

# Immunohistochemical Expression of Cyclin D1 in Human Breast Carcinoma

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## Abstract

**Background:** Breast cancer remains a major health problem in women. The molecular mechanisms of tumor growth and progression are complicated but likely involve the interaction of tumor suppressor genes. Oncogenes, cell cycle regulatory proteins and other factors. Recently some studies showed that Cyclin D1 is a cell cycle regulatory gene emerging as a potentially significant oncogene in invasive breast cancers.

**Objective:** To evaluate immunohistochemical expression of cyclin D1 in women with breast cancer in our population and correlate its expression with different variables such as age, type of tumor and grade.

**Materials and methods:** We retrospectively analyzed data from 76 formalin-fixed of paraffin-embedded tissues diagnosed with breast cancer which were collected from teaching laboratory unit in Baghdad medical city, Iraq, during the period from 2009 till 2013 and compared with positive control. These samples were investigated immunohistochemically, nuclear and cytoplasmic staining of tumor cells was accepted as positive.

**Results:** The results showed that age distribution ranging from (28-67 years) with a mean age of 47.63 years. Regarding tumor types 68 (89.47%) cases were with invasive ductal carcinoma, 6 (7.89%) cases were with invasive lobular carcinoma and 2 (2.63%) cases were recurrent carcinoma. Histologically the tumor grade ranges from well differentiated (grade 1) in 10 (13.15%) cases, moderately differentiated (grade 11) in 52 (68.42%) cases and poorly differentiated (grade 111) in 14 (18.42%) cases. Cyclin D1 expression was positive in 30 (39.47%) cases, while 46 (60%) cases negative. On the other hand most positive cases occurred within age group (41-55 years), invasive ductal carcinoma 26 (86.66%) and moderately differentiated 18 (60%) cases. significant differences noticed between IHC expressions of this marker with age, type of tumor and grade.

**Conclusion:** cyclin D1 is an important regulator of cell cycle progression and overexpression of cyclin D1 has been linked to the development and progression of cancer, Cyclin D1 expression was seen more in invasive ductal carcinoma also is considered a novel and good marker of invasiveness in breast cancer tissue and may be used for treatment.

**Keywords:** Cyclin D; Breast cancer; Immunohistochemistry technique; Breast pathogenesis

## Introduction

Breast cancer most commonly develops in cells from the lining of milk ducts and the lobules that supply the ducts with milk. Cancers that are developing from the ducts are known as ductal carcinomas, while those are developing from lobules are known as lobular carcinomas [1]. In addition, there are more than 18 other sub-types of breast cancer. Some cancers develop from pre-invasive lesions such as ductal carcinoma *in situ* [2].

Signs of breast cancer may include a lump in the breast, a change in breast shape, dimpling of the skin, fluid coming from the nipple, or a red scaly patch of skin [1]. In those with distant spread of the disease, there may be bone pain, swollen lymph nodes, shortness of breath, or yellow skin [3].

There are several well-established risk factors for breast cancer (early onset of menarche, a late age both for a first complete pregnancy and for menopause, the presence of atypical hyperplasia, a positive family history of breast cancer, and exposure to ionizing radiation), other factors contributing to the development of breast cancer likely exist [4].

Cyclins are a family of proteins that control the progression of cells through the cell cycle by activating cyclin-dependent kinase (Cdk)

enzymes, such as Cdk2, 4, 5, and 6 [5]. Most human cancers contain overactive CDK4/6-cyclin D, and CDK4/6-specific inhibitors are promising anti-cancer therapeutics [6].

Cyclin D1 is important for the development and progression of several cancers including those of the breast, esophagus, bladder and lung [7]. Overexpression of cyclin D1 has also been linked to the development of endocrine resistance in breast cancer cells [8,9].

Indeed, *in vitro* study done by Li et al. clarified that cyclin D1 gene encodes the regulatory subunit of a holoenzyme that phosphorylates the retinoblastoma protein (pRb) and nuclear respiratory factor (NRF1) proteins. The abundance of cyclin D1 determines estrogen-dependent gene expression in the mammary gland of mice [10].

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So for all the above this study was designed to explore the role of cyclin D1 in pathogenesis of women with breast cancer and to evaluate the significance of its expression in breast cancer cells regarding prognosis and virulency of tumor cells that express Cyclin D1.

## Materials and Methods

This study is retrospectively designed in which 76 cases which were diagnosed having breast cancer in teaching laboratory unit in Baghdad medical city, Iraq, during the period from 2009 till 2013, were evaluated in terms of age, tumor type and grade.

Breast tissue sections were cut at 4  $\mu$ m and placed on positively-charged slides; one section was stained with hematoxylin and eosin (H&E) and second used for the detection of cyclin D1 by immunohistochemistry technique (IHC). Also we study relationship between cyclin D1 overexpression and different variables.

Immunohistochemistry for Cyclin D1 was performed according to manufacturer instruction [anti-cyclin D1 antibody (ab16663) and Rabbit specific HRP/DAB (ABC) Detection IHC Kit (ab64261)], and interpreted as positive when >10% of the tumor cells expressed the marker with a moderate to strong intensity of staining.

## Statistical Analysis

Was performed using the t-test with Fischer exact test for quantitative parameters such as age of patient, also used chi-Squared test for qualitative parameters, such as histological grade.

## Results

All cases belong to women; age distribution was ranging from (28-67 years) with a mean age of 47.63 years. Regarding age group of patients, we divided the cases into three age groups as shown in (Table 1). Majority of breast cancer was occurring in the age group 41-55 years, while the lowest percentage occurs at age group 56-70 years. There are no significant differences showed among them.

There was a highly significant difference noticed among tumor type's as shown in Table 2, 68 (89.47%) cases were invasive ductal carcinoma, 6 (7.89%) cases were invasive lobular carcinoma and 2 (2.263%) cases were recurrent carcinoma (Table 2).

Histologically the tumor grade ranges from well differentiated in 10 (13.15%) cases, moderately differentiated in 52 (68.42%) cases and poorly differentiated in 14 (18.42%) cases. However, the frequency of breast cancer was found to be commonly of moderately differentiated type of cancer than other types, as shown in (Table 3).

In this study it is observed that the cyclin D1 expression was positive in 30 (39.47%) cases while 46 (60.52%) cases were negative, on the other hand. Statistical analysis shows non-significant difference ( $P>0.05$ ) as shown in (Table 4 and Figure 1).

Table 5 which demonstrated correlation between expressions of cyclin D1 with different variables. The results showed that the age of patients with positive results had ranged between 25 to 70 years as. In the age group 41-55 years were 18 cases out of 76, which show high percent compare to the other. However there was statistical significant differences noticed among them ( $P<0.01$ ). The number of Invasive ductal carcinoma with positive expression of Cyclin D1 were 26 out of 68 cases while the cases with invasive lobular carcinoma that are positively expressing Cyclin D were 4 cases out of 6, also statistical analysis revealed significant differences. According to tumor grade statistical analysis revealed significant differences between expressions of cyclin D1 with each one.

## Discussion

The cyclin D1 proto-oncogene is a vital regulator of G1 to S phase progression in several different cell types. Together with its binding associates cyclin dependent kinase 4 and 6 (CDK4 and CDK6), cyclin D1 form active complexes that promote cell cycle progression by phosphorylating and inactivating the retinoblastoma protein (RB) [11]. Further studies have demonstrated that cyclin D1 also functions as transcriptional modulator by regulating the activity of several transcription factors and histone deacetylase (HDAC3). This activity is independent of CDK4 activity [12].

The current study had demonstrated that cyclin D1 was over

Age (years)	Number	Percentage	Comparison of Significance	
			P-value	Sig.
(25-40)	24	31.57%	1.68	( $P>0.05$ )
(41-55)	34	44.73%		
(56-70)	18	23.68%		
Total	76	99.98%		

**Table 1:** Distribution of patients according to their age strata.

Tumor types	Number	Percentage	Comparison of Significance	
			P-value	Sig.
Invasive ductal carcinoma	68	89.47%	107.7	$P<0.01$
Invasive lobular carcinoma	6	7.89%		
Recurrent carcinoma	2	2.63%		
Total	76	99.99%		

Highly Sig ( $P<0.01$ )

**Table 2:** Distribution of patients according to tumor types.

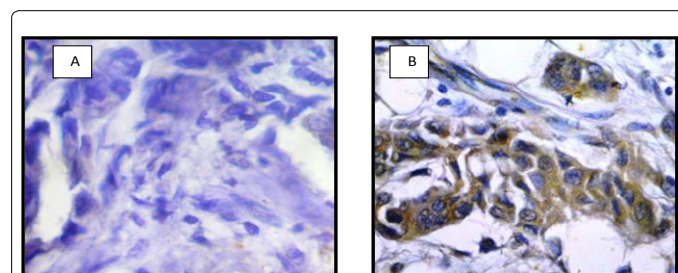
Tumor grade	Number	Percentage	Comparison of Significance	
			Chi <sup>2</sup> -value	Sig.
Well differentiated	10	13.15%	20.6	Non Sig. ( $P>0.05$ )
Moderately differentiated	52	68.42%		
Poorly differentiated	14	18.42%		
Total	76	99.99%		

\*Non-Significant ( $P>0.05$ )

**Table 3:** Distribution of patients according to tumor grade.

Result of Immunohistochemistry	Positive	Negative	Total	Comparison of Significance	
				p-value	Sig.
Patients with breast cancer	30 (39.47%)	46 (60.52%)	76 (99.98%)	6.73	Non Sig ( $P>0.05$ )

**Table 4:** The Expression of Cyclin D in women with breast cancer.



**Figure 1:** Immunohistochemistry for Cyclin D1 in breast cancer section, stained by DAB chromogen and counter stained with hematoxylin is shown as brown in positive cases (magnification power, 400X). A- Negative expression; B- positive expression.

Variables	Positive	Negative	Comparison of Significance	
			Chi <sup>2</sup> - value	Sig.
<b>Age</b>				
25-40 year	8 (26.66%)	16 (34.78%)	50.22	P<0.01
41-55 year	18 (60%)	16 (34.78%)		
56-70 year	4 (13.33%)	14 (30.43%)	Highly Sig.	
<b>Types of tumour</b>				
Invasive ductal carcinoma	26(86.66%)	42(91.30%)	53.5	P<0.01
Invasive lobular carcinoma	4(13.33%)	2(4.34%)		Highly Sig.
Recurrent carcinoma	0	2(4.34%)		P<0.01
<b>Grade</b>				
Grade I	3(13.33%)	6(13.04%)	43.24	
Grade II	18(60%)	34(73.91%)		
Grade III	8(26.66%)	6(13.04%)		
Highly Sig (P<0.01)				

**Table 5:** Immunohistochemistry expression of positive and negative cyclin D1 and related with different variable of patients with breast cancer.

expressed in women with breast cancer; this was in agreement with findings of John, who reported that increased levels of cyclin D1 in several cancers [11]. Also agreement with result of other studies [8,9].

Study done by Liu et al. who reported that both cyclin D1 and the transcription factor C/EBP $\beta$  are required for mammary epithelial cell differentiation; however, the pathway in which they operate is uncertain. Previous analyses of the patterns of gene expression in human tumors suggested a connection between cyclin D1 overexpression and C/EBP $\beta$ , but whether this represents a cancer-specific gain of function for cyclin D1 is unknown [13].

Other study done by Wu et al. demonstrated that the inhibition of cancer cell growth was associated with G1-phase cell cycle arrest and down-regulation of the NF-kB pathway leads to activation of the mitochondrial apoptotic pathway. It was also found that PPLGM significantly decreased the expression of p-Akt, p70S6K1, 4E-BP1, cyclin D1, Bcl-2, p53 and increased expression of Bax, cytochrome c in human triple-negative breast cancer cells And suggest that PPLGM may be an effective therapeutic agent for the treatment of human triple negative breast cancer [14].

Study of Migliaccio et al. who did find the retinoblastoma tumor suppressor (Rb) pathway is frequently deregulated in breast cancer and strategies to target this pathway have recently been proven to be effective in breast cancer patients and suggest that CDK4/6 inhibitors might be particularly useful in patients with hormone-receptor-positive or HER2-positive tumors, whereas the role of such inhibitors in triple-negative breast cancer is still controversial [15].

Other study done by Dickson who refer that the development of selective CDK4 inhibitors launched the first successful efforts to target the pathway for cancer therapy, mechanisms of resistance, and develop rational combinations of CDK4 inhibitors with chemotherapy and other targeted drugs [16].

In this study, the results showed significant differences between cyclin D1 expression and age groups. In general cancer is an age-associated disease. Although the mechanisms of age-associated increase in cancer incidence are not completely understood, it is believed that the tumor stromal environment significantly influences epithelial malignancy. Fibroblasts are a major cell type in the stroma and, under normal circumstances; fibroblasts reside in the quiescent state. Cellular

quiescence is a reversible process where cells enter into the proliferative cycle and then exit back to quiescence [17].

Age at first pregnancy is another aspect of reproductive history that is associated with breast cancer risk. Women who have their first full-term pregnancy at a relatively early age have a lower risk of breast cancer than those who never have children or those who have their first child relatively late in life [18].

Regarding to type of tumor, the results of present study revealed that most cases occur within invasive ductal carcinoma, an agreement with study done by Ameli who reported that among 51 cases of breast cancer, invasive ductal was (30 cases) and lobular (21) carcinomas [19].

According to the histological grade the result revealed that cyclin D1 expression was found to be higher in grade II than other grade as recorded in Table 5. The results were in agreement with other studies which showed high frequency of moderately differentiated breast cancer [20,21].

In conclusion, cyclin D1 is an important regulator of cell cycle progression and overexpression of cyclin D1 has been linked to the development and progression of cancer, Cyclin D1 expression was seen more in invasive ductal carcinoma also is considered a novel and good marker of in breast cancer tissue and may be used for treatment.

## References

- NCI (2014) Breast Cancer Treatment. 05-23.
- World Cancer Report (2014) Chapter 5.2.
- Saunders C and Jassal S (2009) Breast cancer (1stedtn) Oxford University Press.
- Mathilde B, Jean-Marc G, Elisabeth K, Virginie G, Ellen B, et al. (1999) Detection of Epstein - Barr virus in Invasive Breast Cancers. Journal of the National Cancer Institute 91: 1367-1381.
- Galderisi U, Jori FP, Giordano A (2003) Cell cycle regulation and neural differentiation. 22: 5208-5219.
- The I, Ruijtenberg S, Bouchet BP, Cristobal A, Prinsen MB, et al. (2015) Rb and FZR1/Cdh1 determine CDK4/6-cyclin D requirement in C. elegans and human cancer cells. Nat Commun 6: 5906.
- Yamamoto M, Tamakawa S, Yoshie M, Yaginuma Y, Ogawa K (2006) Neoplastic hepatocyte growth associated with cyclin D1 redistribution from the cytoplasm to the nucleus in mouse hepatocarcinogenesis. Mol Carcinog 45: 901-913.
- Kenny FS, Hui R, Musgrove EA, Gee JM, Blamey RW, et al. (1999) Overexpression of cyclin D1 messenger RNA predicts for poor prognosis in estrogen receptor-positive breast cancer. Clin Cancer Res 5: 2069-2076.
- Hodges LC, Cook JD, Lobenhofer EK, Li L, Bennett L, et al. (2003) Tamoxifen functions as a molecular agonist inducing cell cycle-associated genes in breast cancer cells. Mol Cancer Res 1: 300-311.
- Li Z, Chen K, Jiao X, Wang C, Willmarth NE, et al. (2014) Cyclin D1 integrates estrogen-mediated DNA damage repair signaling. Cancer Res 74: 3959-3970.
- Alao JP (2007) The regulation of cyclin D1 degradation: roles in cancer development and the potential for therapeutic intervention. Mol Cancer 6: 24.
- Coqueret O (2002) Linking cyclins to transcriptional control. Gene 299: 35-55.
- Liu Q, Boudot A, Ni J, Hennessey T, Beauparlant SL, et al. (2014) Cyclin D1 and C/EBP $\beta$  LAP1 operate in a common pathway to promote mammary epithelial cell differentiation. Mol Cell Biol 34: 3168-3179.
- Wu Y, Kim J, Elshimali Y, Sarkissyan M, Vadgama JV (2014) Activation of Akt1 accelerates carcinogen-induced tumorigenesis in mammary gland of virgin and post-lactating transgenic mice. BMC Cancer 14: 266.
- Migliaccio I, Di Leo A, Malorni L (2014) Cyclin-dependent kinase 4/6 inhibitors in breast cancer therapy. Curr Opin Oncol 26: 568-575.

16. Dickson MA (2014) Molecular pathways: CDK4 inhibitors for cancer therapy. Clin Cancer Res 20: 3379-3383.
17. Sarsour EH, Goswami M, Kalen AL, Lafin JT, Goswami PC (2014) Hydroxytyrosol inhibits chemokine C-C motif ligand 5 mediated aged quiescent fibroblast-induced stimulation of breast cancer cell proliferation. Age (Dordr) 36: 9645.
18. Henderson BE, Ross R, Bernstein L (1988) Estrogens as a cause of human cancer: the Richard and Hinda Rosenthal Foundation award lecture. Cancer Res 48: 246-253.
19. Ameli F, Rose IM, Masir N (2015) Expression of DDR1 and DVL1 in invasive ductal and lobular breast carcinoma does not correlate with histological type, grade and hormone receptor status. Asian Pac J Cancer Prev 16: 2385-2390.
20. Hussein AA (2013) Molecular detection of Epstein Barr Virus in Women with Breast cancer. J Fac Med Baghdad 55: 144-148.
21. Phukan JP, Sinha A, Deka JP (2015) Cytological grading of breast carcinoma on fine needle aspirates and its relation with histological grading. South Asian J Cancer 4: 32-34.

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