Original Article

Correlation of Serum Concentration Cyclin-Dependent Kinase 4 and Cyclin-Dependent Kinase 6 with Breast Cancer in Babylon Province

Dalya Shakir Al-Owaidi, Moaed E. Algazally[1], Alaa Sadeq Alawaad[2]

Departments of Biochemistry and [2]Internal Medicine, College of Medicine, University of Babylon, Babylon Province, [1]Department of Clinical Biochemistry, University of Al-Ameed, Karbala, Iraq

Abstract

Objectives: The goal of this study to estimate the correlation between breast cancer and cyclin-dependent kinase 4 (CDK4)\CDK6 enzyme concentrations in the occurrence of breast cancer and breast cancer subtypes. **Methods:** A total of 80 breast cancer patients are subdivided according to molecular classification into four groups, Luminal A, Luminal B, Her2/neu enriched, and TPN and 80 healthy individuals as control were enrolled; biochemical tests and body mass index were assayed. **Results:** No significant differences were found between patients and control groups and no significant differences were found among breast cancer subtypes in the concentrations of CDK4 ($P > 0.05$). There were no significant differences found between patients and control groups and no significant difference in the CDK6 concentration among subtypes groups ($P > 0.05$). The correlation between age, CDK4, and CDK6 in cases and control revealed that the correlation was absent between age, CDK4, and CDK6 concentration, except a weak positive correlation was found between CDK4 and CDK6 concentrations. **Conclusion:** There was no significant difference in serum levels of CDK4 and serum level of CDK6 between case and control and among patient subtypes.

Keywords: Breast cancer, cell cycle, cyclin-dependent kinase 4 and cyclin-dependent kinase 6, immunohistochemistry classification immunohistochemistry, retinoblastoma protein

Introduction

Breast cancer begins when cells in the breast start to grow out of control. The tumor considers cancer when the cells can invade surrounding tissues and spread to the distant areas of the body. Breast cancer occurs mostly in women, but rarely men can get breast cancer. Most breast cancers are (ductal cancers) and begin in the ducts that carry milk to the nipple. Some breast cancers are (lobular cancers) and start in the glands that make breast milk.[1]

Breast cancer is the first most common cause of cancer death in Iraq,[2] in Babylon province the number of patients with breast cancer that are admitted in Babylon oncology center exactly in years 2017 and 2018 about 242 and 187, respectively. breast cancer considers as the main cause of death in women ages 40–59.[3]

The cell cycle is a complex process that included in the growth, proliferation of cells, regulation of DNA damage, and repair. The cell cycle involved a variety of regulatory proteins that manage the cell through numbers of phases G1, S, G2, and M phase leading to mitosis and synthesis of two daughter cells. Central to this process are cyclin-dependent kinase (CDKs) and the cyclin protein that regulates the cell progression through the stage of the cell cycle.[4] The cyclin-CDK complexes are controlling on the progression through cell cycle that leads to (G0) the resting state, (G1) growth phase, through the (S) phase of DNA replication, and finally to (M) phase when cell division will occur.[5]

Cyclin D1 and CDK4 are very important in luminal epithelial proliferation in normal mammary tissue and for

Access this article online

Quick Response Code:

Website: www.medjbabylon.org

DOI: 10.4103/MJBL.MJBL_22_20

Address for correspondence: Dr. Dalya Shakir Al-Owaidi, Department of Biochemistry, College of Medicine, Babylon University, Babylon, Iraq.
E-mail: dalyashakir@yahoo.com

Submitted: 21-04-2020 **Accepted:** 03-05-2020 **Published Online:** 17-06-2020

How to cite this article: Al-Owaidi DS, Algazally ME, Alawaad AS. Correlation of serum concentration cyclin-dependent kinase 4 and cyclin-dependent kinase 6 with breast cancer in Babylon province. Med J Babylon 2020;17:199-203.

sustained proliferation in luminal breast cancers.[6] CyclinD1 binds to CDK4 forming a complex converted to an active holoenzyme, this active holoenzyme will hyperphosphorylate of retinoblastoma protein (pRb) and releasing of E2F transcription factors leading to the expression of a variety of genes that facilitate the transition from G1 to S phase.[7,8] Blocking the phosphorylation of pRb and promote G1 cell cycle arrest and cellular senescence is accomplished by CDK4/6 inhibitors.[9] The administration of anti-CDK4/6 agents, therefore, suppresses the bioavailability of the E2F transcription factor, resulting in decreased transcription and translation of S phase-related gene sets, causing breast cancer cells to undergo G1 arrest and/or death.[10]

Materials and Methods

Patients

The breast cancer patients who participated in this study about (80), their age between (30 and 80) years old were divided according to the molecular classification of breast cancer by immunohistochemistry technique that depends on the expression of ER, PR, and Her2-enriched proteins into four groups:

- Luminal A: 37 female patients with breast cancer
- Luminal B: 19 female patients with breast cancer
- Her2\neu+ enriched: 15 female patients with breast cancer
- Triple-negative: 9 female patients with breast cancer.

All patients of these groups were not treated with any type of treatment (hormonal or chemotherapy), that's mean that the blood samples were collected predose to exclude the effect of these drugs on the biochemical result.

Control group

The control group who participated in the study includes (80) healthy females. They obtained from consult clinics of early detection of breast cancer in Hilla teaching hospital their age was ranged between (30 and 80) years.

Ethical consideration

The study was conducted in accordance with the ethical principles that have their origin in the Declaration of Helsinki. It was carried out with patients' verbal and analytical approval before the sample was taken. The study protocol and the subject information and consent form were reviewed and approved by a local ethics committee (College of Medicine/University of Babylon). The project achieves the permission of research ethics in Babylon Center of Oncology/Marjan medical city at the date 15/4/2018.

Collection of the blood samples

Five ml of venous blood were withdrawn from patients and control by venipuncture pushed slowly into gel tubes. Blood was allowed to clot at room temperature for 30 min and centrifuged at 2000 ×*g* for 5 min, then the serum was divided into small Eppendorf tube and kept at (−20°C) to be used later for biochemical estimation of the CDK4 and CDK6 enzymes concentrations by ELISA technique.

Determination of CDK4 and CDK6 concentrations

Sandwich-ELISA kits by Sunlong (China) Company was used in this study as a method. Known concentrations of Human CDK-4 Standard and its corresponding reading OD are plotted on the log scale (x-axis) and the log scale (y-axis), respectively, Figure 1. The concentration of Human CDK-4 in the sample is determined by plotting the sample's OD on the Y-axis. The original concentration is calculated by multiplying the dilution factor.

Also known concentrations of Human CDK-6 Standard and its corresponding reading OD are plotted on the log scale (x-axis) and the log scale (y-axis), respectively, Figure 2. The concentration of Human CDK-6 in the sample is determined by plotting the sample's O. D. On the Y-axis. The original concentration is calculated by multiplying the dilution factor.

Statistical analysis

The general statistical parameters such as mean, standard deviation, percentage, and descriptive plots were done using the Microsoft® Excel 2010 software, while phenotypic means and standard deviation were compared by student *t*-test and one way ANOVA by employing SPSS version 21 (SPSS, IBM Company, Chicago, IL 60606, USA), and *P* value was determined to be significant.

Results

The range of age in this study was about (30–80) years, and the result showed that the control group matches a patient group in age $P > 0.05$; also the results showed that there were no significant differences in the age among different breast cancer subtypes ($P > 0.05$).

The results revealed that there were no significant differences in the body mass index among patients subtypes $P > 0.05$. No significant differences in the concentrations of CDK4 between patients and control groups ($P > 0.05$), the means, standard deviation, and statistical parameters are listed in Table 1.

Furthermore, the results displayed that there was no significant difference in CDK4 concentration between patients subtypes ($P > 0.05$), as shown in Table 2 and Figure 3].

Our results showed that there were no significant differences in the concentrations of CDK6 between patients and control

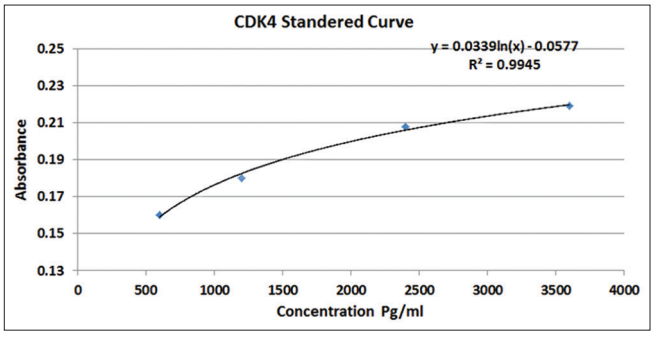

Figure 1: Human cyclin-dependent kinase 4 standard curve

groups ($P > 0.05$). The means, standard deviation, and statistical parameters are listed in Table 3.

Table 4 and Figure 4 list the CDK6 concentration of patients' subtypes. The results revealed that there was no significant difference in CDK6 concentration among patients subtypes ($P > 0.05$).

In Table 5, we found that the correlation was absent between age and CDK4 concentration $P = 0.51$, $r = -0.052$. There is no correlation between age and CDK6 concentration $P = 0.5$, $r = 0.054$. Weak positive correlation was found between CDK4 and CDK6 concentrations $P = 0.027$, $r = 0.175$*, [Figure 5].

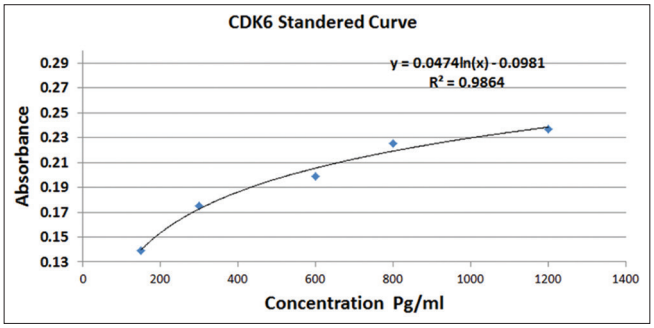

Figure 2: Human cyclin-dependent kinase-6 standard curve

Discussion

CDK4 has a close relationship with CDK6 and plays pivotal roles in mammalian cell proliferation. Cyclins are assembling with CDK4 and CDK6 to form enzymatically active holoenzyme complexes to drive cells progression into the (S) phase of DNA synthesis in the cell-division cycle.[11]

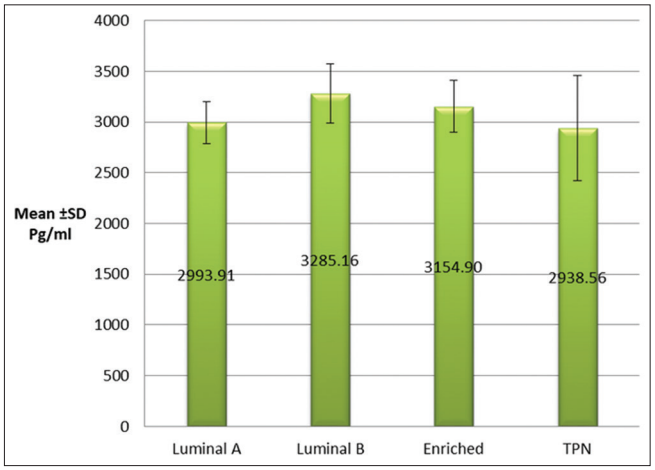

Figure 3: Comparison between Breast cancer groups in mean and standard deviation ofcyclin-dependent kinase 4 concentration

Table 1: Mean and standard deviation of cyclin-dependent kinase 4 concentration for patients and control groups

	Group	*n*	Mean±SD (Pg/ml)	*T*	*P**
Cyclin-dependent kinase 4	Case	80	3956.7±5494.08	1.13	0.19
	Control	80	3133.14±1031.56		

**P* value of Student's test. SD: Standard deviation

Table 2: Mean and standard deviation of CDK4 concentration in patients subtypes

n	(I) group	Mean±SD (Pg/ml)	(J) group	Mean difference (I-J), mean±SD (Pg/ml)	*P*
37	Luminal A	2993.91±1273.46	Luminal B	-291.24-	0.420
			Her2-enriched	-160.98-	0.675
			TPN	55.35	0.906
19	Luminal B	3285.15±1238.0	Luminal A	291.24	0.420
			Her2-enriched	130.25	0.767
			TPN	346.6	0.499
15	Her2-enriched	3154.90±986.10	Luminal A	160.98	0.675
			Luminal B	-130.25-	0.767
			TPN	216.34	0.683
9	TPN	2938.55±1554.0	Luminal A	-55.35-	0.906
			Luminal B	-346.6-	0. 499
			Her2-enriched	-216.34-	0.683
Total	80	3084.53±1233.42			

**P* value of ANOVA test. SD: Standard deviation

Table 3: Cyclin-dependent kinase 6 concentration for patients and control groups

	Group	*n*	Mean±SD (Pg/ml)	*t*	*P**
CDK6	Case	80	2700.29±4438.49	1.35	0.17
	Control	80	1788.46±4094.06		

**P* value of Student's test. SD: Standard deviation

In this study, we measured the actual concentration of CDK4 and CDK6, but the results revealed that there were no significant differences between patients and control groups ($P > 0.05$), and there was no significant difference in CDK4 concentration among patient groups ($P > 0.05$). Furthermore, results revealed that there was no significant difference in the CDK6 concentrations between cases and control ($P > 0.05$), the results also revealed that there was no significant difference in CDK6 concentration among patient groups ($P > 0.05$). Some study reported that the enzymatic activity of CDK4 and CDK6 may be elevated in the breast cancer patient and that leads to the continuous cell proliferation of breast tissue and help in occurrence of breast cancer phenotype. Therefore, the enzymatic activity and enzyme kinetics of both enzymes should take the preposition considering in future studies in breast cancer in Iraq. Furthermore, the measurement of both enzymes by another technique such as gene expression profiling instead of measuring concentration by Eliza technique perhaps explain the cell cycle disorders in both breast cancer and its subtypes.

Recently, drugs that are inhibiting of CDK4/CDK6 activities have high significant efficacy in cancer treatment.[12,13] The mechanism by which how signal transduction pathways in various tumor types activate CDK4/6 may pave the way for producing combinatorial therapies that target both cyclin D and CDK4/6 to improve therapeutic responses.[11]

Our results showed that the correlation was absent between age and CDK4, CDK6 concentrations except for a very weak significant positive correlation between CDK4 and CDK6 concentrations.

In human cancers, the inactivation of p16INK4a is the most common lesion of this pathway and that leads to phosphorylation of pRB and related proteins by (CDK4/6) cyclin D complex that allows the increased synthesis of genes important for DNA replication and thus progression through the cell cycle. CDKs activity in cancer cells may be increase through a variety of mechanisms, and they presumably promote tumorgenesis by suppressing senescence in cancer cells.[14] This observation had brought up the idea that compounds able to enhance the levels of CDK inhibitors or drugs that inhibit CDKs activities may be used for pro-senescence therapy for cancer.[15]

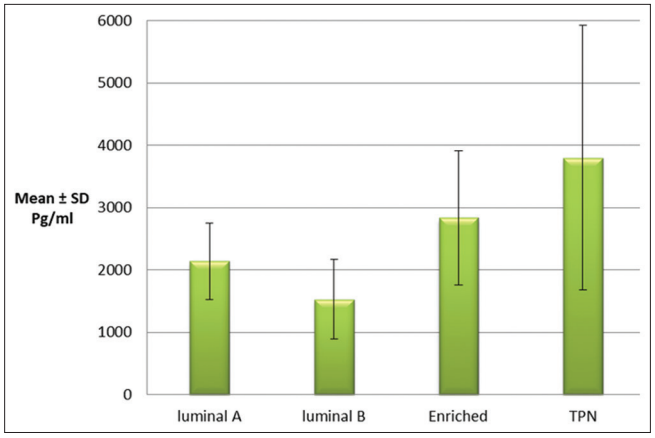

Figure 4: Comparison between breast cancer groups in mean and standard deviation of cyclin-dependent kinase 6 concentration

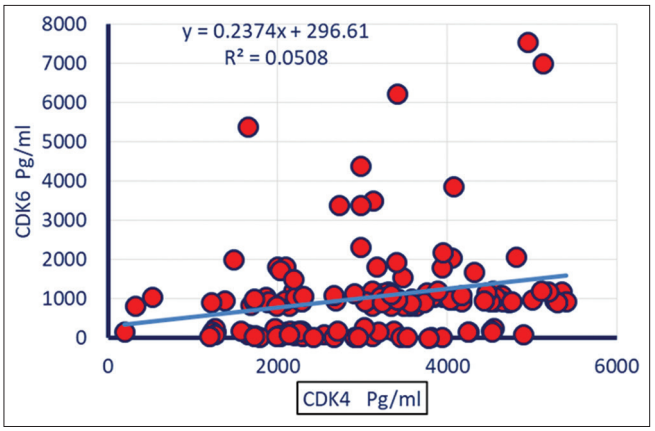

Figure 5: Correlation between cyclin-dependent kinase 4 and cyclin-dependent kinase 6

Table 4: Mean and SD of cyclin-dependent kinase 6 concentration of breast cancer groups

n	(I) group	Mean±SD (Pg/ml)	(J) group	Mean difference (I-J) (Pg/ml)	*P*
37	Luminal A	2141.90±3717.15	Luminal B	612.86	0.587
			Her2-enriched	-697.08-	0.569
			TPN	1658.56--	0.266
19	Luminal B	1529.04±2780.91	Luminal A	612.86--	0.587
			Her2-enriched	-1309.94-	0.344
			TPN	-2271.42-	0.163
15	Her2-enriched	2838.99±4177.82	Luminal A	697.08	0.569
			Luminal B	1309.94	0.344
			TPN	-961.48-	0.568
9	TPN	3800.47±6355.36	Luminal A	1658.56	0.266
			Luminal B	2271.42	0.163
			Her2-enriched	961.48	0.568
80	1481.8903	2313.64±3963.33			

*P value of ANOVA test. SD: Standard deviation

Table 5: Correlation between age, cyclin-dependent kinase 4, and cyclin-dependent kinase 6 in patients and control

Correlations	Age	CDK4	CDK6
Age (years)			
r	1	0.052	0.054
P		0.510	0.500
n	160	160	160
CDK4 (pg/ml)			
r	0.052	1	0.175*
P	0.510		0.027
n	160	160	160
CDK6 (pg/ml)			
r	0.054	0.175*	1
P	0.500	0.027	
n	160	160	160

*Correlation is significant at the 0.05 level (two-tailed)

From our results and explanations above, further study needed to assess the following in breast cancer women, cyclin D1 concentration, p16INK4 concentration, and CDK4 and CDK6 activity.

Conclusion

There was no significant difference in serum levels of CDK4 and serum level of CDK6 between case and control. There is a weak positive correlation between CDK4 serum concentration and CDK6 serum concentration. We think that the measurement of serum enzyme activity for CDK4 and CDK6 is more accurate than the measurement of concentration; therefore, the study of enzyme kinetic of CDK4 and CDK6 was recommended before and after the use of CDK4/CDK6 inhibitor drug to the assessment of the affectivity of these drugs in the survival of women with breast cancer in the Iraqi population.

Financial support and sponsorship

Nil.

Conflicts of interest

There are no conflicts of interest.

References

1. American Cancer Society guidelines. Medical Review," no. 1 August, 2017.
2. Ministory of Health of Iraq. Annual Stastical Report; 2016.
3. Morgan JG. Position paper of the American council on science and health on the risk factors of breast cancer. Breast 2013;4:177-97.
4. Alberts B, Bray D, Lewis J, RaffM, Roberts KW. The Cytoskeleton. New York: Mol. Biol. Cell, Garl. Publ.; 1983. p. 549-607.
5. Malumbres M1, Barbacid M. Mammalian cyclin-dependent kinases. Trends Biochem Sci 2005;30:630-41.
6. Jeselsohn R, Brown NE, Arendt L, Klebba I, Hu MG, Kuperwasser C, *et al*. Cyclin D1 kinase activity is required for the self-renewal of mammary stem and progenitor cells that are targets of MMTV-ErbB2 tumorigenesis. Cancer Cell 2010;17:65-76.
7. Sherr CJ, Roberts JM. CDK inhibitors: Positive and negative regulators of G1-phase progression. Genes Dev 1999;13:1501-12.
8. Narasimha AM, Kaulich M, Shapiro GS, Choi YJ, Sicinski P, Dowdy SF. Cyclin D activates the Rb tumor suppressor by mono-phosphorylation. Elife 2014;3:2014;3: e02872.
9. Knudsen ES, Witkiewicz AK. The strange case of CDK4/6 inhibitors: Mechanisms, resistance, and combination strategies. Trends Cancer 2017;3:39-55.
10. Syn NL, Wee I, Ann Wong AL, Goh RM, Wei Ow SG, Lambertini M, *et al*. Cyclin-dependent kinase (CDK) inhibitors for hormone receptor-positive advanced breast cancer. Cochrane Database Syst Rev 2018;2018:CD012919.
11. Sherr CJ, Beach D, Shapiro GI. Targeting CDK4 and CDK6: From discovery to therapy. Cancer Discov 2016;6:353-67.
12. Asghar U, Witkiewicz AK, Turner NC, Knudsen ES. The history and future of targeting cyclin-dependent kinases in cancer therapy. Nat Rev Drug Discov 2015;14:130-46.
13. Dickson MA. Molecular pathways: CDK4 inhibitors for cancer therapy. Clin Cancer Res 2014;20:3379-83.
14. Qin S, Schulte BA, Wang GY. Role of senescence induction in cancer treatment. World J Clin Oncol 2018;9:180-7.
15. Calcinotto A, Kohli J, Zagato E, Pellegrini L, Demaria M, Alimonti A. Cellular senescence: Aging, cancer, and injury. Physiol Rev 2019;99:1047-78.