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 (66.6%) 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 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 virulence of tumour 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 µm 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 qualitative parameters such as age of patient, also used chi-Squared test for quantitative 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 (2.63%) 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 1: Distribution of patients according to their age strata.

| Age (years) | Number | Percentage | Comparison of Significance |
|------------|--------|------------|---------------------------|
| (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 2: Distribution of patients according to tumor types.

| Tumor types                      | Number | Percentage | Comparison of Significance |
|----------------------------------|--------|------------|---------------------------|
| Invasive ductal carcinoma        | 68     | 89.47%     | 107.7 P<0.01              |
| Recurrent carcinoma              | 6      | 7.89%      |                           |
| Total                            | 74     | 99.99%     |                           |

Table 3: Distribution of patients according to tumor grade.

| Result of Immunohistochemistry   | Positive | Negative | Total |
|----------------------------------|----------|----------|-------|
| Patients with breast cancer      | 30       | 46       | 76    |

Table 4: The Expression of Cyclin D1 in women with breast cancer.

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 expressed 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).

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.
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/EBPβ 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/EBPβ, 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-κB 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.

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