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Relation Expression of Eestrogen Reseptor and Ki67 in Invasive Ductal Carcinoma Breast Cancer

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Abstract

Estrogen are potent mitogenic stimuli that share important properties in the control of cellular proliferation. Increased expression and/or activity of estrogen reseptor (ER) result in aberrant activation of downstream signaling pathways including cAMP/PKA, MAPK/ERK and PI3K/AKT. The importance of estrogen and as a signaling axis in cancer development, progression and metastasis is highlighted by its effects on cancer cells, notably proliferation. But, all mechanism of this proliferation action ER and Ki67 not complete clear yet. The study aims to explore the coupling between the signaling cascades of estrogen receptors to celular proliferation (Ki67). This research used an exploration analitik with cross sectional design, 59 specimen pathologi were explore with Immunohistochemia (IHC) examine to asses expression of Estrogen reseptor and Ki67 in invasif ductal breast cancer. To asses corelation beetwen ER and Ki67 with Chi-Square Test. Result found that the IHC examation show that propotion ER. : 35/ 55 (63 %), Ki67 :32/55 (58 %) in invasive ductal breast cancer. Correlated beetwen ER and Ki67 expression no significant p>0.05 in invasif ductal breast cancer. In conclution, ER does not play a significant role in celluler proliferation in Invasive breast cancer.

Keywords: Expression ER and Ki67; Carcinoma breast cancer.

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1. Introduction

The number of new cases in the USA in 2013 an estimated 232 340 newly diagnosed invasive breast cancer [1, 2]. The incidence of breast cancer in Asia each year 3-4% [3]. Obtained 20-30% of breast cancer have metastatic or locally advanced disease, and another 30% have recurrent or metastatic [4]. The role of estrogen in breast cancer carcinogenesis focused on the production of estrogen in breast tissue [5]. The high plasma estradiol levels significantly associated with breast cancer risk in postmenopausal especially tumor ER / PR +, while ER + / PR- intermidiet, ER- / PR- estradiol no significant effect [6].

Tumor cell proliferation due to the involvement of estrogen / ER in mammary gland development and breast cancer is focused on the action of estrogen on the control of cell cycle G1 phase development / S. Activation of cyclin D1 gene expression is an essential feature of these hormones work [7]. Cyclin D1 is a key regulator of cell cycle proteins that exhibit oncogenic activity in a variety of malignancies. Cyclin D1 mRNA and protein were expressed in about 50% of primary breast carcinoma [8]. The existence of fast track / nongenomic estradiol-regulated protein kinase C (PKC) and the path of signal transduction extracellular signal-regulated kinase (ERK), and cross-talk, and who played in the synthesis of DNA and gene transcription of cyclin D1 [7], as a result of the activation of ER changes in transcriptional activity and gene expression profile of the target. A number of genes, including cathepsin D, cyclin D1, c-Myc and regulated by the progesterone receptor ER-alpha [9]. Tumor cell proliferation due to activation of estrogen / ER of the cell cycle, can be identified by the core antigen Ki67. Ki-67 is a core protein associated with cellular proliferation, Ki-67 is a nuclear antigen identified in 1983 where the Ki-67 appears in the nucleus of cells in all phases of the cell cycle when mitosis S, G1, G2, and M, except on the GO [10].

2. Materials and Methods

2.1 Research Design

This study is a cross-sectional study Ekxplorasi with analytic methods.

1. Laboratory procedure

a. The biopsy section of breast cancer patient were processed into formalin-fixed paraffin-embedded (FFPE) block, then Examined with immunohistochemistry stain of Estrogen Receptor (ER) and Ki67 as a target sample. The answer sheet analyze to take the datas. The FFPE blocks were selected to the make sure the blocks were standarized and the tumor was representative. Every block was cutted by microtome with 4 mm in size and placed in glass object. The immunohistochemistry stain using standard technique. From every block, one slide was stained with haematoxyln-eosin for histopathology, one slide for ER and one slide for Ki67. The stained slides were evaluated microscopically to Determine cancer types, and the immunohistochemistry expression of ER and Ki67.

b. Immunohistochemistry stain methods: using avidin-biotin-peroxidase complex

methods. Before the staining process, every slides must deparaffinized with xylene (twice for 10 minutes each), rehydrated through graded alcohol (5 minutes with alcohol 100%, 5 minutes with alcohol 95%, and 1 minutes with alcohol 70%), and washed in phosphate-buffered solution (PBS) liquid for 5 minutes. The antigen retrieval procedure then applied. Place the slides on the rack and rinsed with citrate buffer (10 mM, pH 6.0) using a microwave in a high power level for 5 minutes and a low power level in 5 minutes. After chilled and washed with PBS, block the non-specific binding site activity using Normal Horse Serum for 20 minutes, then incubate overnight using primary mouse monoclonal antibody for ER (DACO), Ki67 (Daco). After washed with PBS, the section incubated with a secondary immunoglobuline antibody / Biotynylated BIOCARE for 30 minutes, washed with PBS, and reincubated with Streptavidine (BIOCARE) for 60 minutes, incubated with chromogen Diaminobenzidine (DAB) in Tris-HCl pH 7.6 for 10 minutes , and then counterstained with weak Lili-Mayer's hematoxylin, dehydrated with graded alcohol, and cleared using xylol. Lastly, the slides covered with mounting medium and de glass. Immunohistochemistry slides were evaluated microscopically by two pathologist and the researcher to the make accurate conclusion.

C. Immunohistochemistry results intrepretation

ER, Ki67 expression are proteins accumulation in the cell membrane and cell cytoplasm, detected using immunohistochemistry methods roomates Expressed using light microscope will positively stained brown in color on antigen location. Reviews these expression were counted using a scoring system based on color intensity and proportion of colored epithelial cells

2.2 Data Processing and Analysis

The collected data are grouped by destination and type of data is then determined appropriate statistical methods, namely:

1. The bivariate analysis used X2 test for unpaired samples for which data are nominal dichotomous scale. The test is to assess the relationship between the nominal scale variables unpaired two groups. In this case comparing the expression of ER and Ki67 expression invasive ductal breast carcinoma.

3. Results

3.1 Characteristics of the sample

ER Immunohistochemical examination results showed 35 samples (63.6%) expressed positive and 20 samples (36.4%) expressed a negative. While the results of Ki67 obtained 32 samples (58.2%) expressed positive, and 23 samples 41.8% expressed a negative.

3.2 Analysis of the relationship between the Expression of ER with Ki67 expression

Table 1: Characteristics of the study sample (n = 55) invasive ductal breast cancer

Characteristics	**Explanation**	n	**%**
Age	<55 years	35	63.6
	≥55 years	20	36,4
Histopalogi degree	Good Diferensiasi	8	14,5
	Middle Diferensiasi	31	56.4
	Bad Diferensiasi	16	29,1
ER Expression	Positive	35	63.6
	Negative	20	36.4
Ki67 Expression	Positive	32	58.2
	Negative	23	41,8

Expalnation n= number; ER= Estrogen Reseptor ; *Reseptor*, Ki67= anti*gen cancer Ki67*

Table 2: Analysis of the relationship between the expression of ER with the expression of Ki67

ER expression	Ki67 expression		Number	x^2 (*p value*)
	Positive (%)	Negative (%)		
Positive	20(57,1)	15(42,9)	35(100)	0, 043
Negative	12(60)	8(40)	20(100)	(0,836)
Total	32(58,2)	23(41,8)	55 (100)	

Table 2 shows the positive expression of ER in breast invasive ductal carcinoma as many as 35 samples (63.6%). While expression of Ki67 positive in 32 samples in invasive ductal carcinoma of the breast. From the analysis using Chi-Square test, the value p = 0836 which means there is no correlation between the expression of ER with significant Ki67 in invasive ductal carcinoma of the breast. However, when viewed from the distribution of proportions, expression of ER positive for Ki67 expression was found that invasive ductal breast carcinoma expressing the positive ER tends expressing Ki67 positive (57.1%) is greater than the expression of Ki67 negative (42.9%). On the other side of invasive ductal breast carcinoma expression of ER negative, obtained expression of Ki67 positive proportion of 60%.

4. Discussion

ER against Ki67 in invasive ductal breast carcinoma

This study shows that the expression of ER positive invasive ductal carcinoma of the breast 35 (63.6%) of 55 samples of invasive ductal carcinoma of the breast. Other studies such as research Shapochka and his colleagues reported the expression of ER + breast cancer 76%, research Mylonas and his colleagues ER expression 45.9% [11]. Research Nishimura and his colleagues showed 63% positive ER expression in primary tumors [12]. The

high plasma estradiol levels significantly associated with breast cancer risk in postmenopausal especially tumor ER / PR +, while ER + / PR- intermediate, and ER + / PR- estradiol no significant effect [6]. Overexpression of ER alpha and ER beta is very significantly higher in women with prior diagnosis of invasive ER + breast cancer [13]. Research estradiol levels in plasma estradiol level in the multiethnic showed no strong relationship to the risk of breast cancer, but the risk is different in ethnicity [14]. The high level of estradiol is associated with increased risk of ER-positive breast cancer [13]. The high serum estradiol levels in postmenopausal also influence the prognosis of ER- breast cancer preoperatively especially, run the risk of metastasis than ER + (fifteenth). This research obtains Ki67 expression was positive 32 (58.2%) of 55 samples of invasive ductal carcinoma of the breast. Other studies such as research shapochka and his colleagues Reported a 47% Ki67 expression [16] while research Mylonas and his colleagues Ki67 36% [11]. Research Nishimura and his colleagues showed expression of Ki67 expression + 12.4% in primary tumors [12]. This study found that the proportion of Ki67 expression (+) of 20 (57.1%) of 35 samples in invasive ductal carcinoma of the breast that express ER (+). From the analysis we found no significant relationship between the expression of ER with Ki67 expression p = 0836. From the results of the analysis showed that the expression of Ki67 (+) tergatung not fully Expression of ER (+), but by multiple factors other signaling pathways contributing to the expression of Ki67 (+). While research Pietilainen and his colleagues showed no significant relationship [17,18]. In this study, there is a negative ER expression showed greater expression of Ki67 positive (60.4%) in invasive ductal carcinoma of the breast so that the implications in the clinic presenting a poor prognosis. This study shows that the expression of ER positive invasive ductal carcinoma of the breast 35 (63.6%) and the expression of Ki67 positive 32 (57.1%). Penilitian Shapochka and his colleagues expression of ER + breast cancer 76%, 47% Ki67 expression (16) while research Mylonas and his colleagues expression of ER and Ki67 45.9% 36% (11). Research Nishimura and his colleagues showed positive expression of ER 63%, and the expression of Ki67 + 12.4% in primary tumors [12]. In this study it was found that the percentage of positive Ki67 expression was greater in the number of ER negative (60%) compared to ER positive (57.1%). The analysis showed there was no significant relationship between the expression of ER with Ki67 expression p = 0836. while research Pietilainen and his colleagues showed no significant relationship [17,18]. There is a tendency that showed greater expression of Ki67 positive (60.4%) in breast carcinoma expression of ER negative invasive ductal allegedly caused by cross-talk ER Receptor tyrosine kinases (Estrogen independent) so that the implications of the clinic presented a poor prognosis. Ki67 has been regarded as a prognostic sign of invasive ductal carcinoma of the breast [17-19], and as a factor predictor of response to chemotherapy [20, 21]. Rate Ki67 expression was already a recommendation on breast cancer can be as prognosis, prediction of the patients who received standard therapy, even as biomarkers before, during and after neoadjuvant treatment, especially neoadjuvant endocrine therapy [25]. With ER + breast cancer, can be a biomarker Ki67 not been accepted as standard, but can Ki67 free prognostic factor for disease-free survival. [26]. Likewise, the response can Ki67 as a prognostic factor in patients with ER + breast cancer who had received neoadjuvant tamosifen short term and can be a predictor of recurrence (DFS) [27]. Research Shapochka and his colleagues showed that Ki67 expression based on the subtype of breast cancer is highest in Her-2 type, basal like. [16]. Reported that the decreased expression of ER and Ki67 expression significantly increase the risk of recurrence, while the Expression of ER positive and Her-2 showed no significant change in recurrence rates [12].

5. Conclusion

This study showed that ER does not play a significant role in cellular proliferation in invasive ductal carcinoma of the breast.

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