HORMONAL STATUS (ESTROGEN RECEPTORS - PROGESTERONE) AND HER-2 Neu IN VARIOUS CYCLIN D1 EXPRESSIONS IN BREAST CANCER

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ABSTRACT

Breast cancer is the most common malignancy and leading death cause of malignancy in women in the world. Expression of estrogen receptors (ER) and progesterone receptors (PR) has long been used to determine endocrine therapy patients. Family of human epidermal growth factor receptor, namely (HER-2/neu) has been used as markers to predict prognosis and therapy response. But many ductal breast carcinoma patients showed expression of ER, PR, and HER-2 neu negative (triple negative tumors). There should be further research for patients with triple negative tumors, to find prognostic markers and therapies. The purposed of this study was to analyze the relationship between hormonal status and HER-2 expression with cyclin D1expression in breast ductal carcinoma. This study was an observational analytic study, conducted at the Department of Pathology Faculty of Medicine Airlangga University/Dr. Soetomo. Paraffin blocks of 35 patients with invasive ductal breast carcinoma, which has not been treated with anti-cancer drug, in a variety hormonal status and HER-2 neu expression, followed by immunohistochemical examination. The results obtained, 31.4% patients in the age group 50 to 59 years. Cyclin D1 positive expression in 54.3% samples, and positive in 87.5% samples with ER positive and negative in 55.6% samples with negative ER. In positive PR sample, cyclin D1 positive in 72.7% samples, and cyclin D1 negative in 54.2% samples with negative PR.. Also showed that cyclin D1 positive in 85.7% samples with HER-2 positive and negative in 66.7% samples with HER-2 negative. In conclusion, there is a positive correlation between expression of ER and HER-2 neu by cyclin D1 expression.

Keywords: *breast cancer, ER, PR, HER-2 neu, cyclin D1*

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INTRODUCTION

Breast cancer is the highest malignancy and a major cause of cancer deaths in women in the world, and found more than 1,000,000 cases each year (Rosai 2004, Susilo 2008). WHO report shows that 8-9% of women will develop breast cancer. Invasive ductal carcinoma is breast cancer and most heterogeneous group who showed no specific histological characteristics. Prognostic factors are used up to now still not provide enough information to give accurate risk assessment and therapy, so it is still needed additional prognostic factors and therapy (Stendahl et al. 2004).

Hormonal status of estrogen receptors (ER) and progesterone receptors (PR) has been used to determine the suitability of endocrine therapy. Family of human epidermal growth factor receptor that is (HER-2/neu) has been used on a routine examination that serves as a marker to predict prognosis and response to therapy (Payne et al. 2008). Most patients with breast cancer have ER expression, PR, and HER-2 neu negative (triple negative tumors), so the effort required to find prognostic markers and new therapies.

Role of HER-2/neu protein and estrogen receptors (ER) has been known, which is associated with cell proliferation. Cyclin D1 is a major target protein of the role of HER-2/neu and ER. Cyclin D1 is a proto-oncogene as an important regulator in the G1 phase into S phase in cell cycle (Alao 2007, Susilo 2010). Cyclin D1 binds to CDK 4 and CDK 6, and then became an active complex and lead to phosphorylation and inactivation of retinoblastoma protein (Rb). These conditions trigger the G1 to S phase progression in cell cycle (Alao 2007). Expression of cyclin D1 increased, associated with the progression of cell proliferation (Holley et al. 2001).

The purpose of this study was to explore the relationship between hormonal status and HER-2/neu expression of cyclin D1 with invasive ductal breast carcinomas using immunohistochemical methods to ER, PR, HER-2, and cyclin D1. From this study, expected to

be a new marker for prognosis or therapy of invasive ductal breast carcinoma.

MATERIALS AND METHODS

The method is analytical observational research with cross-sectional sample used by as many as 35 of all breast cancer patients by immunohistochemical examination using antibodies to ER, PR, HER-2/neu in the Department/SMF/Installation Pathology Faculty of Medicine Airlangga University/Hospital. Dr. Soetomo, the period 1 January to 31 December 2009. Inclusion criteria were: microscopic according to the criteria of invasive ductal breast carcinoma, can be evaluated clearly, not a lot of necrosis and hemorrhage, and patients have not received chemotherapy or radiotherapy.

The samples are simple random sampling, which was followed by immunohistochemical examination using antibodies against cyclin D1. Next step is the degree of histopathological reassessment of the sample based on the classification of 'Nottingham Modification of Bloom-Richardson System. Paraffin block samples cut 4 microns thick, and then made deparafinisasi with xylol and alcohol. Immunohistochemical examination with standard streptavidin-biotin technique using monoclonal antibodies to ER (Dako) dilution 1/100, PR monoclonal antibody (Dako) dilution 1/100, HER-2/neu polyclonal antibody (Dako) dilution 1/500, and monoclonal antibody cyclin D1 (Dako) in dilution 1/100. Expression of ER, PR, and cyclin D1 is considered as the color brown in the nucleus, whereas HER-2/neu on the cell membrane. ER and PR expression was calculated with 400x magnification, the cut off point of 10%.

Expression of cyclin D1 is calculated with 400x magnification, the cut off point of 25%. While the expression of HER-2/neu is calculated with 400x magnification, using the scoring: 0 (negative): no Stained cells, or membrane tercat vague <30% tumor cells; +1 (negative): Stained membrane slightly/vaguely in > 30% tumor cells, the cell membrane only partially tercat; +2 (weak positive): Stained membrane complete with weak to moderate intensity in> 30% tumor cells; +3 (positive): Stained cell membrane complete with strong intensity at 30% tumor cells. In this study, HER-2/neu positive 2 (weakly positive) were considered as HER-2 negative. The data obtained are then tested statistically using non-parametric chi square statistic.

RESULTS

Data obtained from the Department of/SMF/Installation Pathology Faculty of Medicine Airlangga University/Dr. Soetomo period January 1 - December 31, 2009 obtained by examination of 176 samples immunohistokimia ER, PR, and HER-2/neu in breast malignancy specimens, two of them with a diagnosis of malignant phyllodes. Of 174 breast cancer cases, which fulfilled the inclusion criteria 55 cases living. Calculation by the formula produces the minimum sample size of 35. Surveillance data indicate that the age range of cases of invasive ductal carcinoma between 30-87 years, with a mean of 51 years, the largest age group 50-59 years, as many as 11 patients (31.4%) (Table 1).

ER positive expression in 54.3% samples, while cyclin D1 positive in 7 out of 8 samples with ER positive (87.5%) and negative in 15 of 27 samples with negative ER (55.6%). Statistical test using the Chi Square test, a score x2 = 4.61, phi coefficient = 0.363, and p = 0.032 (p <0.05) which means obtained a significant correlation between expression of cyclin D1 with ER.

Table 1. Characteristics of Research Subjects

Characteristics	Σ	%
Age (years)		
30-39	7	20
40-49	10	28.6
50-59	11	31.4
60-69	6	17.2
70-79	0	0.0
80-89	1	2.8
Cyclin D1 Expression		
Positive	19	54.3
Negative	16	45.7

Expression of positive PR in 31.4% of invasive ductal breast carcinoma samples. Cyclin D1 positive in 8 of 11 samples with positive PR, and negative in 13 of 24 samples with negative PR. Looks like there is a correlation between the expression of both, but obtained Chi Square value of x2 = 2.198, phi coefficient = 0.251, and p = 0.138 (p> 0.05) which means that the relationship between cyclin D1 and PR was not statistically significant. HER-2/neu positive in 40% of the sample, and cyclin D1 positive in 12 of 14 (85.7%) samples with HER-2/neu positive and negative in 14 of 21 (66.7%) samples with HER- 2 negative.

The relationship was analyzed using Chi Square test and obtained the value of x2 = 9.287, phi coefficient = 0.515, and p = 0.002 (p <0.05), so it can be concluded that there was a significant correlation between cyclin D1 expression and expression of HER-2/neu.

Table 2. The relationship between Hormonal status (ER, PR) and HER-2 with the expression of cyclin D1 Breast Invasive ductal carcinoma

	Cyclin D1		Total	*p*
	+	–	(%)	
ER				0.032
+	7 (87.5)	1 (12.5)	8 (22.9)	
–	12 (44.4)	15 (55.6)	27 (77.1)	
PR				0.138
+	8 (72.7)	3 (27.3)	11 (31.4)	
-	11 (45.8)	13 (54.2)	24 (68.6)	
HER-2				0.002
	12 (85.7)	2 (14.3)	14 (40)	
+	7 (33.3)	14 (66.7)	21 (60)	
-				
Total	19 (54.3)	16 (45.7)	17 (100)	

DISCUSSION

Cyclin D1 is a protein located on chromosome 11q13, which plays a role in controlling the cell cycle at G1 phase. Cyclin D1 gene (CCND1), which encodes cyclin D1 protein that will be expressed in response to mitogenic signals promoting transition is limited to the G1 phase. Expression of cyclin D1 increased, associated with an increase in cell proliferation (Holley et al. 2001). In previous research has been submitted that cyclin D1 is a major target protein of the role of HER-2/neu and the main mediator of proliferative effects of estrogen (Lee et al. 2007). The results of this study showed that 54.3% of samples, which showed overexpression of cyclin D1. The results are consistent with some previous research which states that in invasive breast carcinoma, overexpression of this protein has 50-74% of cases (Rudas et al. 2008, Wang et al. 2005, Park et al. 2001).

After statistical analysis found that there was a significant correlation between hormonal status (ER) with the expression of cyclin D1 in breast invasive ductal carcinoma (p <0.05). The results in accordance with several previous studies stating that there is a strong relationship between ER expression with cyclin D1 (Park et al. 2001, Hwang et al. 2003). Cyclin D1 is the main mediator of the proliferative effects of estrogen. While the ER as an important mitogen activator in breast cancer through cyclin D1 (Lee et al. 2007). There was a mention that besides a role in cell cycle, cyclin D1 can directly bind and activate ER without estrogen stimulation (Lee et al. 2007, Rudas et al. 2008). Co-expression of both show a good prognosis breast cancer, with overall survival and disease free survival was longer (Wang et al. 2005, Hwang et al. 2003).

From this study does not gotten a significant correlation between hormonal status (PR) with cyclin D1 expression (p> 0.05). Not much research has been conducted to reveal the relationship with cyclin D1 PR. While some existing research shows that there is no correlation between them (Wang et al. 2005, Bilalovic et al. 2005). The linkage between HER-2/neu with cyclin D1 has been much studied previously. HER-2/neu protein activates RAS oncogene point further affect the cell cycle through cyclin D1 (Chodosh 2002, Susilo 2008). The results of this study indicate that the obtained significant correlation between HER-2/neu expression with cyclin D1 in breast carcinoma (p <0.05). Report Ahnstrom et al. indicate that over-expression of HER-2/neu and cyclin D1 in breast cancer patients associated with high recurrence and poorer prognosis. But not the same as the results of research Lee et al. which states that patients with HER-2/neu positive and cyclin D1 overexpression have better endurance than the expression of cyclin D1 is low (Lee et al. 2007).

CONCLUSION

It was found that there was a significant correlation between expression of ER and HER-2 with cyclin D1 in breast carcinoma showing that cyclin D1 plays a role in carcinogenesis via both routes.

ACKNOWLEDGMENTS

The author would like to thank Prof. Dr. H. Sarmanu, drh., MS., On his willingness as a statistical consultant in this manuscript.

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