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Correlation of CD44 and CD24 expression with the positive response after neoadjuvant chemotherapy in stage IIIB breast cancer patient at Dr. Saiful Anwar Hospital, Malang, Indonesia

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ABSTRACT

Background: Breast cancer is a type of cancer due to abnormal cell growth of breast tissue. CD44 and CD24 is a potential target in breast cancer therapy. This study aims to evaluate the responsiveness of stage 3 breast cancer to chemotherapy by measuring the expression levels of CD44 and CD24 molecules.
Methods: We conducted an observational study with pre-and –post-intervention or test research types. This study was performed on 49 Luminal Stadium IIIB subtype breast cancer patients who received 3 series of neoadjuvant chemotherapy at RSUD dr. Saiful Anwar Malang, immunohistochemical examination, and painting of tissue specimens from the biopsy results. Data were analyzed using SPSS version 20 for Windows.
Results: Based on the Paired t-test and discriminant analysis, there were no significant differences between CD44 and CD24 expression before and after chemotherapy (p=0.501 and p=0.097, respectively). Therefore, the results of the study prompt that the expression of CD44 and CD24 could not be a predictor of the chemotherapy response.
Conclusion: There are different expressions of CD44 and CD44 in primary breast cancer before and after chemotherapy, but no significant difference was found.

Keywords: CD44, CD24, Expression, Breast Cancer.
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INTRODUCTION

Breast cancer is a type of cancer due to abnormal cell growth of breast tissue. The breast tissue comprises the mammary gland, adipose tissue, lactiferous duct, and sinus & lymph nodes. Abnormal cells can grow in any part of the breast and cause slow but inevitable destruction.[1] The incidence of breast cancer has increased consistently and has risen by 50% between 1965 and 1985.[2] In Indonesia, based on the national database Basic Health Research data, breast cancer was the most common cancer in women in 2013, with a prevalence of 17 – 25 cases out of 100.000 population.[3] It was the most admitted cancer cases compared to other cancers (28.7%). Based on the same database, the prevalence of breast cancer reached 0.5 in 1000 Indonesian women.[3]

CD44 is a multifunctional transmembrane Class 1 glycoprotein found in normal cells and highly expressed in cancer cells.[4] CD44 marks cell migration, proliferation, growth, progressivity, and the tumor on the cell membrane.[4] CD44 expression initiates the development of the tumor by binding with receptors such as tyrosine kinase.[4,5] This cell marker has been used to evaluate the effectiveness of drugs targeting progenitor cells.[5] CD44 is also a type of adhesive molecule. Meanwhile, CD24 is a glycosyl-phosphatidyl-inositol protein molecule responsible for the proliferation of tumor cells and an increase of this protein can cause metastasis.[5] Hence, this protein is linked to cancer cell metastasis and chemotherapeutic resistance. CD24 expression is correlated with carcinogenesis and tumor progression.[6] CD24 is said to be a potential target therapy for osteosarcoma, gastric cancer, and breast cancer. So, the expression levels of CD24 can be used as a marker to estimate the sensitivity of these cancer cells.[7]

Based on those mentioned above, the purpose of this study is to evaluate the responsiveness of stage 3 breast cancer to chemotherapy by measuring the expression levels of CD44 and CD24 molecules. Since stage 3 breast cancer is inoperable, it requires neoadjuvant chemotherapy to reduce the size or lymph node metastasis (tumor-free margin). As another reason, this study is conducted on breast cancer with lymph node metastasis where CD44 and CD42 easily can be differentiated so the correlation between chemotherapy and both CD44 and CD24 levels can be measured.

METHODS

This is an observational study with pre and post test observation. The population of this study was all stage IIIB breast cancer patients treated at RSUD dr. Saiful Anwar Malang from December 2019 to April 2020. The data collection process was conducted using total sampling with all patients who were diagnosed with stage IIIB breast cancer subtype luminal (A and B) based on pathology anatomy examination and immunohistochemistry, who were maximum 65 years old with Karnofsky scale >70%, and agreed with consent to undergo biopsy and neoadjuvant chemotherapy combination for 3 series. Patient with the presence of distant metastasis based on a physical and radiologic examination, comorbid that contraindicate to do chemotherapy such as congestive heart failure, history of acute myocardial infarction, chronic liver disease, chronic kidney disease; patient with a history of previous tumor resection and undergo other chemotherapy and administration of hormonal therapy were all excluded in this study. Gender, age, cause, operation, duration of treatment, condition of the patient on discharge were the variables studied.

This study data were the result of biomolecular measurements in the form of expression of CD44 and CD 24 in patients with locally advanced stage IIIB breast cancer Luminal subtype where breast cancer tissue is taken from the biopsy results. The study sample was divided into 2 large groups namely groups with CD24 and CD44 expressions. The CD24 expression group is further divided into 2 small groups namely the group with CD24 expression before neoadjuvant chemotherapy and CD24 expression after neoadjuvant chemotherapy. The CD44 expression group is also further divided into 2 small groups namely the group with CD44 expression before neoadjuvant chemotherapy and CD44 expression after neoadjuvant chemotherapy.

The data were analyzed with the SPSS version 20 for Windows using a Paired t-test and multivariate test for the expression of CD44 and CD24 before and after neoadjuvant chemotherapy.

Table 1. Classification of breast cancer based on molecular subtype

Subtype	Prevalence (%)	Tendency	Prognosis
Basal-like	15.0-20.0	ER/PR/HER2 negatif	Shortest Survival
HER-2+	10.0-15.0	ER(-), PR(-), HER(+)	Shortest Survival
Luminal A	40.0	ER and/or PR(+), HER2(-) low, and Ki67	Longest Survival

Table 2. Average number of CD44 and CD23 expression before and after chemotherapy

Variables	Minimum	Maximum	Mean±SD	Mean Difference	P
CD24					
Before	3.00	20.00	11.53±3.94	1.592	0.097
After	3.00	19.00	9.93±4.56		
CD44					
Before	3.00	20.00	11.87±4.06	0.306	0.501
After	2.00	19.00	11.57±4.05		

RESULTS

According to the breast cancer classification, most of the respondents were in the Luminal A subtype (40.0%), which had the most prolonged survival based on the prognosis, followed by the Basal-like subtype (15.0-20.0%), and HER-2+ subtype (10.0-15.0%) (Table 1).

The following data were from CD44 and CD24 expression in 49 patients with locally advanced stage IIIB breast cancer Luminal subtype (Table 2). Minimum CD44 expression was 2 and maximum CD44 expression was 19 in patients with locally advanced breast cancer IIIB Luminal subtype before chemotherapy. The minimum and maximum CD24 expression were 3 and 20 in patients with locally advanced breast cancer IIIB Luminal subtype after chemotherapy, respectively (Table 2).

The mean cut-off values, from the highest values of CD44 and CD24 before chemotherapy and after chemotherapy, were compared with the lowest values to evaluate whether there were significant differences between before and after chemotherapy. The average data of CD44 and CD24 expressions found that the mean expression of CD44 and CD24 before chemotherapy was higher than the mean expression of CD44 and CD24 after chemotherapy, with the lowest CD44 and CD24 expression on CD24 expression after chemotherapy. Simultaneously, the mean expression of CD44 and CD24 was highest in CD44 expression before chemotherapy. From the data on the average expression of CD44 and CD 24, it was seen that there was a decrease in the mean expression of CD44 and CD 24 before chemotherapy was performed compared with after chemotherapy (Figure 1).

Based on the results in Table 2, it was found that the mean between CD44 and CD24 expressions before chemotherapy with CD44 and CD 24 expressions after chemotherapy was given was 0.306 and 1.592, which showed a tendency to change the expression of CD44 and CD24 with an average of 0.306 and 1.592.

Evaluation of CD44 and CD24 was assessed by pathologists blindly, randomly, and without knowledge of the study and was examined with a 100 times magnification light microscope in 10 visual fields to assess the intensity and quantity of immunostaining. Immunostaining is grouped based on the following staining reactions: (1) shows the intensity of weak cytoplasmic staining, (2) shows the intensity of moderate cytoplasmic staining, (3) shows the intensity of strong cytoplasmic staining, and (4) shows the intensity of very strong cytoplasmic staining. (Figure 2A-2D). Data obtained were qualitatively assessed and calculated by the reaction of strong intensity and very strong only because of the reagent's reactivity that shows the nature of the tumor surface cells. After selection, count the number of 100 times the visual field of the staining results seen microscopically.

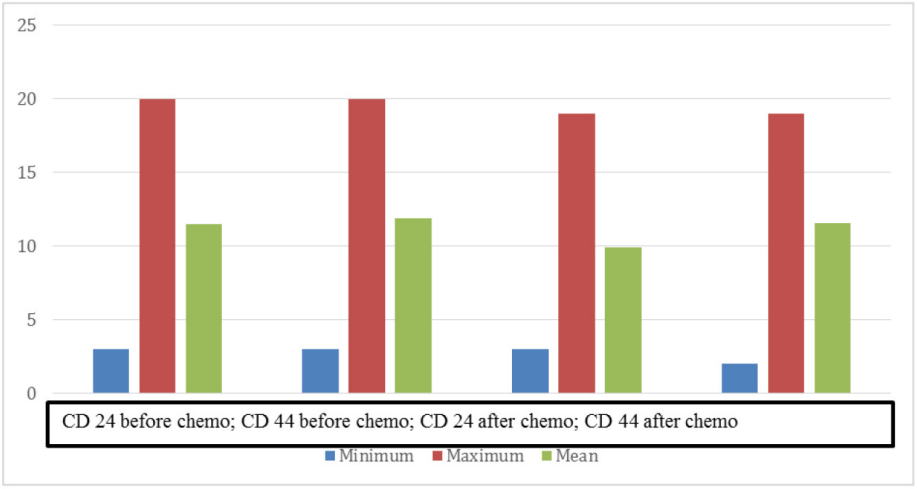

Figure 1. The expression of CD44 and CD24 in graphic

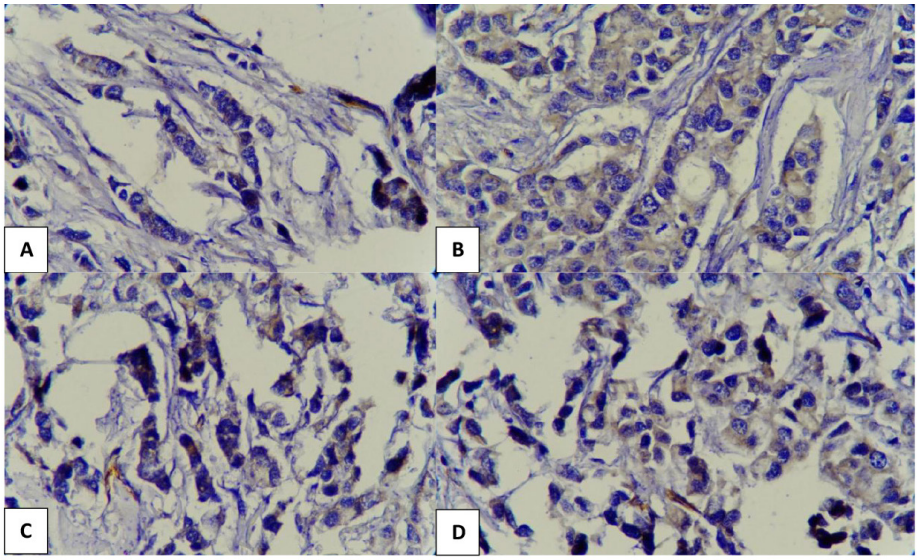

Figure 2. A) CD24 before chemotherapy; B) CD24 after chemotherapy; C) CD44 before chemotherapy; and D) CD44 after chemotherapy

Based on the Pearson correlation test, there was no statistically significant correlation between CD24 expression before and after chemotherapy (r=-0.190; p=0.191) (Table 3). However, there was a significant strong positive correlation between CD44 expression before and after chemotherapy (r=0.697; p=0.000) (Table 3).

DISCUSSION

In this study, from 49 patients with locally advanced stage IIIB luminal subtype, the minimal expression for CD44 and CD24 was 3.00. The maximum CD44 and CD24 expressions were 19.00 and 20.00, respectively. Cancer development starts from a growing cell population that will emerge from the body that can be distinguished as tumorigenic (tumor trigger) cells from nontumorigenic cancer cells based on cell surface markers, including the CD44 and CD24 differentiation clusters.[8,9] Breast cancer is a small population of cells with the classic features of cancer stem cells and becomes tumorigenic cells by accumulating mutations.[10] Early identification of breast cancer stem cells is based on a combination of CD44 and CD24; in particular, the CD44 +/CD24−/low phenotype has stem cell properties.[8,9]

A higher CD44 expression is significantly correlated with smaller tumor size, while axillary lymph node metastasis is at the lower stage.[11] Myoepithelial cells express CD44 in normal breast epithelium and are involved in the early stage of breast carcinogenesis.[12] Increased levels of CD44 expression correlate with its role in the development of breast cancer, poor prognosis, and the involvement of the axillary lymph node metastases in breast cancer.[12]

In this study, patients have been evaluated clinically and parametrically using immunohistochemistry in which the assessment component consists of CD44 and CD24 expression before chemotherapy and after chemotherapy.

The data analysis result using a Paired t-test found correlation coefficient value is positive (r 0.697) for the difference between CD44 expression before and after chemotherapy and -0.190 for the difference between CD24 expression before and after chemotherapy. The result can be interpreted as a strong correlation between CD44 expression before and after chemotherapy, while the correlation for CD24 expression before and after chemotherapy is not strong. From the results above, it was found that the p-value between CD44 and CD24 expression before chemotherapy with after chemotherapy was given was statistically significant, but it showed that there was no significant difference between CD44 and CD24 expression before and after chemotherapy in each group.

CD24 expressions were correlated significantly with HER2-positive status, while CD44 was associated with HER2-negative.[13] According to Horiguchi K et al, CD24 and CD44 did not significantly correlate and were not associated with pathological response rates by neoadjuvant chemotherapy.[11] Thus, further research is needed in terms of chemotherapy sensitivity concerning cancer stem cell markers.

Various studies describe CD44 as a tumor marker that is commonly expressed in various types of cancer. The majority of cancer cell lines express high CD44 levels. Unlike their expression in many different cancer subtypes, ambiguity in the classification and distribution of

Table 3. A Pearson correlation test between CD24 and CD44 expression before and after chemotherapy

Variables	r	P
CD24		
Before	-0.190	0.191
After		
CD44		
Before	0.697	0.000*
After		

CD24 persists. The conclusions from several studies regarding their expression, role in tumor initiation, metastasis, and membrane distribution appear different.[14] Previous studiy has found a significant relationship between tumor size and CD44 or CD24 expression.[11] A positive CD44 level is higher in smaller-sized tumors than in larger-sized tumors.[11]

CONCLUSION

CD44 and CD 24 are transmembrane glycoprotein complex weighing 80-85 kDa, which has a role in physiological and pathological processes, including cell adhesion, inflammation, and tumor development. The character of cells expressing CD44 / CD24 shows slow behavior, which has a high level of resistance to primary chemotherapy and radiation. It was found that the difference between the two expressions was not significant. The prognostic implications of the chemotherapy response of CD24 and CD44 expression in primary breast cancer are still unknown.

ETHICAL CLEARANCE

The Institutional Ethical Committee and Review Board of the General Hospital Saiful Anwar Malang with registration number 400/029/K.3/302/2019. Informed and written consent to voluntarily participate was received from every study participant and a clear explanation of the research was provided to them.

CONFLICT OF INTEREST

The author reports no conflicts of interest in this work.

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AUTHOR CONTRIBUTIONS

Muhammad B. Budianto designed and conceptualized the study, made critical revisions to the draft, and gave final approval to the submitted version. Artono Isharanto collected data, partially contributed in writing. Andry Haris collected data, partially contributed to writing and preparing the manuscript.

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