RESEARCH ARTICLE

Editorial Process: Submission:06/26/2022 Acceptance:04/24/2023

The Potential of Expression of Cyclin-D1 on Neoadjuvant Chemotherapy in Invasive Breast Carcinoma

Primariadewi Rustamadji[1]*, Elvan Wiyarta[2], Ineke Anggreani[1]

Abstract

Background: Patients undergoing neoadjuvant chemotherapy (NC) for invasive breast cancer (IBC) need indicators to track their progress during treatment. The goal of this research is to learn how cyclin D1 works in conjunction with taxane and non-taxane therapy for people with IBC. **Methods:** There were 31 examples divided into two groups, based on: those using a different type of NC (taxane- or non-taxane-based), and NC administration time (before or after). Tumor grade, age, PR, ER, Ki-67, HER2, and Cyclin D1 expression were among the factors considered. Using immunohistochemical labeling, we were able to categorize cyclin D1 levels according to a threshold value, and we supplemented this with data we found in our databases. To analyze the data, we used a modified linear model. **Results:** The expression of Cyclin D1 decreased after NC delivery (p=0.086). Cyclin D1 expression was reduced in the taxane group (p=0.792). The non-taxane group also saw no differences in outcomes (p = 0.065). There was a larger decrease in Cyclin D1 expression in the non-taxane group compared to the taxane group, but the difference was not statistically significant (p=0.200). **Conclusion:** Cyclin D1 expression, even if the differences are not statistically significant, may be a prognostic indicator of NC reaction in IBC. The involvement of Cyclin D1 in NC warrants more research with bigger IBC sample sizes.

Keywords: Cyclin-D1- taxane- chemotherapy- breast cancer- invasive

Asian Pac J Cancer Prev, **24 (4)**, 1131-1136

Introduction

Cancer deaths from breast cancer are the leading cause of mortality among women. According to Global Burden of Cancer (GLOBOCAN) statistics, women will be diagnosed with breast cancer at 11.7% of all cases and 6.9% of all deaths linked with the disease in 2020 (GLOBOCAN, 2020). Breast cancer can be classified as invasive (IBC) or non-invasive (nIBC) (GLOBOCAN, 2020). There are several subtypes of IBC, which is the most frequent form of breast cancer. Because of the high variation of IBC, the treatment must also be done early and aggressively (Rustamadji et al., 2021; Rustamadji et al., 2021; Sharma et al., 2010).

Neoadjuvant chemotherapy (NC), which is administered before surgery, is a crucial part of modern IBC care (Lee et al., 2011). Currently, NC is the gold standard for patients with locally progressed breast cancer and is the therapy of choice for early-stage, possibly treatable diseases (Lee et al., 2011). There are two primary categories of NC: those founded on taxanes and those that do not (Zhang et al., 2019). In breast cancer therapy, the taxane is one of the most influential and extensively used systemic therapies. Resistance to NC, on the other hand, impacts breast cancer treatment (Zhang et al., 2019).

A mechanism of self-protection has been devised by cancer cells in order to fight the effects of NC, for example, the NFkB activation pathway (Biliran et al., 2005). One of the NFkB protein complex's most important functions is regulating gene expression(Biliran et al., 2005). By controlling many anti-apoptotic genes, NFkB may help cancer cells withstand chemotherapy(Biliran et al., 2005). Survival factors like Cyclin D1 are among them (Biliran et al., 2005; Garg et al., 2016; Pacifico et al., 2006). The involvement of cyclin D1 in the NC resistance pathway makes it a candidate for use as a prognostic indicator (Mohammadizadeh et al., 2013). Predictive indicators are ultimately used in therapy to improve total mortality after NC administration in IBC. Both taxane-based and non-taxane-based NC for IBC will be compared for their effects on cyclin-D1 expression before and after treatment. We hypothesized that cyclin-D1 expression is associated with NC delivery in IBC patients.

Materials and Methods

Data Collection and Design of Study

This research was carried out between January and May of 2022 in the Pathological Anatomy Laboratory of the University of Indonesia's Faculty of Medicine.

*[1]Department of Anatomic Pathology, Faculty of Medicine, Universitas Indonesia, Dr. Cipto Mangunkusumo National Hospital, Jakarta, Indonesia. [2]Department of Medical Science, Faculty of Medicine, Universitas Indonesia, Dr. Cipto Mangunkusumo National Hospital, Jakarta, Indonesia. *For Correspondence: primariadewi.rustamadji@ui.ac.id*

The Universitas Indonesia Institutional Review Board approved the 21-11-1252 testing protocols in November 2021. Each participant gave a written agreement and understood the goal of the research. The research adheres to Declaration of Helsinki (Rickham, 1964). The data collection period ran from January 2014 through June 2016, and the five-year monitoring phase will run from January 2019 through May 2021. Data were collected, including tumor grade, age, tumor size, HER2 status, Ki-67, underarm lymph node spread, lymphovascular penetration, and NC type (taxane-based or non-taxane-based). Quantitative analysis of the IHC labeling findings on the paraffin sample was also performed to collect data on cyclin D1 expression.

Samples

Primary tumor paraffin samples were taken from female breast surgery patients who had initially been identified with IBC histopathologically. Specimens from individuals with non-IBC diseases, systemic illnesses, and damaged paraffin blocks are discarded. Both the NC treatment status (before or after) and the type of NC used (non-taxane or taxane) were used to categorize the data. The selected group represents the largest representative selection from the available records in the department. To avoid any potential for prejudice, only one researcher (E.W.) had access to the final groupings. Researchers didn't have access to the studies' classifications until after the analysis was done.

Preparation of Samples

Kusmardi et al., Wiyarta et al., and Primariadewi et al. all use this staining method (2022). In xylol (Brataco.inc, Bogor, Indonesia), we deparaffinized the paraffin block, then rehydrated it in 96%, 70%, and purified water for 5 minutes, as per protocol. Heat-induced antigen recovery in pH 9.0 Tris EDTA (Merk, Jakarta, Indonesia). was performed in a 96°C chamber for 20 minutes. There was a 15-minute period of peroxidase block (Merk, Jakarta, Indonesia) after antigen retrieval, then followed by 15 minutes of PBS pH 7.4 (Brataco Inc., Bogor, Indonesia) rinses. Post-primary and Novolink polymer incubations were conducted after anti-cyclin D1 antibodies (ab134175, Abcam, Cambridge, UK) were incubated for one hour. Hematoxylin and 5 percent lithium carbonate were used to counterstain the tissue slices before they were examined under a microscope for DAB staining (Abcam, Jakarta, Indonesia).

Quantification of Cyclin D1 Expression

Histopathology experts P.R. and I.A. assessed the immunohistochemistry stainings. A Leica DM750 microscope with a 400x total magnification was used to examine each specimen. Five sites were chosen at random, each containing 500 tumor cells, and their Cyclin D1 expression was analyzed. Each location had at least one hundred malignant cells. Cyclin D1 expression was detected by a dark smear in the tumor cytoplasm (Fusté et al., 2016). Cell counter were used to assess the brown hues and to classify the staining intensity as negative (0), weak (1+), moderate (2+), or strong (3+)(O'Brien et al., 2016). In order to measure the expression of cyclin D1, Peurala et al. developed a quantification formula (Ortiz et al., 2017). The percentage values of the very positive, positive, and low positive categories are combined to quantify the cyclin D1 expression (Ortiz et al., 2017). In addition, the cyclin D1 expression group was separated in to high and low expressions based on the prior quantification values. Samples with values higher than 0.4 were classified as having strong cyclin D1 expression (Ortiz et al., 2017). Two raters independently collected all of the results for the group. Until the entire sample has been examined, the results of the calculations that have already been performed are merged and sent to additional scholars (E.W.). The combined rating from the two raters will serve as the basis for further study.

Statistical Analysis

All data was been tabulated in Microsoft Excel (Microsoft Corp, Redmond, WA, USA) before research. Using SPSS (Statistical Package for the Social Sciences) 20, we examined and portrayed the collected data (IBM Corp, Armonk, NY, USA). To categorize cyclin D1 expression, the number 0.4 was used (Ortiz et al., 2017). The combined ratings from the two raters were then compared to the threshold to determine the overall rating (high or low). Each sample's Cyclin D1 expression level is represented these overall rating.

Results

All thirty-one samples were stained for cyclin D1 by immunohistochemistry. Before and after administration of NC, each specimen exhibits the clinicopathologic features outlined in Tables 1 and 2. Figure 1 depicts the results of exemplary IHC staining. Negative, low positive, positive, and high positive samples of tumor cells are shown in each image, respectively. The images are composites of different parts of a single sample. These samples are analyzed further after the strength of the brown color has been measured and assigned a number. All 31 samples were assessed separately by two experts (I.A and P.R.).

Following NC therapy, as shown in Figure 2 and Table 3, Cyclin D1 expression dropped, albeit not significantly ($p=0.086$). Both Table 4 and Figure 3 demonstrate the data's separation into taxane and non-taxane group. Cyclin D1 was downregulated in the taxane-treated cohort. However, the numbers were too low to be considered significant ($p=0.792$). Similar results were observed in the control sample ($p=0.065$) that did not include taxanes. The level of cyclin D1 was also compared between the two groups. Cyclin D1 levels were significantly lower in the non-taxane group than in the taxane group. The difference between the two groups was not statistically significant ($p=0.200$).

Discussion

Cyclin D1 expression was significantly changed in individuals with IBC who received NC. These alterations are detectable on an individual and collective level. Pre-NC administration, cyclin D1 expression was often

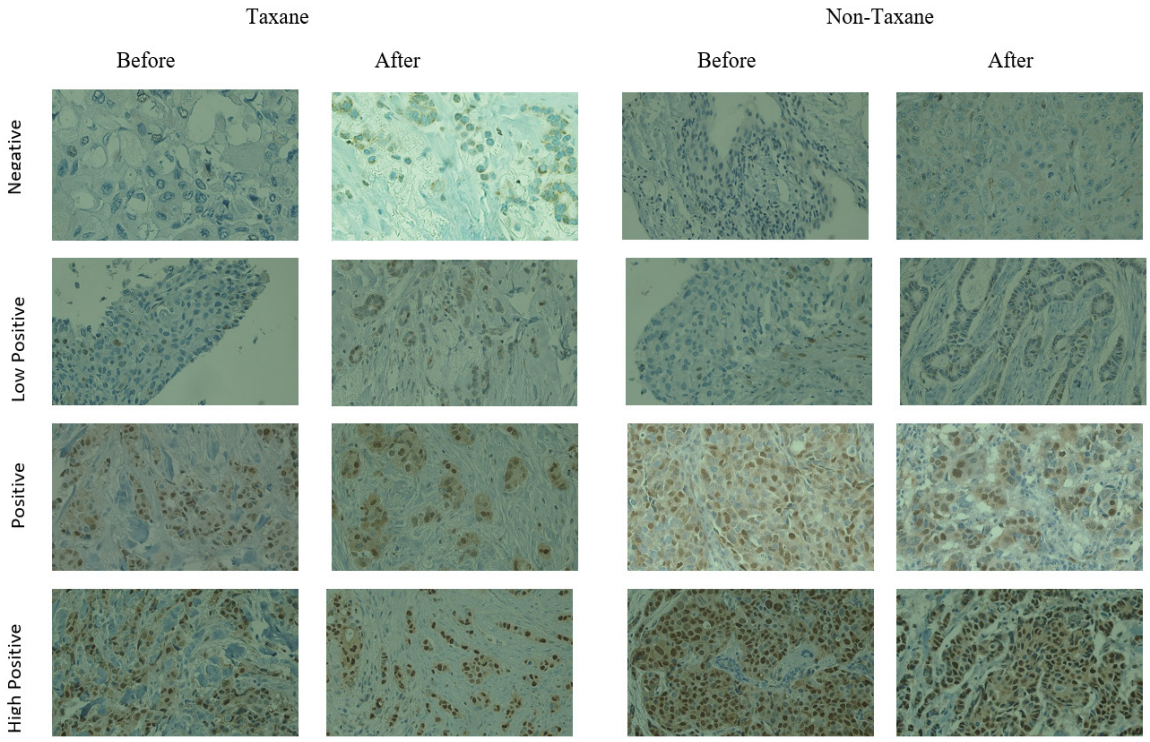

Figure 1. IHC Staining for cyclin-D1 Expression in IBC Tumor Cells at 400x Magnification before and after NC Administration. The scale bar represents 50 μm for all images.

higher than after administration of NC. This provides evidence for a causal relationship between NC and a reduction in cyclin D1 in IBC cells. Possible explanations for these observations involve the role of Cyclin D1 in drug resistance and the impact of it on the efficacy of therapy (Pysz et al., 2014). Consequently, several kinds of NC were created to target this biomarker (Villegas et al., 2018). Numerous investigations have shown that some anticancer drugs function by reducing cyclin D1 synthesis (Grillo et al., 2006). This is consistent with Grillo et al., (2006), which demonstrate that siRNA-mediated suppression of cyclin D1 in MCF-7 breast cancer cells has anticancer drug target potential. Overexpression of cyclin D1 was also linked to resistance to NC. Overexpression of cyclin D1 increases tumor cell proliferation and imparts resistance to cisplatin-mediated apoptosis, as reported by Biliran et al., (2005). All of these factors explain the involvement of NC in cyclin D1 expression modification.

Table 1. Clinicopathological Characteristics before Neoadjuvant Chemotherapy Administration

Variables	Category	Cyclin-D1 Expression		p-value
		High (%)	Low (%)	
Age	≥50 y.o.	14 (83.9%)	2 (12.5%)	0.654
	<50 y.o.	12 (80.0%)	3 (20.0%)	
Tumor grade	3	8 (88.9%)	1 (11.1%)	0.581
	2	15 (78.9%)	4 (21.1%)	
	1	3 (100.0%)	0 (00.0%)	
ER status	Positive	14 (93.3%)	1 (6.7%)	0.165
	Negative	12 (75.0%)	4 (25.0%)	
PR status	Positive	12 (85.7%)	2 (14.3%)	0.8
	Negative	14 (82.4%)	3 (17.6%)	
HER2 status	Positive	11 (78.6%)	3 (21.4%)	0.467
	Negative	15 (88.2%)	2 (11.8%)	
Ki67 status	Positive	4 (80.0%)	1 (20.0%)	0.797
	Negative	22 (84.6%)	4 (15.4%)	
Taxane	With	9 (90.0%)	1 (10.0%)	0.522
	Without	17 (81.0%)	4 (19.0%)	

ER, estrogen receptor; PR, progesterone receptor, HER2, human epidermal growth factor receptor 2; Univariate analysis was performed using the chi-square test with continuity correlation;* p-value less than 0.05 is considered statistically significant

Table 2. Clinicopathological Characteristics after Neoadjuvant Chemotherapy Administration

Variables	Category	Cyclin-D1 Expression		p-value
		High (%)	Low (%)	
Age	≥50 y.o.	14 (87.5%)	2 (12.5%)	0.57
	<50 y.o.	12 (80.0%)	3 (20.0%)	
Tumor grade	3	10 (90.9%)	1 (9.1%)	0.732
	2	12 (80.0%)	3 (20.0%)	
	1	4 (80.0%)	1 (20.0%)	
ER status	Positive	14 (93.3%)	1 (6.7%)	0.165
	Negative	12 (75.0%)	4 (25.0%)	
PR status	Positive	12 (85.7%)	2 (14.3%)	0.8
	Negative	14 (82.4%)	3 (17.6%)	
HER2 status	Positive	11 (78.6%)	3 (21.4%)	0.467
	Negative	15 (88.2%)	2 (11.8%)	
Ki67 status	Positive	4 (80.0%)	1 (20.0%)	0.797
	Negative	22 (84.6%)	4 (15.4%)	
ALNM	Yes	12 (80.0%)	3 (20.0%)	0.57
	No	14 (87.5%)	2 (12.5%)	
LVI	Yes	12 (75.0%)	4 (25.0%)	0.165
	No	14 (93.3%)	1 (6.7%)	
Taxane	With	9 (90.0%)	1 (10.0%)	0.522
	Without	17 (81.0%)	4 (19.0%)	

ALNM, Axillary lymph node metastasis; ER, estrogen receptor; HER2, human epidermal growth factor receptor 2; LVI, Lymphovascular invasion PR, progesterone receptor; Univariate analysis was performed using the chi-square test with continuity correlation; * p-value less than 0.05 is considered statistically significant

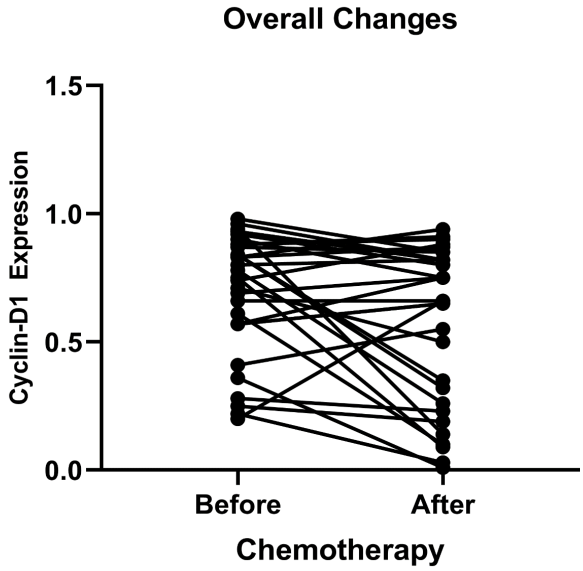

Figure 2. Individual before-after Line Showing Overall Changes in Cyclin-D1 Expression before and after Administration of Neoadjuvant Chemotherapy

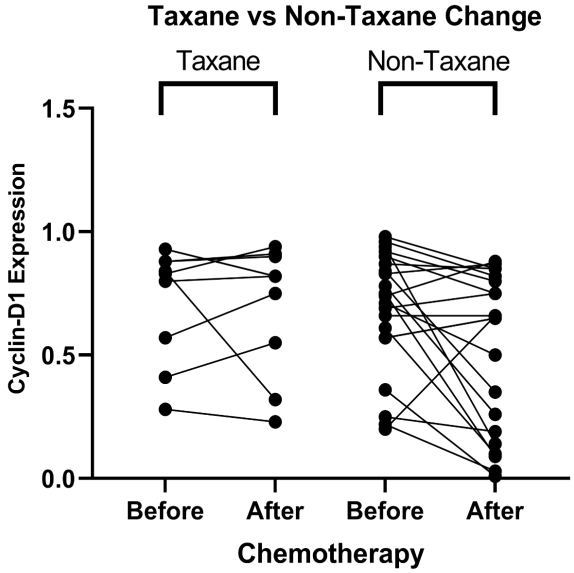

Figure 3. Individual before-after Line Showing Changes in Cyclin-D1 Expression before and after Administration of Neoadjuvant Chemotherapy in Taxane-based vs. non-Taxane-based Group

Table 3. Overall Changes in Cyclin-D1 Expression before and after Administration of Neoadjuvant

Category	n	Cyclin-D1 Expression	P value
Before	31	0.700 ± 0.238	0.086
After	31	0.578 ± 0.308	

Statistical analysis was performed using the generalized linear model

Pre- and post-NC groups and taxane and non-taxane groups showed differences in cyclin D1 expression. This research found that the non-taxane group experienced a greater shift in cyclin D1 than the taxane group. Although these findings are not statistically significant, they suggest a possibly more prominent decreasing tendency in the

Table 4. Changes in Cyclin-D1 Expression before and after Administration of Neoadjuvant in Taxane vs non-Taxane Group

Group	Category	n	Cyclin-D1 Expression	P value	Cyclin-D1 Expression Mean Difference	P value
Taxane	Before	10	0.735 ± 0.231	0.792	0.17 ± 0.29	0.2
	After	10	0.706 ± 0.253			
Non-Taxane	Before	21	0.683 ± 0.245	0.065	0.03 ± 0.19	
	After	21	0.517 ± 0.318			

Statistical analysis was performed using the generalized linear model

group using non-taxane-based NC. Some research may account for this discovery. Overexpression of cyclin D1 was also linked to IBC in patients who underwent surgery followed by anthracycline-based treatment, as was previously reported by Reis-Filho et al., (2006). In a separate investigation, cyclin-dependent kinase was linked with a more accurate prognostic model and lower pathological complete response rates (Wachter et al., 2013). This observation may be because anthracyclines (such as epirubicin and pirarubicin) interact with topoisomerase II and inhibit DNA transcription (Fisher et al., 1997; Li et al., 2011). In the context of neoadjuvant treatment, combining these two drugs is one of the most often reported chemotherapy regimens (Li et al., 2011; Tiezzi et al., 2007).

Even though participants were recruited from an IBC referral center, the study's sample size was insufficient. It's possible that this is the case because NC has not been extensively adopted in the area where the research is taking place. Consequently, the expression of cyclin D1 was very minimally changed. Despite the need to consider clinical and laboratory importance, cyclin D1 expression is decreasing across all categories. As part of this initiative, more research on cyclin D1's role in IBC and NC will need to be done.

In conclusion, in IBC, cyclin D1 expression may be a prognostic indicator of NC response and clinical outcome, despite the fact that the differences are not statistically significant, as determined by an analysis of clinical and biochemical data. The involvement of cyclin D1 in NC warrants more research with bigger IBC sample sizes.

Author Contribution Statement

Conceptualisation, P.R., E.W., I.A.; methodology, P.R., E.W.; software, E.W.; validation, P.R., I.A.; formal analysis, P.R., E.W.; investigation, E.W., I.A.; resources, P.R.; data curation, P.R., E.W.; writing—original draft preparation, P.R., E.W.; writing—review and editing, all authors; visualization, E.W.; supervision, P.R.; project administration, E.W.; funding acquisition, P.R. The published version of the work has been reviewed and approved by all authors.

Acknowledgements

None.

Ethics approval and consent to participate

In November 2021, the Universitas Indonesia Faculty of Medicine Ethics Committee authorized the report procedures, with protocol number 21-11-1252. All participants engaged in the research provided informed permission for participation.

Consent for publication

All participants engaged in the research provided informed permission for publishing.

Availability of data and materials

This published paper includes all data produced or analyzed during the research.

Competing interests

The authors state that they have no conflicts of interest.

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