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RESEARCH ARTICLE

Expression of cyclin D1 in breast carcinoma and its relation to other prognostic markers in Saudi female patients

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Abstract

Cyclin D1 expression has been implicated in breast cancer pathogenesis with prognostic impact which still controversial. We aimed to examine the frequency of cyclin D1 expression and to find the relationship between its expression and some well-known clinicopathologic prognostic determinants in breast invasive ductal carcinoma in Saudi females. This study included 100 cases of invasive ductal carcinoma. Immunohistochemistry was performed for estrogen receptors (ER), progesterone receptors (PR), Her2/neu and cyclin D1. Cyclin D1 expression was assessed and compared to the tumor histological grade, nodal status, tumor size and ER, PR, Her2/neu immunostaining results. Cyclin D1 was positive in 68%, and negative in 32%. There was a statistically significant reverse relationship between cyclin D1 and tumor grade. A statistically significant association between cyclin D1 expression and ER and PR. Our data strengthen the importance of cyclin D1 measurement in invasive duct carcinoma and its strong association with estrogen and progesterone receptor status.

INTRODUCTION

Breast cancer is the most common cancer among women, and is the most likely cause of death due to malignancy worldwide (Ferlay et al., 2013). The recent increased knowledge in molecular mechanisms of this cancer and consequent targeted treatments have improved its outcome (Jiang et al., 2011).
Various prognostic parameters are described and validated, but the search for newer prognostic factors continues as the existing parameters do not provide sufficient information for accurate risk assessment and treatment planning (Ravikumar &Ananthamurthy 2014).

Cyclin D1 is a known cell cycle regulator which acts by binding with cyclin dependent kinase (CDK 4/6) in the cell cycle and inactivating the retinoblastoma (Rb) protein, helping in the progression of the cell cycle. Binding of cyclin D1 to CDK4 and CDK6 induces hyper phosphorylation of retinoblastoma protein (Rb). Hyper phosphorylated Rb loses its ability to bind to the E2F family of transcription factors. This leads to the activation of E2F and transcription of several genes required for the G1 to S phase transition, thereby promoting cellular proliferation (Ishii et al., 2006).
The gene encoding Cyclin D1 is located on chromosome 11q13, which is commonly amplified in many human cancers including breast cancers. Cyclin D1 overexpression has been reported in up to 50% of human breast cancers (Ishiiet al.,2006, Eeckhoute et al., 2006). This aberrant over expression of Cyclin D1 is known to drive breast carcinogenesis by cell cycle mediated action (Mohammadizadeh et al., 2013).
Cyclin D1 can also affect the cell cycle progression independently of CDK mechanisms by forming potential further interactions with many other molecules including estrogen receptors, androgen receptors , signal transducer

and activator of transcription (STAT3) and nuclear factor kappa B (NFKB1) as well as histone acetylase and deacetylase (Sutherland &Musgrove, *2004)*.
Some previous studies have shown that over expressed cyclin D1 in breast cancer patients acts by binding directly to the estrogen receptors (ER) and propagate the downstream effects of estrogen in a CDK independent and Rb independent fashion (Arnold & Papanikolaou, 2005). Moreover, cyclin D1 overexpression has been linked to estrogen and progesterone receptor (PR) status, possibly by its role in regulating progesterone receptor gene expression (Chuanwei , 2010).
There is some evidence that over expression of cyclin D1 is a prognostic factor for better outcome in ER positive invasive breast cancer. However, other studies have shown that over expression and amplification of cyclin D1 is a predictor of poor response to anti-estrogen treatment (Sutherland &Musgrove, *2004)*.

As the role of cyclin D1 in breast cancer is unclear, we aimed to examine the frequency of cyclin D1 expression and to find the relationship between cyclin D1 expression and some well-known clinicopathologic prognostic determinants in breast invasive ductal carcinoma in Saudi females.

Material and methods

A total of 100 cases of breast cancer were included in this study from various surgical clinics at Taif, KSA, during the period from October 2013 to January 2015. Their mean age ± SD was 55.21±5.8 years. Patients were eligible if they had histologically confirmed primary invasive ductal carcinoma and had undergone mastectomy and axillary lymph node dissection. Patients who had undergone diagnostic core biopsies, lumpectomy without axillary clearance and neoadjuvant chemotherapy were excluded from the study. The study protocol was approved by the ethical committee of Faculty of Medicine, Taif University, and informed consent for the use of specimens was obtained from all participants.
The studied variables included age, tumor grade, tumor stage, ER, PR, human epidermal growth factor receptor 2 (HER2-neu) and cyclin D1 status.
As regards tissue samples, four µm thick sections from formaline-fixed, paraffin embedded tissue blocks were stained with hematoxylin–eosin for morphological assessment.
The status of ER, PR and HER2-neu had been determined immunohistochemically using the formalin fixed and paraffin embedded tissue samples of the primary tumor. Breast cancer cases were graded according to the modified criteria as described by Bloom and Richardson (Bloom & Richardson, 1957).
Expression of ER and PR was considered as negative if lesser than 1% of nuclei were stained and as positive if 1% or higher of nuclei were stained. The antibodies used were against ER (monoclonal mouse anti-human; Clone: 1D5; isotype: IGg1, kappa; Dakocytomation, Denmark) and against PR (monoclonal mouse anti-human; Clone: 1A6; isotype: IGg1, kappa; Dakocytomation, Denmark).

The antibody used for HER2-neu study was (polyclonal rabbit antihuman antibody against c-erbB-2 oncoprotein, Dakocytomation, Denmark)(Brunelli et al., 2008).

The histopathology slides were reviewed and a suitable block was chosen to perform immunohistochemistry (IHC) for Cyclin D1. IHC was performed using polymer technique on tissue sections of 4- 5 µm thickness, floated on salinized slides and incubated overnight at 60 C°. The slides were deparaffinized with xylene and rehydrated with ethanol. The slides were then placed in hydrogen peroxide solution. Antigen retrieval was done by steam treatment in Tris ethylenediaminetetraacetic (Tris-EDETA) acid buffer. The slides were coated and incubated at room temperature for 30 min with primary rabbit monoclonal antibody to Cyclin D1, clone EP12 from Dako. Subsequently, the slides were incubated with secondary antibody. Reactivity was detected using diaminobenzidine as chromogen and was counterstained with Harris' hematoxylin. The staining for Cyclin D1 was interpreted as positive when at least 10% or more of the tumor cells showed nuclear expression of the marker with a moderate to strong intensity of staining (The intensity of immunohistochemical reaction by light microscopy was recorded as 0 (negative) when no staining of the nuclei was seen even at high magnification, 1 (weak) if staining was visible only at high magnification, 2 (moderate) when staining was readily visible at low magnification and 3 (strong) if staining was strikingly positive even at low power magnification) [6]. The clinicopathological parameters like tumor grade, tumor size, LN metastasis, ER, PR and Her2/neu status were compared and correlated with Cyclin D1 expression.

Statistical analysis

Correlation of Cyclin D1 expression with the other recorded clinicopathological parameters were performed by SPSS version 16 software and EPI info 6.0 using Chi-square & Fischer's exact tests. *P* value was considered significant at P≤0.05.

Results:

The clinicopathological features and immunohistochemical findings of the 100 breast cancer patients included in the study are summarized in Table 1. The mean age of patients (±SD) was 55.21±5.8 years. Regarding the tumor size 28%, 44%, 20% and 8% of the tumors were T1, T2, T3, and T4 respectively. 6%, 68% and 26% of the tumors were Grade I, II and III, respectively. ER, PR and HER2-neu were positive in 60 %, 56% and 82% of the cases, respectively. Cyclin D1 was positive in 68%, and negative in 32% (Figures 1). Cyclin D1 staining was strong (S), intermediate (I), weak (W) in 20.1%, 43.8%, 4.1% cases, respectively.

We investigated the relation between cyclin D1 immunostaining with tumor size, grade, axillary lymph node metastasis and hormone receptor expression. There was a statistically significant reverse relationship between cyclin D1 and tumor grade ($P = 0.0001$). The relationship between cyclin D1 and ER was also statistically significant ($P = 0.0001$). A statistically significant relationship was found between cyclin D1 and PR ($P = 0.0001$). There was no statistically significant relationship between cyclin D1 and age, stage or HER2-Neu ($P > 0.05$).

Table 1: The Clinicopathological features of breast cancer patients (n=100).

Patient characteristic	***no***	***%***
Tumor size		
T1 (≤2 cm)	28	(28)
T2 (>2 cm–5 cm)	44	(44)
T3 (>5 cm)	20	(20)
T4 (tumor of any size that has broken through (ulcerated) the skin, or is attached to the chest wall)	8	(8)
Lymph node status		
N0	42	(42)
N1	32	(32)
N2	8	(8)
N3	18	(18)
Histological grade (modified Bloom-Richardson score)		
I (Well differentiated)	6	(6)
II(Moderately differentiated)	68	(68)
III(Poorly differentiated)	26	(26)
estrogen receptor status		
ER-positive	60	(60)
ER-negative	40	(40)
Progesterone receptor status		
PR-positive	56	(56)
PR-negative	44	(44)
HER-2		
positive	82	(82)
negative	18	(18)
CyclinD1		
Positive	68	(68)
negative	32	(32)

Table 2: ***relation of cyclin D1 immunostaining with tumor size, grade, axillary lymph node metastasis and hormone receptor expression***

Patient characteristic	***no***	***Cyclin D1 positive 68***	***Cyclin D1 negative 32***	***P value***

Tumor size				
T1	28	20	8	0.08
T2	44	28	16	
T3	20	10	10	
T4	8	2	6	
Lymph node status				
N0	42	30	12	
N1	32	14	18	0.07
N2	8	6	2	
N3	18	12	6	
Histological grade*				
I (Well differentiated)	6	6	0	
II(Moderately differentiated)	68	60	8	0.0001
III(Poorly differentiated)	26	2	24	
estrogen receptor status				
ER-positive	60	54	6	
ER-negative	40	14	26	0.0001
Progesterone receptor status				
PR-positive	56	52	4	
PR-negative	44	16	28	0.0001
HER-2				
positive	82	58	24	
negative	18	10	8	0.2

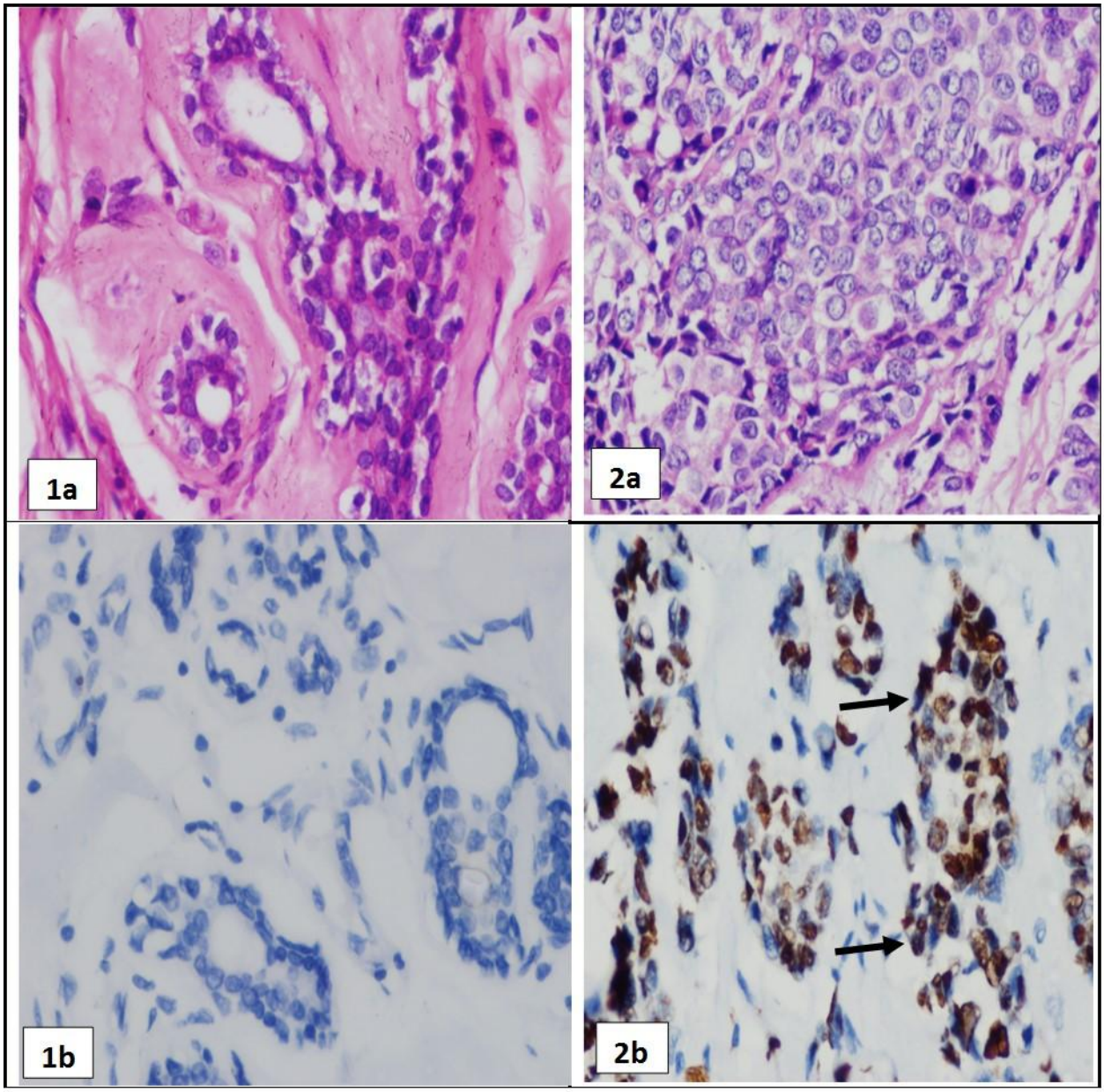

Figure (1): showing histopathological sections of invasive duct carcinoma. Negative immunosatining for cyclin is detected in (1b). Strong positive nuclear reaction for cyclin (arrows) is detected in 2b. [1a,2a : H&EX400; 1b,2b cyclin immunohistochemistry X400]

Discussion

Breast cancer is a heterogeneous disease arising from multiple genetic changes. ER and PR represent the most acceptable factors for predicting prognosis, response or resistance to treatment (Arafah, 2012).

The interaction of these known prognostic markers with newer molecular markers is a subject of intense investigation in predicting outcome in breast cancers.

Cyclin D1 is a cell cycle regulatory protein encoded by the CCND1 gene located on chromosome 11q13. 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 development of breast carcinoma [Yu et al, 2001, Sutherland&Musgrove,2002). The oncogene CCND1 is amplified in 10-20% of breast carcinomas (Arnold &Papanikolaou, 2005). While the overexpression of its product cyclin D1

is more frequent and seen in about 65-81% of breast carcinomas as detected by IHC (Gillett,et al., 1994, Zhang et al., 1994). Cyclin D1 expression was positive in 68% of our studied cases. Similar results was detected by ravikumar and ananthaumurthy, 2014.

Cyclin D1 overexpression occurs in the early stages of breast oncogenesis and plays a crucial role in the further progression of the tumor (Zhang et al., 1994). In our study, a statistically significant relationship was found between cyclin D1 and tumor grade .This observation is the same as the finding of some other studies in this field (Arnold& Papanikolaou, 2005). The grade of invasive ductal carcinoma is estimated by histological evaluation of tubular formation, mitosis and pleomorphism. Low grade tumors are well-differentiated and show histological features closer to their original tissue. The reverse relationship observed between cyclin D1 overexpression and tumor grade suggests that higher expression of cyclin D1 may directly or indirectly result in maturation of tumor cells. Some of the past researches support this opinion (Alao, 2007). However, cyclin D1 expression did not show significant correlation with tumor size or LN metastasis. However McIntosh et al.,1995 showed a high expression of Cyclin D1 with smaller tumor size.

Regarding the hormone receptor status, our results demonstrated that ER, PR and HER2-neu were positive in 60 %, 56% and 82% of the cases, respectively.

We found a statistically significant relationship between cyclin D1 expression and ER as well as between cyclin D1 expression and PR. While N status was mostly seen in patients with negative PR. These findings further confirm the results of some previous studies in this field, which have stated a positive relationship between hormone receptor status and cyclin D1 overexpression in breast carcinoma (Lee et al., 2007). This is in favor of the effect of cyclin D1 on cell maturation and differentiation. There was no statistically significant relationship between cyclin D1 overexpression and HER2-neu status. However, some studies reported overexpression of HER2-neu to be associated with the high expression of cyclin D1 (Lee et al., 2007).

A number of studies report cyclin D1 overexpression to be a predictor of worse prognosis (McIntosh, 1995, Kenny et al., 1999) while others have found an association with an ER-positive phenotype and a better clinical outcome (Michalides et al, 1996, Stendahl et al., 2004). Cyclin D1 is a factor, known to interact with ERα and independently of oestrogen, activate the receptor and potentially modify oestrogen/anti-oestrogen responses (Zwart et al., 2009, Zwijsen et al., 1997). Overexpression of cyclin D1 changes the antagonistic effect of tamoxifen to an agonistic effect. Therefore tamoxifen resistance might be predicted with cyclin D1 overexpression (Zwijsen et al., 1997, Rudas et al., 2008). it is also suggested that direct targeting of the cyclin D1 gene or gene products may prove more successful than approaches that rely on arguably incomplete knowledge of the oncogenic mechanisms of cyclin D1. CDKIs have been tried as monotherapy and combination therapy with favorable results in progression free survival in ER positive breast cancer (Serkan et al., 2014).

In conclusion, the high rate of expression of cyclin D1 in breast cancer in our study and the strong positive association with ER status high lightened the importance of measurement of cyclin D1 expression in line with other factors and especially in hormone resistant breast cancers. Also the presence of negative correlation between cyclin D1 expression and tumor garde support previous finding of cyclin D1 expression in well differentiated, slowly growing breast cancer which could be related to better outcome. Further follow up studies are necessary to clarify the prognostic value of cyclin D1 in Saudi females with breast cancer.

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