% of Total	12.80%	31.90%	44.70%	

	Present	Count	6	20	26	
		% of Total	12.80%	42.60%	55.30%	
Histopathological type	Encapsulated papillary carcinoma with invasion	Count	0	1	1	0.788
		% of Total	0.00%	2.10%	2.10%	
	IDC with medullary features	Count	1	1	2	
		% of Total	2.10%	2.10%	4.30%	
	IDC with squamous differentiation	Count	0	1	1	
		% of Total	0.00%	2.10%	2.10%	
	Invasive ductal carcinoma	Count	11	29	40	
		% of Total	23.40%	61.70%	85.10%	
	Invasive lobular carcinoma	Count	0	2	2	
		% of Total	0.00%	4.30%	4.30%	
	Metaplastic carcinoma	Count	0	1	1	
		% of Total	0.00%	2.10%	2.10%	
HP Grade	1	Count	1	10	11	0.237
		% of Total	2.10%	21.30%	23.40%	
	2	Count	7	12	19	
		% of Total	14.90%	25.50%	40.40%	
	3	Count	4	13	17	
		% of Total	8.50%	27.70%	36.20%	
DCIS/ LCIS	Absent	Count	5	24	29	0.098
		% of Total	10.60%	51.10%	61.70%	
	Present	Count	7	11	18	
		% of Total	14.90%	23.40%	38.30%	

Association between Cyclin D1 and Clinicopathological parameters is shown in Table 2.

Cyclin D1 positivity is seen in 35/47 (74.4%) cases of invasive breast carcinomas. Cyclin D1 and tumour size association revealed most of the tumors were of size 2 cms to 5 cms (32 cases, 68.1%) in which 25 cases exhibited cyclin D1 overexpression.

Cyclin D1 associated with histopathological type revealed predominant type to be Invasive ductal carcinoma of which 29 cases exhibited Cyclin D1 overexpression and 11 cases exhibited weak expression. Association between Cyclin D1 and nodal staging revealed 20 cases of N0 stage in which 14 cases exhibited overexpression of cyclin D1.

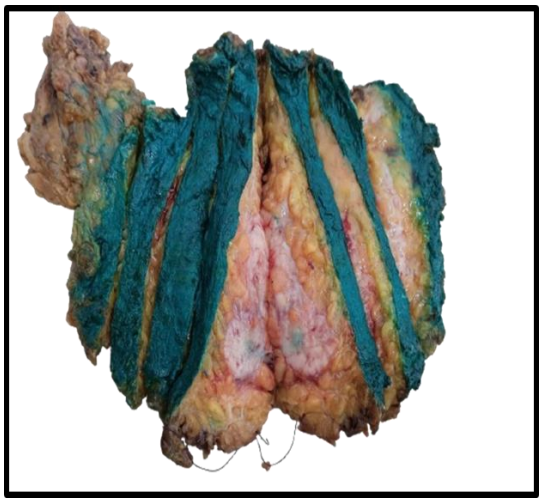


Figure 1: Gross image of Invasive breast carcinoma measuring 4x 2 x 2cms. (HP-S-1029/21)

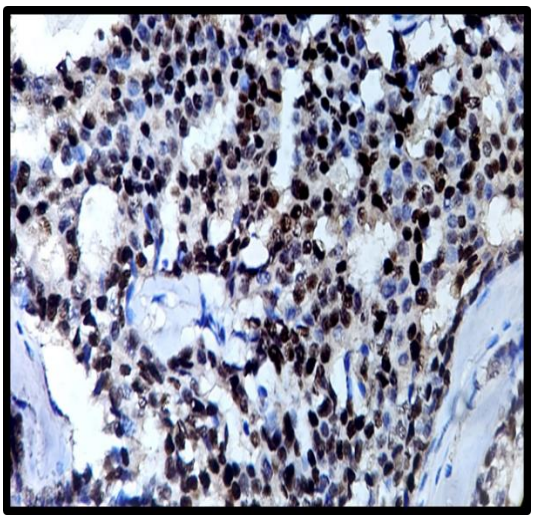


Figure2: - Strong expression of Cyclin D1, Invasive ductal carcinoma, Grade 1(Cyclin D1, 10x)

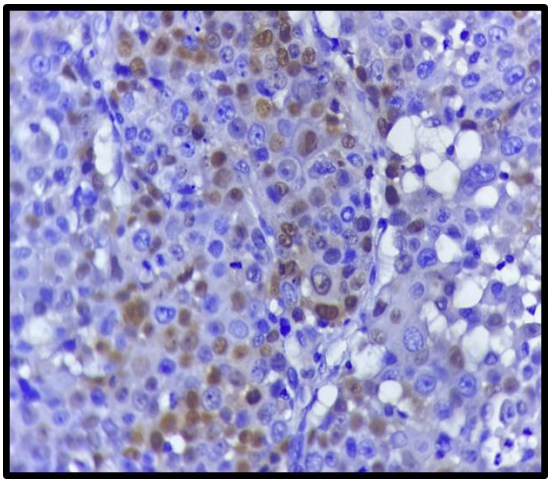


Figure3: - Intermediate expression of cyclin D1, Invasive ductal carcinoma, Grade 2 (Cyclin D1, 10x)

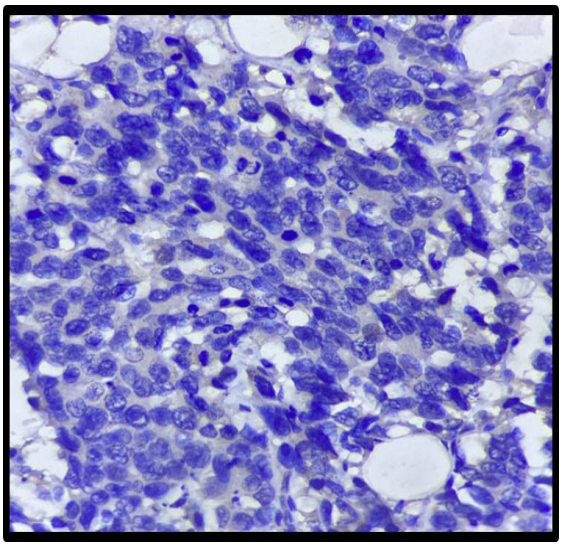


Figure 4: Weak expression of Cyclin D1,Invasive ductal carcinoma, Grade 3 (Cyclin D1, 10x)

DISCUSSION

Breast cancer has become the second most common cancer in the world and the most common cancer among women. The amplification of Cyclin D1, which results in overexpression, is one such fundamental genetic change in the development of breast cancer by its cell mediated action. The present study attempted to study the role of Cyclin D1 with various clinicopathological parameters which included 47 patients who were diagnosed with breast carcinoma and undergone Modified radical mastectomy without a history of chemotherapy or radiotherapy.

Perimenopausal and postmenopausal age groups had a higher incidence of breast carcinomas with mean age of 54.6 years (range: 31 to 79 years). This result was consistent with the study conducted by Rao et al[7].Most of cases belonged to upper quadrant similar to the studies done by Bosompem K et al and Sandhu DS et al[8,9]. The most frequent histologic type for invasive ductal cancer was represented by 40 cases (85.10%) with no specific type. Similar articles by Li Ch et al[10], T.S. Tenea-Cojan et al[11], Zangouri v MD et al[12] also revealed that the most prevalent histologic subtype was invasive ductal carcinoma.

At presentation, 68.1% of the individuals had tumours measuring between 2 and 5 centimetres [T2], and the most frequent nodal status found was pN0. Studies by Grothery et al. and Jung J et al. which was also similar to ours, revealed that pT2 to be the most common tumour stage and pN0 as the most common nodal stage[13,14,15].

Most common tumour grade was Grade 2 which correlated with studies done by Sarkar et al who also reported a higher proportion of patients with grade 2 tumours in keeping with the aggressive nature of breast cancer and late presentation of the disease.[16].

Several studies have investigated the relationship between cyclin D1 overexpression and the histopathological grade of breast cancer. Cyclin D1 is a key regulator of the cell cycle, particularly in facilitating the transition from the G1 to S phase. Similar findings were also seen in the study done by Angela B et al stating that cyclin D1 overexpression in breast cancer cells can lead to loss of growth control and progression through the G1/S transition, contributing to tumorigenesis.[17] High expression of cyclin D1 was also detected in a considerable majority of patients with low to moderate tumour grade and those with positive ER/PR status, which is in line with the results of Western studies.

Particularly, invasive ductal carcinoma (29 cases,61.7 %) and all invasive lobular carcinoma (2 cases) were found to overexpress cyclin D1. According to a study by Tetsunari Oyama et al[18], Cyclin D1 overexpression is very common in a large percentage of invasive lobular carcinoma (ILC) cases, indicating its significance in the initiation and advancement of this subtype of breast cancer. According to a study by Miguel A. Ortega et al[19] there are notable variations in Cyclin D1 expression between ILC and IDC, which supports the drug's potential as a diagnostic and prognostic marker in the treatment of breast cancer. Cyclin D1 expression aids in understanding the distinct mechanisms behind both malignancies.

In the present study, 4 ER-positive, 3 PR positive and 6 Her-2-neu positive cases exhibited Cyclin D1 overexpression similar to Arian Lundberget al[20] stating that Cyclin D1 is more commonly overexpressed in ER-positive tumours, explaining its role in the hormonal pathway that drives these cancers.

Concerning breast carcinoma of 14 cases analysed in the study, Cyclin D1 immunoreactivity was weakly or negatively expressed in 4 triple negatives.

Maximum cases showing Cyclin D1 expression were of low grade (90.9%) though not statistically significant in this study. It would be reasonable to conclude that increased Cyclin D1 expression was linked to the most well-established favourable prognostic variables in light of the aforementioned findings. A statistically insignificant correlation was found between negative Cyclin D1 expression and intermediate tumour grade and greater stage, positive lymph node status, and lymphovascular invasion in TNBC cases.

LIMITATIONS :

The limitations found in this study included exclusion criteria with treatment(hormone / chemotherapy/ radiotherapy), hence sample size was limited for this prospective cross-sectional study. Other prognostic markers could not be retrieved for the study. Patient follow-up could be able to document only for few cases. Hence increased sample size with analysis of all the prognostic markers is recommended for the future study.

CONCLUSION :

Cyclin D1 expression in breast carcinomas exhibited over-expression in low-grade breast cancer. Also observed was that the Cyclin D1 expression in triple-negative breast cancers was weakly expressed. In light of the above findings, it would be reasonably safe to conclude that increased Cyclin D1 expression was associated with low-grade and nodal status (N0) of invasive breast carcinoma indicating the prognostic utility of this marker. The study also highlights the significance of targeting Cyclin D1 as a promising potential therapeutic target gene.

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