Original Research Article

Correlation of cyclin D1 between hormones receptors and other clinical parameters in invasive breast carcinoma

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ABSTRACT

Background: Breast carcinoma is the most common malignancy in women. The prognosis of breast carcinoma is determined by different prognostic factors including age of patients, type of tumour, stage of tumours, grade of tumors, presence or absence of metastasis and hormones receptor status. The present study was conducted to assess the expression of cyclin D1 in invasive breast carcinoma and its correlation with age, tumour grade, lymph node metastasis and hormones receptors (estrogen receptors and progesterone receptors).
Methods: Present study was conducted in department of pathology UPUMS, Saifai, Etawah, Uttar Pradesh. This was a prospective cross sectional observation study conducted on 21 mastectomy specimen from January 2019 to June 2020, diagnosed as invasive breast carcinoma on routine haematoxylin and eosin stain. Immunohistochemistery marker was scored by using allred scoring methods. Grading was calculated according to Nottingham grading system.
Results: It was observed out of 21 cases 9 (42.8%) cases were showing cyclin D1 positivity. In our study 14 cases were estrogen receptor and progesterone receptor positive, out of 14 ER and PR positive cases 9 (64.2%) cases showing cyclin D1 positivity. There was significant correlation between cyclin D1 and hormones receptors (ER and PR). No significant correlation between cyclin D1and age, tumour grade and lymph node metastasis.
Conclusions: Present study depicts significant correlation between cyclin D1 and hormones receptors (ER and PR). No significant correlation between cyclin D1and age, tumour grade and lymph node metastasis.

Keywords: Invasive breast carcinoma, Cyclin D1, ER, PR

INTRODUCTION

Breast carcinoma is the most common malignancy in women and has been emerged as leading cause of death. The diagnosis of breast carcinoma is done by clinical examination, radiological examination and cytological examination, diagnosis is confirmed by histopathological examination. About 80% of all breast cancers are infiltrating ductal carcinoma. The prognosis of breast carcinoma is determined by different prognostic factors including age of patients, age of menarche and menopausal, type of tumour, stage of tumours, grade of tumors, presence or absence of metastasis and hormones receptor status.[1]

Mammalian cyclin D1, first identified in 1991, mediates G1 to S phase transition in the cell cycle.[2] Cyclin D1 is a also known as cell cycle regulator which acts by binding with cyclin dependent kinase in the cell cycle and inactivating the retinoblastoma (Rb) protein, helping in the procession of the cell cycle. The gene encoding cyclin D1 is the product of CCND1 gene located on the

chromosome 11q13, which is amplified in 15% of breast cancers.[3] This aberrant over expression of cyclin D1 is known to drive breast carcinogenesis by cell cycle mediated action.

The present study was conducted to assess the expression of cyclin D1 in invasive breast carcinoma and its correlation with hormones receptors and others clinical parameters.

METHODS

Present study was conducted in department of pathology, UPUMS, Saifai, Etawah. This was a prospective cross-sectional observation study conducted on mastectomy specimen diagnosed as invasive breast carcinoma on histopathological examination in department of pathology.

Type and duration of study

Current study is a prospective cross-sectional study conducted from January 2019 to June 2020.

Inclusion criteria

All female patients diagnosed with invasive breast carcinoma were included in the study. Total 21 mastectomy specimens from January 2019 to June 2020 were grossly examined, fixed, processed and paraffin embedding done by routine histological technique. Histopathological diagnosis established on routine haematoxylin and eosin stain. Haematoxylin and eosin satin was done by as per standard protocol. Immunohistochemistery marker was used as per standard protocol. The study included parameters like histological type, histological grade, lymph node metastasis, ER, PR and cyclin D1 status. Grading was calculated according to Nottingham grading system

Expression of estrogen (ER) and progesterone (PR) was considered as positive according to allred scoring guidelines.[4] For ER, PR scoring sections were examined and distinct nuclear staining will be taken as positive(carcinoma breast will be taken as positive control). Primary antibodies used for estrogen receptor, monoclonal rabbit anti-human estrogen receptor α; clone: EP1 and for progesterone receptor, monoclonal mouse anti- human progesterone receptor; clone: PgR 636.

Expression of cyclin D1 was considered as negative if <1% cells showing staining.[6] Primary antibody used for cyclin D1 monoclonal rabbit anti-human cyclin D1; clone: and used secondary antibody poly HRP clone: EP12.

Statistical analysis

A descriptive study will be carried out for all the variables included in the study. Chi-square and Fischer test will be used to compare the categorical values. Spearman's correlation coefficient will be used to assess the relationship between the two variables. p value less than 0.05 was considered as statistically significant.

RESULTS

In current study the youngest patient was 35 years old and oldest was 77 years of age. It was observed that maximum cases (66.6%) were in the age group <50 years, followed by (33.3%) in >50 years (Table 1).

Table 1: The frequency distribution of age of total cases (n=21).

Age groups (years)	N	%
≤50	14	66.6
>50	7	33.3
Total	21	100

Out of the 21 cases of ductal carcinoma breast, majority 12 (57.1%) cases were grade II followed by 7 (33.3%) cases were grade I and II (9.5%) cases were grade III (Table 2).

Table 2: Demonstrate frequency distribution of histological grade of ductal carcinoma breast according to MBR grade.

Grade	N	%
I (WD)	7	33.3
II (MD)	12	57.1
III (PD)	02	9.5
Total	21	100

It was observed that majority of patients (42.85%) showing no lymph node metastasis and 33.3% patients were in N1 stage, 9.5% cases were in N2 stage and there was no case in N3 stage (Table 3).

Table 3: Distribution of ductal carcinoma breast cases according to presence of axillary lymph node metastasis.

Lymph node involved (n=21)		N		(%)	
Present	N1		7		33.3
	N2	9	2	42.85	9.5
	N3		0		0
Absent, N0		9		42.85	
Not accessed, Nx		3		14.28	
Total		21		100	

In ≤50 years age group patients (14) only 6 patients and in >50 years age group patients (7) only 3 patients showing cyclin D1 positivity. There was no significant correlation between cyclin D1 and age, (p=1.000) (Table 4).

Table 4: Correlation of cyclin D1 expression with age.

Clinical parameter (n=21)	N	Cyclin D1 significant positive cases	P value
Age groups (years)			
≤50	14	6	
>50	7	3	1.000
Total	21	9	

Out of 12 cases of grade II (MD), 4 (33.3%) cases showed positive cyclin D1 expression while 8 (66.6%) cases were cyclin D1 negative. Out of 7 cases of grade I (WD), 3 cases (42.85%) show positive cyclin D1 expression and 4 cases (57.14%) were cyclin D1 negative. Out of 2 cases of grade III (PD) (Figure 1), 1 case (50%) showing cyclin D1 expression. No significant correlation was observed in cyclin D1 expression with histological grading (p=0.211) (Table 5).
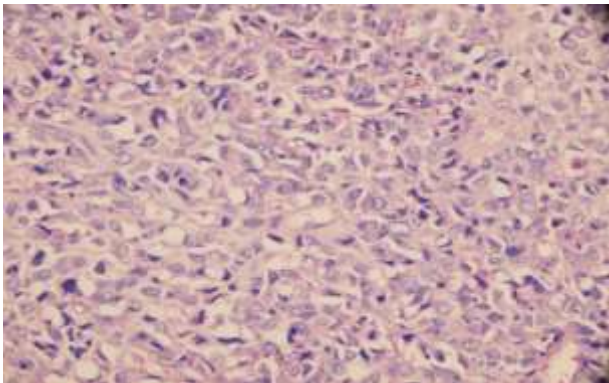

Figure 1: H&E stained section of ductal carcinoma (grade-III).

Table 5: Correlation of cyclin d1 expression with tumour histological grading.

Histological grading	N	Cyclin D1 significant positive	P value
I (WD)	7	3	
II (MD)	12	4	0.211
III (PD)	2	2	
Total	21	9	

Table 6: Correlation of cyclin D1 with lymph node metastasis.

Lymph node metastasis	N	Cyclin D1 positivity	P value
Nx	3	2	
N0	9	4	
N1	7	3	0.747
N2	2	0	
N3	0	0	
Total	21	9	

Out of 9 cases of lymph node metastasis only 3 (33.3%) cases showing cyclin D1 positivity (Figure 2). There was no significant correlation between cyclin D1 and lymph node metastasis (p=0.747) (Table 6).
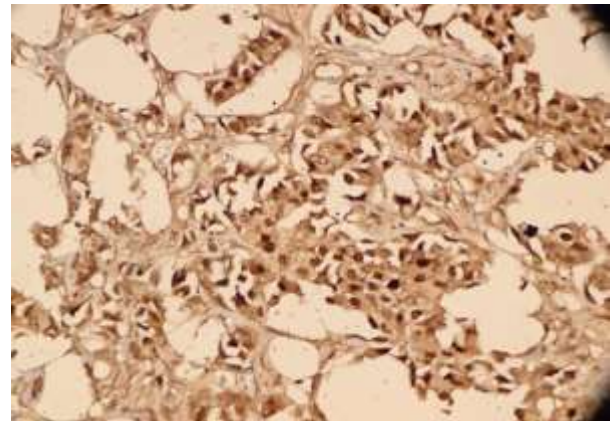
Figure 2: Cyclin D1 positivity in tumor cells.

Out of 21 cases, 14 cases were ER and PR positive and in 14 cases of ER and PR positive cases 9 (64.2%) cases showing cyclin D1 positivity. There was significant correlation between hormone receptors (ER and PR) (p=0.002) (Table 7).

Table 7: Correlation of cyclin D1 expression with ER and PR markers.

Immuno-prognostic marker	Total cases	Significant positive cyclin D1 (>10% of cells)	P value
ER			
Positive cases	14	9	0.002
Negative cases	07	00	
Total cases	21	9	
PR			
Positive cases	14	9	0.002
Negative cases	07	00	
Total cases	21	9	

DISCUSSION

Carcinoma of breast is most common malignancy in women. According to WHO classification, invasive carcinomas are the most common cancer of breast constituting around 90% of all breast cancers. In present study total 21 cases were of invasive breast carcinoma.

Evaluations of prognostic factors are very important for the management and therapeutic decision of breast cancer patients. Different prognostic factors of breast cancer in this study included are age of patients, tumour grade,

lymph node status, cyclin D1 expression, along with hormonal receptor status.

Cyclin D1 is an important cell cycle regulatory protein, is encoded by the CCND1 gene, present on chromosome 11q13. Function of cyclin D1 is regulation of progression of the cell cycle through the G1 to S phase through the formation of active enzyme complexes with cyclin-dependent kinases Cdk4 and Cdk6. These kinase phosphorylate substrate including the retinoblastoma gene product, pRb, thus relieving inhibitory function of pRb on S phase. On overexpression of cyclin D1, loss of normal cell cycle control function, leading carcinogenesis. Cyclin D1 also play important role in development of mammary gland, it is required for normal lobuloalveolar development. Mice lacking Cyclin D1

develop abnormal lobulo-alveolar proliferation of mammary glands.[7]

Age

Women who are <50 years of age at the time of diagnosis have better prognosis. In present study ≤50 years age group was found 66.6% (Table 1, Figure 1). Similar results were seen by Saiditi et al, Lengare et al, Sarkar, Anders and Madani (Table 8).[8-12] This justifies that now a day's breast carcinoma are frequent in younger age group. In current study, youngest female with breast carcinoma was of 35 years and oldest female was of 77 years. Expression of cyclin D1 was no statistically significant with age group in present study (p=1.000) (Table 4).

Table 8: **Comparison of tumor characters with other studies**.

Clinical character	Lengare et al[9]	Mohammadizad[13]	Siadati et al[8]	Sarkar et al[10]	Kumar et al[17]	Present study
Age (years), N (%)						
≤50	28 (56)		220 (73.33)	61 (55.4)		14 (66.6)
>50	22 (44)		Including 50 years; 80 (26.66)	Including 50 years 49 (44.6)		7 (33.3)
Tumour grade, N (%)						
Grade I	10 (20)	(18)	28 (9.33)	29 (26.4)	1 (2.5)	7 (41.17)
Grade II	13 (26)	(41.6)	242 (80.66)	48 (43.6)	27 (69.2)	10 (58.82)
Grade III	27 (54)	(40.4)	30 (10)	33 (30)	11 (28.2)	0 (0)
Lymph node status, N (%)						
Nx	-	-	257 (85.66)		-	2 (11.7)
N0	16 (32)	-		24 (21.8)	17	8 (47.05)
N1	19 (38)	-		<3 lymph node; 26 (23.6)	13	5 (29.4)
N2	9 (18)	-		>3 lymph node; 60 (54.6)	3	1 (5.88)
N3	6 (12)	-			7	1 (5.88)

Table 9: Comparision of immunomarker with other studies.

Immunomarkers	Lengare et al[9]	Sarkar et al[10]	Madani[12]	Mohammadizadeh[13]	Kumar et al[17]	Present study
ER, N (%)	28 (56)	49(44.5)	60 (43.8)	(60.7)	26 (65)	12 (70.5)
PR, N (%)	26 (52)	32 (29.1)	37(27)	(58.4)	26 (65)	12 (70.5)

Tumour grade

Tumour grade is also a most important prognostic factor and provide useful information regarding to response to chemotherapy, therefore tumour grade is predictive as well as prognostic factor. In present study tumour grade-II was frequent (57.1%)(Table 2, Figure 2). Similar results were seen by Saiditi, Sarkar and Mohammadizad.[8,10,13] This indicate that tumours with grade II are more frequent. In this study expression of cyclin D1 with tumour histological grade is statistically not significant in our study (p=0.211) (Table 5).

Lymph node status

Lymph node metastasis is most important prognostic factor for breast cancer patients. Lymph node metastasis status is important for treatment plan and it indicate chronicity of disease, disease free and overall survival of life. In our study 42.85% cases showing no lymph node metastasis, which indicates that maximum case showing no lymph node metastasis. Similar results were seen by Siditi indicating maximum cases showing no lymph node metastasis (Table 6).[8] In present study expression of

cyclin D1 was not significantly associated with lymph node metastasis (p=0.747).

Estrogen receptor

Estrogen is important for development of breast and on other regulatory molecule which play important role in proliferation cancer cells in breast. Estrogen receptor is most powerful predictive marker of breast cancer therapy. The presence of estrogen receptor and progesterone receptor (ER and PR) in breast tissue correlate well with the response of chemotherapy.

Tumours that are ER and PR positive better response to chemotherapy and have good prognosis. In our study 66.6% (14/21) cases are estrogen positive. Similar results were seen by Mohammadizadeh, Lengare et al, Ravi Kumar (Table 9). [9,13,17] It proves that most of the invasive breast cancer are ER positive, there also was significant correlation in our study between ER positivity and cyclin D1 expression (p=0.002) (Table 7). Sarkar, Kumar, Mohammadizdeh, Lengare et al also showed significant correlation between cyclin D1 expression and ER Positivity.[9,10,13,17]

Progesterone receptor

Along with estrogen receptor, progerterone receptor (PR) is also predictive marker of breast cancer but weaker than estrogen receptor. PR is co-dependent marker of functional ER. In our study 66.6% (14/21) cases showing PR positivity (Table 7). Similar results were seen by Kumar and Lengare et al that PR is expressed 60-70% of invasive breast cancer (Table 9).[9,17] PR showing significant correlation between cyclin D1 expression (p=0.002). This finding is similar to some previous studies like Sarkar, Mohammadizadeh, Lengare et al, Ravi Kumar also showed significant correlation between cyclin D1 and PR positivity.[9,10,13,17] This is in favour of the effect of cyclin D1 on cell maturation and cell differentiation.

Limitation of study

Limitation of current study was; sample was small since in rural areas most of patients does not underwent in mastectomy procedure and also it was COVID-19 period that was another reason for small sample size.

CONCLUSION

In Invasive breast carcinoma expression of cyclin D1 was significantly associated with ER and PR positivity and not significantly associated with age, tumour grade AMD lymph node metastasis. Cyclin D1 can be used in routine immunohistochemistery marker as a prognostic and predictive marker. It also can be aid appropriate targeted therapy.

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