The Egyptian Journal of Hospital Medicine (April 2022) Vol. 87, Page 2082-2090

Immunohistochemical Expression of Cyclin D1 in Breast Carcinoma

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ABSTRACT

Background: Cyclin D1 can function as an oncogene that favours a proliferative advantage to tumor cells. Recent evidences strongly suggest the role of Cyclin D1 in breast cancer resistance to therapy and cancer progression.

Objectives: To evaluate the immuno-expression of Cyclin D1 and correlate this with clinicopathological parameters, which are known to be prognostic factors, thus evaluating the role of cyclin D1 as a prognostic marker in breast carcinoma.

Materials and methods: Fifty-two cases of invasive breast carcinoma removed by modified radical mastectomy were enrolled in this study and evaluated for expression of Cyclin D1 by immunohistochemistry. The expression was evaluated by the Allred score that combines the intensity of immunoreaction with the percentage of positive cells.

Results: Cyclin D1 expression appeared as brownish nuclear staining and showed variation in its expression among different types of invasive breast carcinoma. It showed significant correlation with tumor grade (p= 0.004), histologic type (p= 0.014), luminal molecular subtype (A&B) tumors (p= 0.001), estrogen receptors (ER) (p= 0.02), progesterone receptors (PR) (p= 0.007), and tumors of cases were received Neoadjuvant chemotherapy (NCT) before surgery (p= 0.049).

Conclusion: The current data suggests that Cyclin D1protein have a role in breast oncogenesis and is involved in progression of breast carcinoma as its expression is correlated with many prognostic factors. Therefore, it may serve as a prognostic marker for patient outcome and may help in making an appropriate strategy of adjuvant therapy selection.

Keywords: Breast carcinoma, Immunohistochemistry, Cyclin D1.

INTRODUCTION

Breast cancer (BC) is by far the most frequent cancer among women with an estimated 2 million new cancer cases diagnosed in 2018 (23% of all cancers), and ranks second overall (10.9% of all cancers) [(1)]**.** It is the leading cause of cancer related mortality, representing 15% of deaths per year worldwide [(2)]**.** In Egypt, breast cancer is the most common malignancy in females; it accounts for 32 % of cancer in women [(3)]**.** BC is a heterogeneous disease clinically and pathologically [(4)]. Age, tumor size, histological grade, lymph node metastasis are considered as standard prognostic factors whereas hormone receptor; estrogen (ER), progesterone (PR), and human epidermal growth factor 2 (HER2) status are regarded as predictive factors in patients with breast carcinoma [(5)].

Breast cancers are classified with respect to the presence or absence of these hormone receptors into four distinct intrinsic molecular subtypes: luminal A, luminal B, HER2-enriched, and basal-like/triple negative breast cancer (BLBC)/ (TNBC) [(6)].

Cyclin D1 is emerging as a significant biomarker in invasive breast cancer. It is the product of the CCND1 (PRAD1) gene located on chromosome 11q13 and it is the key cell cycle G1/S regulatory protein [(7)]. D cyclins, including cyclins D1, D2, and D3, form active complexes with either CDK4 or CDK6, which, in turn, phosphorylate the retinoblastoma (Rb) protein and drive G1 to S phase progression. D cyclins coordinate cell cycle progression with the extracellular stimulation. Given the role of D cyclins in mediating extracellular cues with cell proliferation, it is not surprising that overexpression of D cyclins or hyper-activation of their cognate CDKs directly contributes to neoplastic growth [(8)].

Cyclin D1 overexpression is commonly found in human breast cancers. Many experimental evidence has suggested that cyclin D1 can function as an oncogene and that increased expression of cyclin D1 accelerates the G1 phase and likely provides a proliferative advantage to tumor cells [(9)]. In addition, recent evidences strongly suggest the role of Cyclin D1 in the resistance to therapy and cancer progression [(10)]**.**

This study was designed to evaluate cyclin D1 expression and its correlation with the available clinicopathological criteria in patients with BC.

MATERIAL AND METHODS

Fifty-two formalin-fixed paraffin embedded tissue blocks of 52 cases of invasive BC were retrieved for this study from Pathology Department, Sohag University Hospitals, and Sohag Oncology Center during the period from January 2018 to December 2020.

Ethical consent:

An approval of the study was obtained from Sohag University Academic and Ethical Committee. Tissue specimens were obtained by modified radical mastectomy in all cases. Nineteen out of fifty-two (19 /52) of included breast cancer patients received neoadjuvant chemotherapy (NCT) before surgery. Every patient signed an informed written consent for acceptance of participation in the study. This work has been carried out in accordance with The Code of Ethics of the World Medical Association

Received: 24/11/2021
Accepted: 22/01/2022

(Declaration of Helsinki) for studies involving humans.

Histological examination:

Sections of 4µm thick were prepared from the tissue blocks and stained with hematoxylin and eosin stains (H&E) and reviewed for confirmation of the original diagnosis, and revision of the histological type and grade of the tumors according to the World Health Organization (WHO) criteria of Breast Tumors published in 2019, fifth edition [(11)].

Immunohistochemical staining:

Sections of 4µm thick were mounted on pre-labeled poly-L-lysine coated slides. The selected sections were de-paraffinized in xylene for 20 minutes, rehydrated in downgraded alcohol (100%, 80%, 70%, and 50%) 2 min for each, then rinsed in distilled water. Tissue sections were incubated in 0.5% hydrogen peroxide/methanol for 10 min to block endogenous peroxidase activity followed by washing twice in phosphate buffer saline (PBS). The antigen was retrieved by boiling tissue sections twice in citrate buffer, pH 6.0, using a microwave at mid-high power for 10 min each, followed by cooling down to room temperature for 20 min.

Following washing twice in PBS, blocking of non-specific protein binding was done by adding two drops of protein-blocking serum (Cat # AEX080-IFU, ScyTec laboratories-USA- ready to use) for 10 minutes at room temperature in a humidity chamber. Tissue sections were incubated overnight at 4 C° in a humid chamber with Monoclonal Rabbit antibody Anti-Cyclin D1 (Dako-USA-clone EP12 Catalog #IR083- USA). The resulting immune-complex was detected by a universal staining kit (Cat # AEX080-IFU, ScyTec laboratories-USA- ready to use). Tissue sections were treated with biotinylated goat anti-polyvalent antibody (Econo Tek, ScyTec laboratories, ready to use), and then peroxidase-labeled streptavidin (Econo Tek HRP, ScyTec laboratories, ready to use) was applied for 30 min at room temperature, rinsed in PBS, incubated with substrate/ chromogen diaminobenzidine (DAB) mixture for 5-15 minutes at room temperature until the positive control showed staining; followed by rinsing with distilled water. The tissue sections were counter-stained in Myer's Hematoxylin, washed in tap water, dehydrated in ascending graded alcohols (70%, 95%, and 100% alcohols), cleared in xylene, left to dry, then mounted by using an aqueous-based mounting medium dibutylphthalate polystyrene xylene (DPX) and cover slipped.

Excess reagent was thrown off and tissue sections were rinsed twice in PBS, incubated with streptavidin for 10 min at room temperature, washed twice in PBS and exposed to a freshly prepared 3, 3′-diaminobenzidine tetrahydrochloride solution (DAB) solution for 5-10 min until the positive control showed brown staining then tissue sections were washed in distilled water and finally, were counterstained with hematoxylin, washed in running water, dehydrated in upgraded series of alcohol (70%, 80%, 90%, and 100%), cleared in xylene for 5 min then mounted with DPX and cover slipped. Each staining run included sections from tonsil as a positive control. Cyclin D1 expression appeared as brownish nuclear staining. Additional tissue sections were stained in parallel, but with omission of the primary antibody as negative controls for immuno-histochemistry, immunohistochemical (IHC).

Immunohistochemical detection of Cyclin D1 and scoring:

Tissue sections were histologically examined by bright-field microscope at low power magnification (X40 and X100) to detect the sites of antibody positivity, then by higher power magnification (X200 and X400) to evaluate immunostaining.

Cyclin D1 immunohistochemical intensity and distribution were semi-quantitatively scored using the Allred score method. Only nuclear staining was considered specific.

With this method, the intensity of the immunohistochemical reaction as viewed under the light microscope was recorded as: 0, negative (no staining of any nuclei even at high magnification); 1, weak (only visible at high magnification); 2, moderate (readily visible at low magnification) or 3, strong (strikingly positive even at low power magnification).

The proportion of positive cells was recorded as; No staining = 0, Positive cells < l% = 1, Positive cells 1–10% = 2, Positive cells 11–33% = 3, Positive cells 34–66% = 4, and Positive cells ≥ 67% = 5. The proportion of positive cells and intensity scores was then added to obtain a total score, which ranged from 0 to 8. Tumours were then categorized into groups: Negative expression (total scores 0–2), Intermediate expression (total scores 3–5), and Strong expression (total scores 6–8) **(12)**.

Statistical Analysis:

Data were statistically analyzed using IBM SPSS Statistics for Windows version 23. Quantitative data were expressed as mean± standard deviation, median, and range. Qualitative data were expressed as numbers and percentages. Chi-Square test, ANOVA test and Spearman's correlation were used to evaluate the statistical significance of various parameters with p-value <0.05 was considered statistically significant and highly significant if <0.001.

RESULTS

Patients' clinical characteristics:

This study included 52 (74.28%) specimens of invasive breast carcinoma (IBC). The age ranged between 25and 83 years with a mean ± SD and a median of 52.27 ± 14.09 and 54.50 years respectively. The tumor size ranged between 1.5and 9 cm with a mean ± SD of 3.54 ± 1.65cm. Regarding tumor size, 42/52 (80.77%) cases were in the (T1-T2 / ≤ 2-5 cm) group and 10/52 (19.23%) cases were in the (T3-T4 / > 5 cm)

group. About 22/ 52 (42.3%) of IBC cases were left-sided tumors whereas 30/52 (57.7%) were right-sided.

From included IBC cases 33/52 (63.5%) of cases did not received NCT before surgery while 19/52 (36.5%) of cases received NCT. Tissue specimens of IB cases (52/70) were obtained by Modified radical mastectomy in 34 (65.4%) cases, by Oncoplastic breast surgery in 14 (26.9%) cases, and by Excision in 4 (7.7%) cases.

Histopathological findings:

Histological types and variants of the studied IBC cases are; 30 (57.7%) infiltrating ductal carcinoma of no special type (IDCNST) **(Figure 1A)**, 2 (3.8%) Carcinoma with Medullary features, 5 (9.6%) Mucinous carcinoma, 5 (9.6%) Invasive lobular carcinoma (classical type) **(Fig 1B)**, 3 (5.8%) Tubulo-lobular carcinoma, and 7(13.5%) Mixed Invasive ductal &lobular carcinoma (Mixed IDC &ILC) **(Figure 1C).**

The clinicopathological features of the IBC cases included in the study are summarized in **(Table 1);** the variants of invasive ductal carcinoma (IDC) and invasive lobular carcinoma (ILC) were grouped for statistical purposes. IDC cases were younger than ILC cases and Mixed IDC & ILC cases, with the highest age incidence between 35-59 years. ILC and Mixed cases were comparable in age.

Lymphovascular invasion (LVI) and perineural invasion (PNI) were evaluated. Histopathologically most of the cases showed the presence of LVI 27/52 (51.9%), by contrast, PNI was absent in most cases 41/52 (78.8%). Tumor size (T) and lymph node status (N) were assessed according to the Union for International Cancer Control (UICC) [13]**.** Axillary lymph nodes (LNs) were involved by tumor cells in 29/52 (55.7%) of cases. IBC was graded according to Nottingham histological grade. Grade II tumors were most prevalent.

Table (1): clinicopathological data of the studied IBC cases

Parameter	N (52)	Histologic type		
		IDC (n=37)	ILC (n=8)	Mixed (n=7)
Age (mean ± SD)		47.4±12.1	61.1±1.4	63.7±1.8
Tumor grade				
• I	8	6 (100%)	0 (0.0%)	0 (0.0%)
• II	27	21(72.4%)	6 (20.7%)	2(6.9%)
• III	17	10 (58.8%)	2 (11.8%)	5 (29.4%)
LVI				
• Present	27	14 (51.9%)	6 (22.2%)	7 (25.9%)
• Absent	25	23 (92.0%)	2 (8.0%)	0 (0.0%)
PNI				
• Present	11	6 (54.5%)	3 (27.3%)	2 (18.2%)
• Absent	41	31 (75.6%)	5 (12.2%)	5 (12.2%)
Axillary LN metastasis				
• N0	23	21(91.3%)	2(8.7%)	0(0%)
• N1	12	3 (25%)	5(41.7%)	4(33.3%)
• N2	12	9(75.0%)	1(8.3%)	2(16.7%)
• N3	5	4(80.0%)	0(0.0%)	1(20.0%)
ER status				
• Positive	30	26(86.7%)	3(10.0%)	1(3.3%)
• Negative	22	11 (50.0%)	5 (22.7%)	6 (27.3%)
PR status				
• Positive	24	21(87.5%)	2(8.3%)	1(4.2%)
• Negative	28	16(57.1%)	6(21.4%)	6(21.4%)
Her-2 status				
• Positive	29	23(79.3%)	2(6.9%)	4(13.8%)
• Negative	23	14(60.9%)	6(26.1%)	3(13.0%)
Molecular subtypes				
• Luminal type A	6	5(83.3%)	1(16.7%)	0(0.0%)
• Luminal type B	24	21(87.5%)	2(8.3%)	1(4.2%)
• Her-2 Enriched	5	2 (40.0%)	0 (0.0%)	3 (60.0%)
• Triple Negative	17	9 (52.9%)	5 (29.4%)	3 (17.6%)

IDC: Invasive ductal carcinoma (NOS, Mucinous, and Medullary), ILC: Invasive lobular carcinoma (Classic and Tubulo-lobular), Mixed: Mixed IDC and ILC

Immunohistopathological findings:

Cyclin D1 protein expression appeared as brownish nuclear staining. It showed variation in its

expression among different types of invasive breast carcinoma. Forty-four out of fifty-two (44/52) (84.6%) cases showed positive cyclin D1 expression. Cyclin D1was strongly expressed in 25/52 (48.0%) of cases of IBC from them 18/52 (72.0%) cases were invasive ductal carcinoma. Cyclin D1was intermediately expressed in 19/52 (36.5%) of cases and negatively expressed in 8/52 (15.5%) of cases **(Figure 2 A, and B)**.

Statistical evaluation of cyclin D1 expression according to age, tumor side, tumor size, PNI, LVI, and Axillary LN metastasis showed no significance (**Table 2).** Expression of Cyclin D1 was detected in different histological subtypes of IBC, with slightly higher expression in IDC compared to other subtypes (p=0.014). In addition, Cyclin D1 expression was significantly higher in low grades of IBC (p= 0.004).

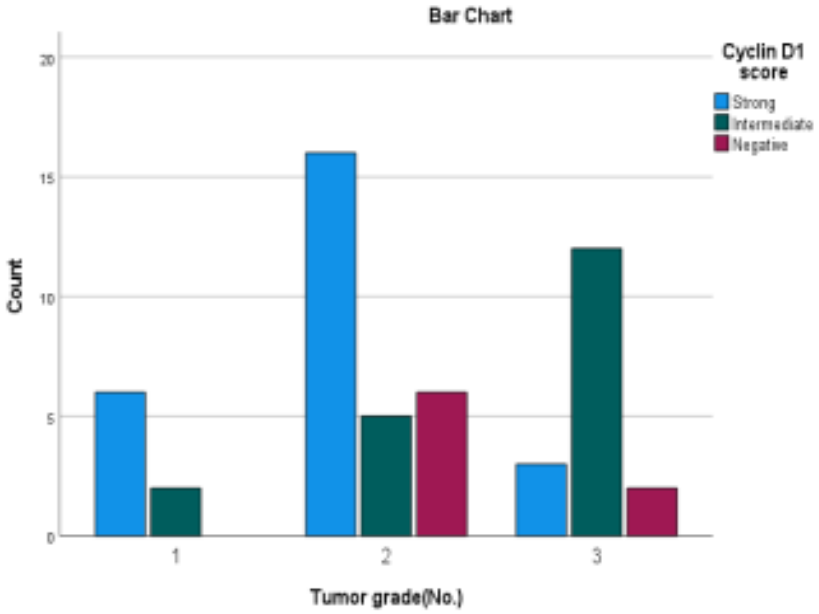
There was a significant correlation between cyclin D1 expression, and ER (p= 0.02), and PR (p= 0.007). The expression was high in ER-positive tumors 29/30 (96.7%), and PR-positive tumors 23/24 (95.8%). In contrast, there is an inverse correlation between cyclin D1 expression and Her-2 status (p= 0.022), 28/29 (96.6%) of Her-2 negative tumors showed positive cyclin D1 expression (Strong & intermediate).

There was a significant relation between cyclin D1 and molecular subtypes (p= 0.001). Among tumors strongly expressing cyclin D1, 20/25(80.0%) were of luminal type (A&B) tumors, while among tumors negatively expressing cyclin D1 8/8 (100%) were of TNBC type tumors. In addition, gradual loss of cyclin D1 expression among TNBC was noticed. The Luminal A &Luminal B subtypes are grouped for statistical purposes. Tumors of cases who received NCT before surgery showed higher Cyclin D1 expression compared to tumors in patients who did not receive NCT before surgery (p= 0.049). All correlations between Cyclin D1 expression and the studied clinicopathological parameters **(Table 2).**

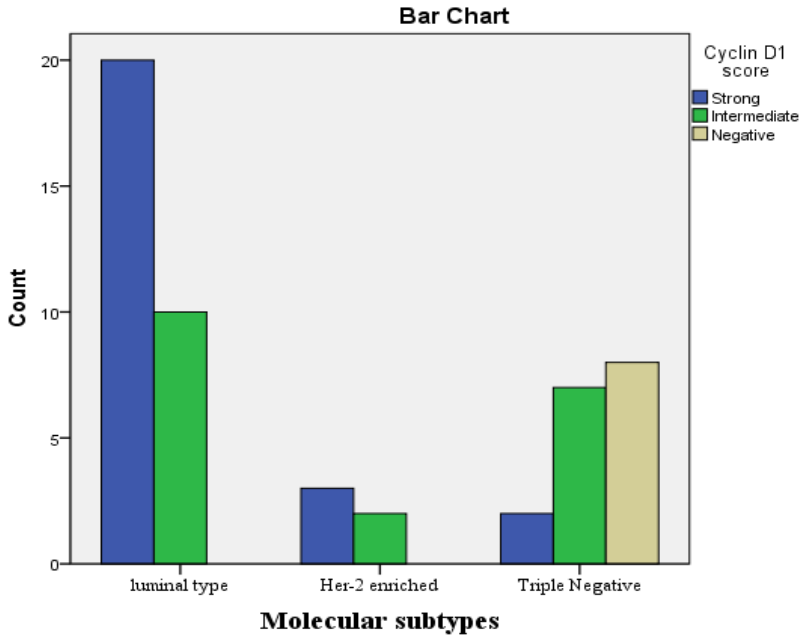
Table (2): Correlation between Cyclin D1 expression and the studied clinicopathological parameters

	Clinico-pathological Parameter	N (52)	CYCLIN D1 Expression			p-value
			Strong IRS (n=25)	Intermediate IRS (n=19)	Negative/weak IRS (n=8)	
Age (years)	25-38	15	8 (53.3%)	6 (40%)	1 (6.7%)	0.469
	39-52	19	9 (47.4%)	8 (42.1%)	2 (10.5%)	**(NS)**
	53-66	17	4 (50.0%)	4 (21.1%)	9 (33.3%)	
Histologic type	IDC	37	18(72.0%)	17 (89.5%)	2 (25.0%)	
	ILC	8	3 (12.0%)	2 (10.5%)	3 (37.5%)	0.014*
	Mixed	7	4 (16.0%)	0 (0.0%)	3 (37.5%)	
Tumor size	T1-T2 (≤ 2-5 cm)	42	21(84.0%)	16 (84.2%)	5 (62.5%)	0.362
	T3-T4 (> 5 cm)	10	4 (16%)	3 (15.8%)	3 (37.5%)	**(NS)**
Tumor Side	Right	30	14(56.0%)	14 (73.7%)	2 (25.0%)	0.063
	Left	22	11(44.0%)	5 (26.3%)	6(75.0%)	**(NS)**
Tumor grade	I	8	6 (24.0%)	2 (10.5%)	0 (0.0%)	
	II	27	16(64.0%)	5 (26.3%)	6(75.0%)	0.004*
	III	17	3 (12.0%)	12(63.2%)	2(25.0%)	
LVI	Present	27	15(60.0%)	7(36.8%)	5(62.5%)	0.254
	Absent	25	10(40.0%)	12(63.2%)	3(37.5%)	**(NS)**
Perineural invasion	Present	11	4(16.0%)	6(31.6%)	1(12.5%)	0.369
	Absent	41	21(84.0%)	13(68.4%)	7(87.5%)	**(NS)**
Axillary LN metastasis	N0-N1	35	14(56.0%)	14(73.7%)	7(87.5%)	0.193
	N2-N3	17	11(44.0%)	5(26.3%)	1(12.5%)	**(NS)**
Neoadjuvant chemotherapy	Not received NCT before surgery	33	13(52.0%)	12 (63.2%)	8 (100%)	0.049*
	Received NCT before surgery	19	12(48.0%)	7 (36.8%)	0 (0.0%)	
ER status	Positive	30	20(66.7%)	9(30.0%)	1(3.3%)	0.002*
	Negative	22	5(22.7%)	10(45.5%)	7(31.8%)	
PR status	Positive	24	17(70.8%)	6 (25.0%)	1(4.2%)	0.007*
	Negative	28	8 (28.6%)	13 (46.4%)	7 (25.0%)	
Her-2 status	Positive	23	8(34.8%)	8(34.8%)	7(30.4%)	0.022*
	Negative	29	17(58.6%)	11(37.9%)	1(3.4%)	
Molecular subtypes	Luminal type (A&B)	30	20(66.7%)	10 (33.3%)	0 (0.0%)	
	Her-2 Enriched	5	3 (60.0%)	2 (40.0%)	0 (0.0%)	0.000*
	Triple Negative	17	2 (11.8%)	7 (41.2%)	8 (15.4%)	

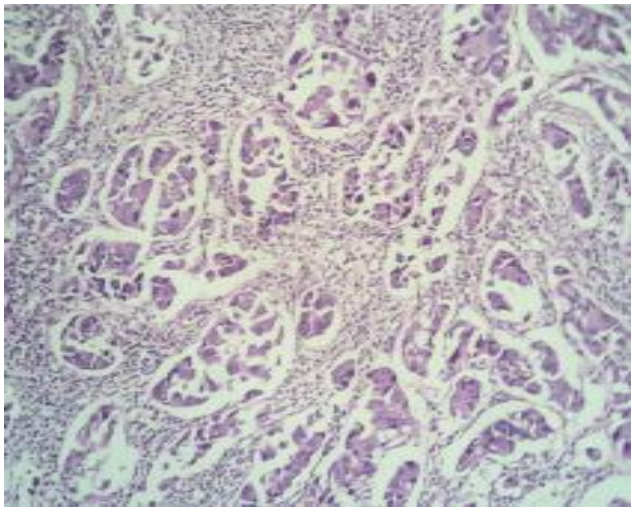
P-Value was calculated by Chi-square test, *=Significant, NS= Non-Significant, IRS: Immunoreactive score.



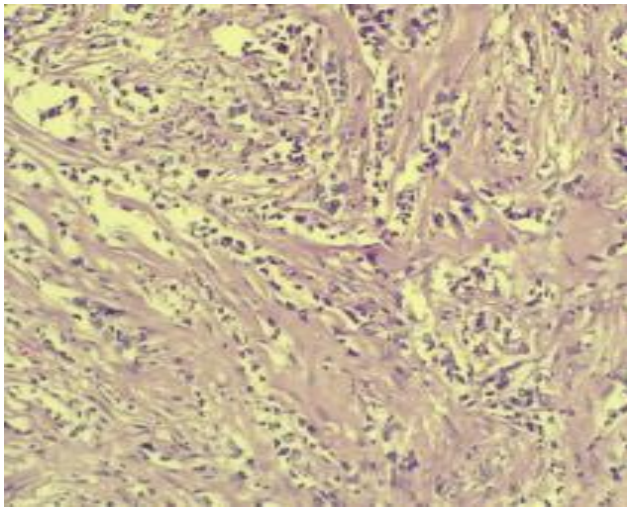
Graph (1): Significant relation between cyclin D1 expression & tumor grades.



Graph (2): Significant relation between cyclin D1 expression & Molecular subtypes.



A



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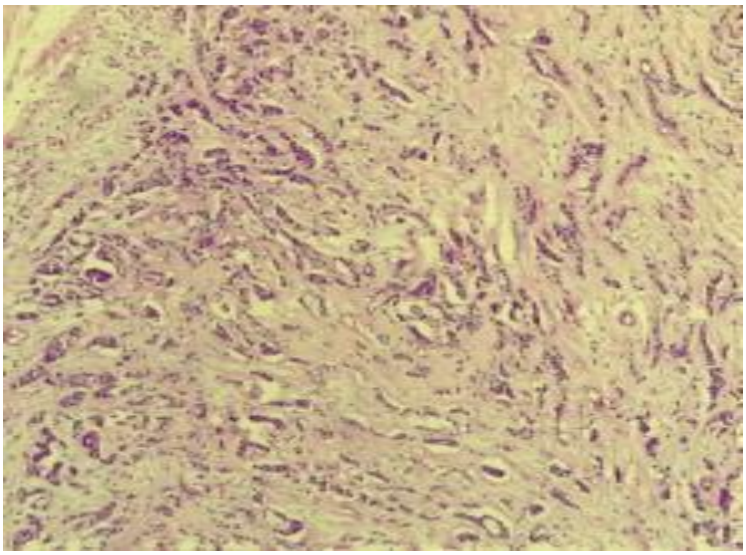
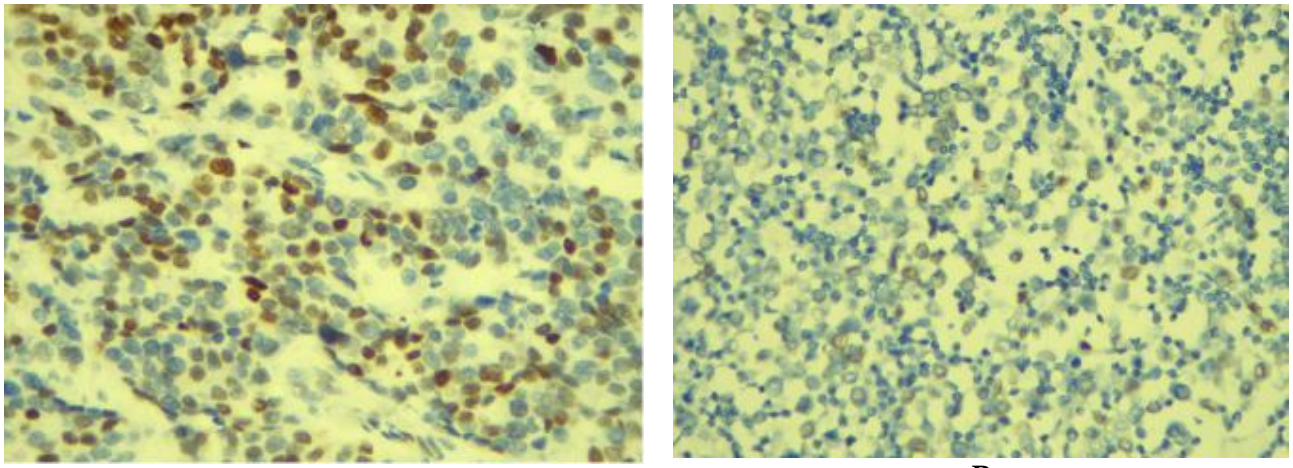


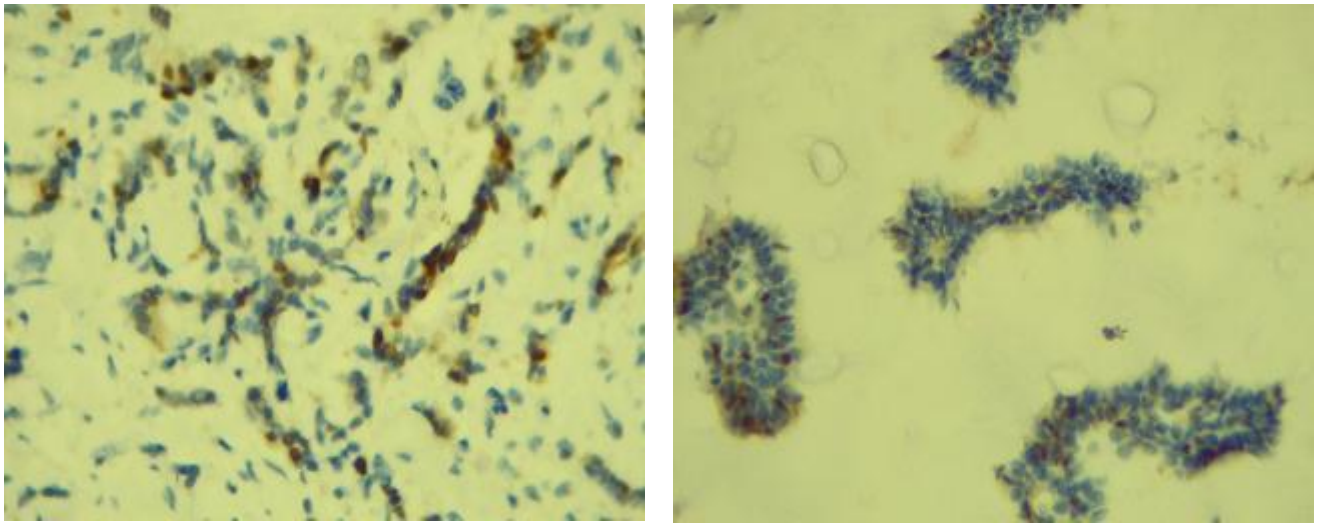
C

Figure (1): Histological types of IBC: A. Infiltrating duct carcinoma, NST (IDCNST), (H&E X200). B. Invasive lobular carcinoma (classical type), (H&E X200).C. Mixed IDC &ILC, (H&E X100)



A **B**

Figure (2): Nuclear Cyclin D1 expression at different types of IBC: A. Strong Cyclin D1 expression in Invasive ductal carcinoma, NOS (X400). B. Negative Cyclin D1 expression in Invasive Lobular Carcinoma (X400).



A **B**

Figure (3): Nuclear Cyclin D1 expression at different types of IBC: A. Cyclin D1 expression in mixed IDC& ILC (X400). B. Cyclin D1 expression in mucinous carcinoma (X400).

DISCUSSION

Breast cancer is the most commonly diagnosed cancer and the leading cause of cancer death among

world women. According to GLOBOCAN 2018, breast cancer accounting for 24% of all incidences of cancer among women. About 630,000 women were estimated to die due to breast cancer (15% of total cancer deaths) [14]. Breast cancer is the second-leading cause of death from cancer in Egyptian women; the overall incidence of cancer is 157.0 per 100 000 Egyptian women with the highest incidence being BC (32%) [15].

Biomarkers capable of predicting progression, risk stratification, and therapeutic benefit are needed. Cyclin D1 is emerging as a significant biomarker in invasive breast cancer, not still used as a routine tool in breast cancer, although it has shown its prognostic value in several studies [12].

The current study included 52 specimens of invasive breast carcinoma. Our study revealed that Cyclin D1 was expressed in (84.6%) of cases; strongly expressed in (48.0%) of cases of IBC from them (72.0%) cases were IDC. Cyclin D1 was intermediately expressed in (36.5%) of cases and negatively expressed in (15.5%) of cases. Similar results were found by **Hadžisejdić *et al.*** [16] where Cyclin D1 was expressed in (91%) of cases and classified into four cyclin-D1 expression groups; (64%) were classified as strong, (27%) as moderate, and (9%) as weak/negative-expressing samples. Strong cyclin D1 expression in the majority of breast cancers, as detected in our study, is not surprising given that this protein plays an important role in the progression from G1 to S phase and can be up-regulated through several different pathways, including EGFR-family protein, ER, c-myc and fibroblast growth factor receptor pathways [17, 18].

This study showed no significant statistical correlation between cyclin D1 expression and patient's age, tumor size, and axillary LN metastasis similar to the findings of **Li *et al.*** [7] and **Parvin *et al.*** [12]. By contrast, a study by **Holah and Hemida** [19] found a highly statistically significant association between faint Cyclin D1 expression, low percentage of cyclin D1 expression (<10% of tumor cells), and larger tumor size. In addition, there is a highly statistically significant association between faint and moderate Cyclin D1 expression, low and moderate percentage of Cyclin D1 expression (<10% and 10–50% of tumor cells), and advanced nodal stage. It may be attributed to the use of different cut-off points to decide small and large tumors or may be related to large sample size or different methods of cyclin D1 protein-expression scoring. Our study showed no significant statistical correlation between cyclin D1 expression and PNI & LVI similar to the study by **Hadžisejdić *et al.*** [16] and **Holah and Hemida** [19].

In our study, cyclin D1 expression was significantly associated with tumor grade of differentiation (p= 0.004), as we noticed decreased cyclin D1 expression in poorly differentiated tumors (grade III) compared to well-differentiated tumors (grade I). These results were in accordance with **Chung *et al.*** [20] who found that overexpression of cyclin D1 was associated with lower histological grade together with **Mylona *et al.*** [21] who found that cyclin D1 might induce growth arrest instead of cell cycle progression. These results could be explained by an interaction between cyclin D1, histone acetylases and Rb such that acetylation of Rb leads to cell cycle exit and induces growth arrest and could be also explained as in normal human mammary epithelial cells, cyclin D1 overexpression causes growth inhibition rather than growth progression, induces differentiation, and enhances apoptosis [21].

Cyclin D1 is necessary for the normal lobulo-alveolar development of the breast as transgenic mice experiments with targeted deletion of the gene encoding Cyclin D1 leads to poor mammary gland development and also protects against the development of breast carcinoma [22]. By contrast, cyclin D1 overexpression in transgenic mammary tissues results in mammary hyperplasia and the development of mammary adenocarcinomas [23].

According to the finding of the current study, cyclin D1 expression shows a statistically significant correlation with studied histological types (p= 0.014). A significant high positive expression of cyclin D1 in our cases of IDC; 94.6% is detected. A study by **Abd El Maqsoud and Aly** [24] found a strong correlation between cyclin D1 overexpression and histological type (p = 0.008) which come in line with our finding. In contrast **Mylona *et al.*** [21] showed no statistical significance between cyclin D1 expression & IDC/ILC histological types, but cyclin D1 overexpression was related to better overall survival of patients with ductal carcinoma (P = 0.001), whereas no correlation was observed within the subgroup of lobular carcinomas. The possible explanation for these discrepancies includes a lack of standard immunohistochemical assays or the use of different techniques to detect Cyclin D1.

Our study showed positive cyclin D1 expression in 75.8% of tumors which did not receive NCT before surgery, and 100% of tumors that received NCT before surgery. If we compare the previous percentages, we found that there is a significant increase in cyclin D1 protein expression after chemotherapy. This agreed with the results of **Penault-Llorca *et al.*** [25] who found a significantly higher percentage of cells that stained positive for cyclin D1 after NCT (p= .016) and **Li *et al.*** [26] who compared the IHC staining pattern between pre-and post-treatment specimens using the Wilcoxon signed-rank test, and found that the expression levels of cyclin D1 (median, 8% vs. 9%; P = 0.016) after NCT treatment was increased significantly.

In contrast, a study by **Villegas *et al.*** [27] included 284 breast tumors received NCT from two clinical trial cohorts GeparTrio and GeparQuattro trials found low levels of Cyclin D1 were more frequent in those tumors; these results are in line with the results of the previous study done by **Assaf *et al.*** [28]. These differences in cyclin D1 expression after NCT among

previous studies may be due to a comparison of different NCT regimens, or due to their studies included a larger number of studied cases. However, changes in protein expression after NCT treatment have been reported by **Bottini *et al.* [29].**

A significant relation between cyclin D1 and molecular subtypes in our study was detected. Among tumors strongly expressing cyclin D1 80.0% were of luminal type (A&B) tumors, while among tumors negatively expressing cyclin D1 87.5% were of triple-negative type tumors. In addition, we found a significant correlation between cyclin D1 expression, and ER, and PR; 96.7% of ER-positive, and 95.8% of PR positive tumors, showed positive cyclin D1 expression (strong & intermediate). In contrast, there is an inverse correlation between cyclin D1 expression and Her-2 status, 96.6% of Her-2 negative tumors showed positive cyclin D1 expression.

This comes in line with findings by **Li *et al.* [7]** who found that Cyclin D1 expression was significantly correlated with positive ER and PR, and negative HER-2. Higher Cyclin D1 expression was found in luminal B tumors, whereas, loss of expression or very weak expression of Cyclin D1 was observed in basal-like (TNBC) tumors. Findings by **Ortiz *et al.* [30]** found that the IHC positivity of cyclin D1 correlated with the expression of ER (p<0,001) and PR (p<0,001) as well as with Luminal type (p<0,001) and Loss of cyclin D1 expression correlated with triple negative subtype (p<0,001) also comes in agreement with our results.

As appears in the current study, cyclin D1 overexpression more frequently in patients with ER-positive tumors, is a consistent finding in all studies to date and may be attributable to the intimate physiological relationship between cyclin D1 and ER. Thus, estrogen has been shown to activate cyclin D1 through increased transcription of CCND1; whereas, conversely, cyclin D1 has been found to activate ER-mediated transcription in the absence of estrogen, possibly through direct attachment to the hormone-binding domain of ER [18].

CONCLUSION

The present study demonstrated that Cyclin D1 overexpression is frequent in breast cancers. Our data have also shown that there were significant correlations between CyclinD1 overexpression and decreased tumor grade, positive hormone receptors (ER/PR) & negative HER-2 status.

We found that majority of Luminal breast cancer subtypes showed increased expression of Cyclin D1, while TNBC showed decreased expression of it, so Cyclin D1 might play an important role in defining malignant behaviors, and its expression associated with better tumor characteristics.

Based on the foregoing, we can consider that cyclin D1 protein has a role in the early stages of breast oncogenesis and continues to have a significant effect throughout the development of malignancy. The previous findings make it possible to use cyclin D1 in the diagnosis and prognosis of BC.

Financial support and sponsorship: Nil.
Conflict of interest: Nil.

REFERENCES

1. **Zaidi Z, Dib H (2019):** The worldwide female breast cancer incidence and survival. Cancer Research, 79: 4191-94.
2. **Bray F, Ferlay J, Soerjomataram I *et al.* (2018):** Global cancer statistics 2018: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. CA: A Cancer Journal for Clinicians, 68: 394-424.
3. **Ibrahim N, Talima S, Makar W (2019):** Clinico-Epidemiological Study of Elderly Breast Cancer in a Developing Country: Egypt. Journal of Cancer Treatment and Research, 7: 23-27.
4. **Hoda S, Brogi E, Koerner F *et al.* (2014):** Rosen's breast pathology, Lippincott Williams & Wilkins, Pp. 855-870. https://www.wolterskluwer.com/en/solutions/ovid/rosens-breast-pathology-3301
5. **Arciero C, Styblo T (2018):** Clinically Established Prognostic Factors in Breast Cancer. In Kirby I. Bland, Edward M. Copeland, V. Suzanne Klimberg and William J. Gradishar (eds.), The Breast (Fifth Edition) (Elsevier). Pp. 711-735. https://www.elsevier.com/books/the-breast/bland/978-0-323-35955-9
6. **Goldhirsch A, Wood W, Coates A *et al.* (2011):** Strategies for subtypes—dealing with the diversity of breast cancer: highlights of the St Gallen International Expert Consensus on the Primary Therapy of Early Breast Cancer 2011. Annals of Oncology, 22: 1736-1747.
7. **Li Z, Cui J, Yu Q *et al.* (2016):** Evaluation of CCND1 amplification and CyclinD1 expression: diffuse and strong staining of CyclinD1 could have same predictive roles as CCND1 amplification in ER positive breast cancers. American Journal of Translational Research, 8: 142-153.
8. **Musgrove E, Caldon C, Barraclough J *et al.* (2011):** Cyclin D as a therapeutic target in cancer. Nature Reviews Cancer, 11: 558-72.
9. **Xu S, Gu G, Ni Q *et al.* (2015):** The expression of AEG-1 and Cyclin D1 in human bladder urothelial carcinoma and their clinicopathological significance', International Journal of Clinical and Experimental Medicine, 8: 222-27.
10. **Montalto F, De Amicis F (2020):** Cyclin D1 in Cancer: A molecular connection for cell cycle control, adhesion and invasion in tumor and stroma. Cells, 9: 2648-53.
11. **Tan P, Allison K, Ellis I *et al.* (2020):** The 2019 World Health Organization classification of tumours of the breast. Histopathology, 77(2):181-185.
12. **Parvin T, Das C, Choudhury M *et al.* (2019):** Prognostic Utility of Cyclin D1 in Invasive Breast Carcinoma. Indian Journal of Surgical Oncology, 10: 167-173.
13. **Eckyen E (2017):** Breast Tumours.' in MB James D. Brierley BSc, FRCP, FRCR, FRCPC, FRCPC Mary K. Gospodarowicz MD, FRCR (Hon) and Christian Wittekind MD (eds.), TNM Classification of Malignant Tumours (UICC) (John Wiley & Sons, Ltd: Oxford, UK). Pp. 135-165. https://www.legeforeningen.no/contentassets/201604933ce448e888a101ab969a4205/tnm-classification-of-malignant-tumours-8th-edition.pdf
14. **Liu L, Kawashima M, Toi M (2020):** Breast cancer in global health: beyond diversity and inequality. IJS Global Health, 3: 32-36.
15. **El-Sheikh N, Mousa M, Tawfeik A *et al.* (2021):** Assessment of Human Papillomavirus Infection and Risk

Factors in Egyptian Women With Breast Cancer. Breast Cancer: Basic and Clinical Research, 15: 279-83.

16. **Hadžisejdić I, Mustać E, Jonjić N *et al.* (2010):** Nuclear EGFR in ductal invasive breast cancer: correlation with cyclin-D1 and prognosis. Modern Pathology, 23: 392-403.
17. **Bai T, Liu F, Zou F *et al.* (2017):** Epidermal growth factor induces proliferation of hair follicle-derived mesenchymal stem cells through epidermal growth factor receptor-mediated activation of ERK and AKT signaling pathways associated with upregulation of cyclin D1 and downregulation of p16. Stem Cells and Development, 26: 113-122.
18. **Feng Y, Spezia M, Huang S *et al.* (2018):** Breast cancer development and progression: Risk factors, cancer stem cells, signaling pathways, genomics, and molecular pathogenesis. Genes & Diseases, 5: 77-106.
19. **Holah N, Hemida A (2020):** Cyclin D1 and PSA act as good prognostic and clinicopathological indicators for breast cancer. Journal of Immunoassay and Immunochemistry, 41: 28-44.
20. **Chung J, Noh H, Park K *et al.* (2014):** Longer survival in patients with breast cancer with cyclin d1 over-expression after tumor recurrence: longer, but occupied with disease. Journal of Breast Cancer, 17: 47-52.
21. **Mylona E, Tzelepis K, Theohari I *et al.* (2013):** Cyclin D1 in invasive breast carcinoma: favourable prognostic significance in unselected patients and within subgroups with an aggressive phenotype. Histopathology, 62: 472-480.
22. **Ravikumar G, Ananthamurthy A (2014):** Cyclin D1 expression in ductal carcinoma of the breast and its correlation with other prognostic parameters. Journal of Cancer Research and Therapeutics, 10: 671-675.
23. **Cai Z, Liu Q (2017):** Cell cycle regulation in treatment of breast cancer. Translational Research in Breast Cancer, 17: 251-270.
24. **Abd El Maqsoud N, Aly M (2010):** Significance of Cyclin D1 overexpression and amplification in ductal hyperplasia, carcinoma in situ and invasive carcinoma in Egyptian female breast. International Journal of Cancer Research, 6: 202-219.
25. **Penault-Llorca F, Abrial C, Raoelfils I *et al.* (2008):** Changes and predictive and prognostic value of the mitotic index, Ki-67, cyclin D1, and cyclo-oxygenase-2 in 710 operable breast cancer patients treated with neoadjuvant chemotherapy. The Oncologist, 13: 1235-1245.
26. **Li X, Liu M, Zhang Y *et al.* (2011):** Evaluation of ER, PgR, HER-2, Ki-67, cyclin D1, and nm23-H1 as predictors of pathological complete response to neoadjuvant chemotherapy for locally advanced breast cancer. Medical Oncology, 28: 31-38.
27. **Villegas S, Darb-Esfahani S, Von Minckwitz G *et al.* (2018):** Expression of Cyclin D1 protein in residual tumor after neoadjuvant chemotherapy for breast cancer. Breast Cancer Research and Treatment, 168: 179-187.
28. **Assaf M, Rashed H, Ibrahim D (2020):** Immunohistochemical Expression of Cyclin D1 and PD-L1 With TILs Density in Prediction of the Response to Neoadjuvant Chemotherapy Treatment in Invasive Ductal Carcinoma of the Breast. Prensa Med Argent., 106: 1-7.
29. **Bottini A, Berruti A, Bersiga A *et al.* (2000):** p53 but not bcl-2 immunostaining is predictive of poor clinical complete response to primary chemotherapy in breast cancer patients. Clinical Cancer Research, 6: 2751-2758.
30. **Ortiz A, Garcia D, Vicente Y *et al.* (2017):** Prognostic significance of cyclin D1 protein expression and gene amplification in invasive breast carcinoma. PloS One, 12: e0188068.