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Survival Rate of Luminal Subtype Breast Cancers after Neoadjuvant Treatment Based on Variant E-Chaderin and Vimentin Expression

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ABSTRACT

Breast cancer is the most common cancer among women worldwide,with million new cases diagnosed every year.. In Indonesia, there are currently no exactly data on the number of incidence rate of breast cancer. Overall, more than 70% of patients breast cancer were diagnose at advanced stage. who have just come to doctors have shown the metastatic characteristics and 50% of post-chemotherapy treatment patients have experienced chemotherapy resistance and recurrence in primary tumor localization. Neoadjuvant chemotherapy treatment to breast cancer patients required biomolecular indicator as medical consideration for treatment and selection of chemotherapy types to patients. Therefore, the effectivity of neoadjuvant chemotherapy cannot be predicted by biomolecular approachment yet. This research was aimed to analyze the potential expression of vimentin and e-cadherinin order to find the biomolecular predictor of neoadjuvant chemotherapy response in breast cancer stage III. Samples were collected from luminal subtype breast cancer stage IIIB patients in Dr. Saiful Anwar General Hospital of Malang, Indonesia. The expression of vimentin ande-cadherin and was examined and analyzed by immunohistochemically. The data obtained by immunohistochemistry examination and analysis were statistically processed using analysis of variance (ANOVA) generated by SPSS program for Windows. This study obtained that neoadjuvant chemotherapy treatment can decrease vimentin and increased e-cadherin expression. This study concluded that vimentin and e-cadherincan further utilized as the predictor of neoadjuvant chemotherapy response based on anthracycline in the patients of luminal subtype breast cancerstage IIIB.
KEYWORDS: breast cancer,vimentin, e-cadherin, neoadjuvant chemotherapy.

INTRODUCTION

Breast cancer is a mutational disease in women with the mortality rate that increases years to years. It caused by gene mutation and disruption of cell death program which leads to uncontrollable harmful cells development. The difference of biological behaviors between breast cancer cells required different medical treatment interventions. Steroid hormone receptor, such as estrogen receptor (ER), progesterone receptor (PR),andoncogene ErbB-2/reseptor human epidermal growth factor-2 (HER-2) are the important factors for the breast cancer differentiation to select the suitable response towards therapies and prognosis the patients should have [1].

Breast cancer luminal A subtype is indicated with the expression of ER (+), PR (+), Bcl-2 (+), cytokeratin CK8/18 (+),HER2 (-), and low-expression of Ki67,andhigh-expression of GATA3. Breast cancer subtype luminal B is indicated withthe expression ER (+) and/or PR (+),HER-2 (+), high-expression of Ki67 (>13,25%). To treat the breast cancer patients, hormonal therapy is not sensitive enough, because of that, the therapy based on patients biomolecular condition will improve the medical chemotherapy for breast cancer patients[2].

Until now, the selection of breast cancer therapy is still based on the anatomical disease extension. However, the biology molecular mechanism which involves in biological behavior of breast cancer did not catch adequate attention. The standard of breast cancer therapy stage III starts with neoadjuvant systemic therapy, either chemotherapy or hormone therapy, sometimes combined with radiation therapy[3]. There are many combinations of chemotherapy medicine used as neoadjuvant, but anthracycline-based medicine (epirubicin/adriamycin/doxorubicin) is often used as the first line in chemotherapy, such as the combination of *Fluorouracil-Epirubicin-Cyclophosphamide* (FEC). The patient response towards chemotherapy is examined first before further medical treatment, such as surgery or the medical procedures[4]. In this decade, neoadjuvant chemotherapy has been developed and involved in the

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medical treatment of cancer, either downstaging and downsizing of breast cancer. However, there are so many chemoresistance cases in breast cancer patient that decrease the chemotherapy effect to the tumor cells and increase the harmful side effects [5].

It is well known that breast cancer pathomechanism shows an increase in progression due to the activation of *Epithelial Mesenchymal Transition* (EMT) pathway which affects the ability of cancer cells to be more invasive, and exhibits high motility allowing to enter in the circulatory system and, if finding a conducive environment, will shape a secondary cancer progression[6].

EMT itself is basically a series of processes regulated by several transcription factors such as SNAIL, ZEB or Twist causing characteristic changes of epithelial cells into unstable mesenchymal cell characters. Research on the molecular regulation of EMT has been proposed through some tissue culture studies responded by cellular communication pathway between the activator and repressor of EMT, either on the pathway of TGF-β or signaling Wnt. The activation of EMT pathway has several endpoints including a decreased E-cadherin expression and an increased expression of EMT-associated genes such as vimentin [7]. EMT has established and named for its role in cancer metastasis. It turns out to be related to the ability of cancer cells as the EMT activation is able to penetrate the circulation system through the mechanism of diapedesis due to the bond with β1 integrin in which the circulation of cancer cells will express CD44 and TrkB that can help protect cancer cells from phagocytic or anionic mechanism that allows the cancer cells to be home on the secondary area away from the site of the primary tumor and to form a new tumor progression [2].

E-cadherin is the phenotype protein marker of epithelial cells, while vimentin is phenotype protein marker of mesenchymal cells. These proteins can be examined using immunohistochemical approachment by using breast cancer tissue collected from biopsy and surgery from patients in order to observe the expression of e-cadherin and vimentin which highly expected to be related to chemoresistance case. In addition, besides of the increase of vimentin and decrease ofe-cadherin expressionare considered as anindicator of EMT case and chemoresistance cases [7].

Neoadjuvant chemotherapy procedures have no obvious biomolecular indicator as consideration of chemotherapy treatment selection. The effectivity of neoadjuvant chemotherapy still can not predictexactly yet. Therefore, this study was aimed to analyze the expression of vimentin, e-cadherinin order to discover the predictor of biological cells changes as the response towards neoadjuvant chemotherapy to breast cancer stage III patients.

MATERIALS AND METHODS

This research was conducted using experimental research design. Samples were collected from breast cancer patients subtype Luminal Stage IIIB in Dr. Saiful Anwar General Hospital of Malang, Indonesia. Immunohistochemical examination and analysis of vimentin and e-cadherin were performed in the Anatomical Pathology laboratory of Dr. Saiful Anwar General Hospital of Malang, Indonesia, and Faculty of Medicine, Brawijaya University, Malang.

Immunohistochemistry (IHC)

In this research, expression of vimentin and e-cadherin was examined based on immunohistochemical methods with specific monoclonal antibodies. The tissue specimen was fixed with a 10% formalin buffer. The tissue preparation to be observed was first dephorned with xylol for 5 minutes. Then, it was dehydrated with 96% ethanol, 80% ethanol, and 70% ethanol each for 5 min. The tissue was then washed using distilled water. The tissue was washed with PBS (Phosphate Buffer Saline), fixed with 1% paraformaldehyde in PBS, and washed. Then, the tissue was incubated for 1 hour with 8% BSA in PBS and washed twice with PBS 5 min each. The antibody was then diluted in 1% BSA and incubated for 1 hour at ambient temperature, then washed with PBS for 5 minutes 3 times, the step repeated. Then, it is incubated for 1-2 hours at ambient temperature in the dark to prevent the cells from drying out. Cells were washed in the dark using PBS three times for 5 minutes. The cell then observed its expression by a light microscope. Cells expressing vimentin ande-cadherin will be appeared in brown or dark color, while the cells that do not express will appear in purple color under light microscope examination (M=400x).

Data Analysis

The data were analyzedusing a pairedt-test since the vimentin and e-cadherin expression variables were quantitative. The research hypothesis was analyzed by a nonparametric statistical test for both nominal and ordinal scale data and using parametric statistics for interval and ratio data. The analysis was generated by SPSS program for Windows software.

RESULTS AND DISCUSSION

Immunoreactivity results showed a positive result marked by brown membranous staining and circling cancer cells (red arrow) (Fig 1). In the sample group of luminal subtype breast cancer stage IIIB patients who responded to neoadjuvant chemotherapy treatment of 19 people, with a mean value of vimentin 18.68%, and limit between 9.57% - 27.79% (table 1).

Based on the results of statistical analysis, there was a significant difference ($p = 0.000 <$) mean ± SD of vimentin between groups that showed response as neoadjuvant chemotherapy effect with groups with no response to chemotherapy effects. Groups that indicate neoadjuvant chemotherapy effect response have lower vimentin expressions than those indicating a response. This suggests that neoadjuvant chemotherapy treatments may decrease vimentin in patients who may respond to the chemotherapy treatment.

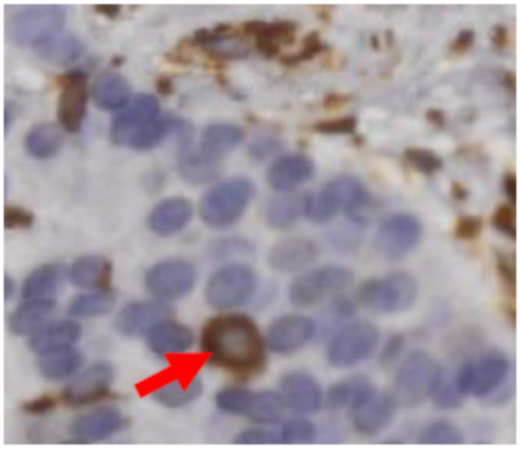

Figure 1. Expression of vimentin in breast cancer tissue. The positive results of immunoreactivity are characterized by brown membranous staining encircling cancer cells (M=400x) (red arrows).

Table 1. Vimentin examination results

Groups	n	Mean (%)	Confidence interval 95%	
			Lower limit (%)	Upper limit(%)
Response	19	18.68	9.57	27.79
No response	16	44.81	35.51	54.11

Figure 2 shows the expression of e-cadherin in breast cancer tissue from luminal subtype breast cancer stage IIIB patients prior to neoadjuvant chemotherapy treatment. Immunoreactivity examination results showed positive results, indicated with brown membranous staining circling cancer cells. Figure 2C shows the negative reaction of membranous staining on the breast cancer tissue of the patient. In figure 2 there is no brown color that circles around the cancer cell.

Patients who responded after neoadjuvant chemotherapy were 19 patients, resulting e-cadherinexpression mean valueof 69.16, and statistical limit between 48.75 - 89.57 (table 2). This indicates that if breast cancer patients can respond well to neoadjuvant chemotherapy treatment 3 times, it will show a higher e-cadherin expression than in those patients who did not respond.

E-cadherin is a protein marker of epithelial cell phenotype, whereas vimentin is a protein marker of mesenchymal cell phenotype, either of these proteins can be observed and measured using an immunohistochemical examination of breast cancer tissue from biopsy or surgery. Therefore, the expression of protein vimentin and e-cadherin is expected to be related to the chemoresistance cases [7]. Supported also by the results of research [8], which states that in addition to an increase of vimentin expression, it will also occure-cadherin decrease. Some pathological conditions can occur in the EMT process, that pathological condition including cellular junctional instability, cytoskeleton actin reorganization, increased motility and cell invasion capability, decreased regulation and relocation

of e-cadherin, decreased regulation causing β-catenin translocation from cell membranes to cell nuclei, removal of molecular markers from mesenchymes, such as vimentin, fibronectin, and e-cadherin[9][10][11].

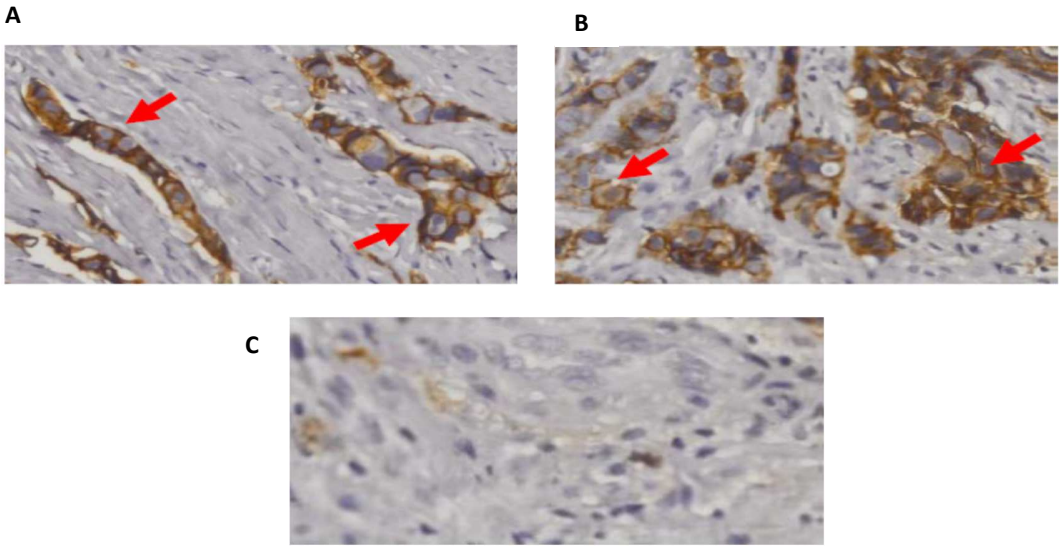

Figure 2. Expression of e-cadherin in breast cancer tissue.

Figures A and B show positive results of immunoreactivity characterized by brown membranous staining encircling cancer cells (red arrows). Figure C foci cancer with negative reaction results membranous staining.

Table 2. Results of the e-cadherin examination

Groups	n	Mean Value	Confidence interval 95%	
			Lower limit (%)	Upper limit (%)
Response	19	69.16	48.75	89.57
No response	16	24.81	14.24	35.38

CONCLUSION

The conclusions from this study were vimentin and e-cadherin can be used as a predictor of anthracycline-based neoadjuvant chemotherapy in luminal subtype breast cancer stage IIIB.

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