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# Relations of Human Epidermal Growth Factor Receptor 2 (HER2) Expression and E-Cadherin (CDH1) Expression in Breast Cancer Patients

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**ABSTRACT**

The aim of this study is to analyze the relations of Human Epidermal Growth Factor Receptor 2 (HER2) expression and E-Cadherin (CDH1) expression in breast cancer patients. To date, the synergistic effect of this CDH1/HER2 complex is not well clarified. The design of this study was cross-sectional with a total sample of 56 formalin-fixed paraffin tissue blocks that had been examined for HER2. Furthermore, CDHI expression was examined using the Immunohistochemistry staining technique with the Labeled Streptavidin Biotin Complex (LSAB) method. Bivariate analysis was performed using the Spearman correlation test with abnormally distributed data ($p>0.05$). Of the 56 data on breast cancer patients, most of the patients (87.5%) were diagnosed at the age of $\geqslant$40 years. The majority of cancer staging was IIIB, which was 42.9% of the total 56 patients. The study results shows that 80.0% of HER2-positive patients were in the strong CDH1 group. From these data, there is evidence of correlation between HER2 expression and CDH1 expression in breast cancer patients, however this correlation was not significant ($p>0.05$).

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## INTRODUCTION

Breast cancer is the leading cause of cancer-related deaths in women in both developed and developing countries (WHO, 2018). Every year, there are 2.1 million cases of female breast cancer worldwide. In 2018, an estimated 627,000 women died from breast cancer, which is about 15% of all cancer deaths among women (UICC, 2019).

In 2018, in Indonesia there were 22,692 people who died from breast cancer (UICC, 2019). In the Department of Surgery, M. Djamil Hospital Padang, there were 509 patients between 2008 and 2017 (Harahap & Khambri, 2018). The low survival rates in developing countries are mainly due to the lack of adequate early detection and diagnosis programs and care facilities, so that many patients are found already at the end-stage and have metastasized. In addition, inadequate therapy can also lead to relapse (White et al., 2014).

Human Epidermal Growth Factor Receptor 2 (HER2) is a proto oncogene that functions to stimulate cell proliferation by activating tyrosine kinase. Physiologically, HER2 will stop working if the cells needed are sufficient. However, in cancer cells HER2 amplification occurs. This will activate the Ras/Raf/mitogen-activated protein kinase (MAPK) and phosphoinositide 3-kinase/Akt (PI3K/Akt) pathways (Dey, Leyland-Jones, & De, 2016; Fink & Chipuk, 2013). Activation of these pathways causes proliferation, survival, differentiation, angiogenesis, and invasion of tumor cells (Baker, Zlobin, & Osipo, 2014; Iqbal & Iqbal, 2014; Vu & Claret, 2012).

Elastin-Cadherin/E-Cadherin/CDH1 is expressed in the epithelial tissue of the breast and functions as an adhesive (adhesion) between epithelial cells. E-cadherin binds with β-catenin to form the E-cadherin-β-catenin complex to maintain cell adhesion. E-cadherin is thought to have tumor suppressor properties (tumor suppressor gene) where its absence is associated with carcinogenesis and metastasis. Down regulation of E-cadherin releases free β-catenin which activates the Wnt signaling pathway. At the same time, the reduction in E-cadherin will trigger an epithelial-to-mesenchymal transition (EMT). EMT is a process of changing the epithelium into mesenchyme which plays a role in carcinogenesis and metastasis (Darwin, Elfi, & Elvira, 2017). In addition, this cell adhesion system can be disrupted by the tyrosine kinase c-erbB-2/HER2/neu (Januardi, Pualilin, Kadir, & Prihantono, 2019).

This synergistic effect of the E-cadherin/HER2 complex is not well clarified. In several studies that have been done, it was found that there was no relationship between e-cadherin expression and HER2 (Januardi et al., 2019; Panigoro, Karsono, & Sari, 2017). This conclusion is supported by Ingthorsson *et al.* (2016) who found that HER2 could trigger EMT directly without the involvement of E-cadherin. Thus, this allows E-cadherin expression to remain strong in the event of HER2 overexpression. However, in another study it was found that E-cadherin inactivation led to overexpression of HER2 with a worse impact on cancer prognosis. (Corso, Bonanni, & Veronesi, 2018). Recently, a study in China has formulated a HER2-ATF4-ZEB1-e-cadherin pathway. They proved that there was a real reciprocal relationship between HER2 status and E-cadherin expression. It was stated that an increase in HER2 would result in downregulation of E-cadherin (Zeng, Sun, Li, Xiao, & Chen, 2019).

This study aims to analyze the relationship between HER2 expression and E-cadherin expression in breast cancer patients. While in other previous studies the expression of CDH1 examined only from HER2 positive, this study analyze CDH1 expression from both HER2 positive and negative. The results of this study are expected to increase knowledge about the role of E-cadherin in the diagnosis and prognosis of HER2-Positive breast cancer patients. Furthermore, clinicians may also consider routine E-cadherin screening for breast cancer patients, particularly HER2-Positive, to predict recurrence and patient prognosis.

## MATERIALS AND METHODS

### Study Design

This study is a cross-sectional study, in which the independent and dependent variables are examined at one particular time.

### Population and Samples

The population of this study was the results of breast cancer biopsy/surgery in the 2018-2020 period. The sample is a part of the population that has exclusion and inclusion criteria. The inclusion criteria in this study were: i) breast cancer cases of women who had undergone biopsy/surgery in the 2018-2020 period; ii) have a histopathological examination result and have a medical record at RSI Ibnu Sina Padang or RSB Ropanasuri Padang; iii) the biopsy/surgery results are stored in the form of a paraffin block; and iv) have had the results of HER2 Immunohistochemistry (IHC) tests. The exclusion criteria in this study were: i) incomplete medical record; ii) paraffin block not found; and iii) no IHC staining results were obtained even though they had been cut twice. To determine the sample size (n), this study used the cross sectional research sample formula as follows:

$$n = \frac{Z_{1-\frac{\alpha}{2}}{}^2 P(1-P)}{d^2}$$

where P is the proportion in previous studies, which is 17% (He, Lv, Song, & Zhang, 2019); *Za* = 1,96 and *d* is absolute precision = 0,1.

After entering these numbers into the above formula, the result was 54.02 ≈ 54. Thus, in this study the minimum sample size was 54 tissue paraffin blocks. To anticipate the possibility of dropping out, 70 blocks of paraffin were collected.

### Research Procedure

During the 2018-2020 period there were 332 cases of breast cancer at RSI Ibnu Sina Padang and RSB Ropanasuri Padang. From this number, 70 cases had complete medical records, biopsy/surgery tissue results were stored in the form of paraffin blocks, and had IHC examination results, especially HER2. Furthermore, examination of CDH1 expression (antibody: Santa Cruz, USA) was performed using the IHC staining technique.

The CDH1 IHC staining technique using the Labelled Streptavidin Biotin Complex (LSAB) method was performed using a manual procedure. After the procedure, the preparation is viewed under a binocular light microscope (Olympus CX22 series) to assess CDH1 expression. A total of 14 paraffin blocks did not get stained even though they had been cut twice. Thus, only 56 paraffin blocks remained that could be used as research samples.

### Data Analysis

At the beginning of the study, a data normality test was carried out to determine whether the data was normally distributed or not normally distributed using the Kolmogorov Smirnov test (n>30). If the p value >0.05, the data is normally distributed and continued using parametric analysis and vice versa. The presentation of categorical research variables is presented in the form of a frequency distribution table and narrative.

Bivariate analysis was conducted to see the relationship between variables, based on the purpose of this study, the bivariate analysis carried out was the Pearson correlation test if the data were normally distributed, and the Spearman if the data were not normally distributed. The research data analysis was carried out at the confidence level of 95% CI (α = 0.05), if the results obtained were p value <0.05, there was a significant relationship. Data processing and analysis was carried out with the SPSS 25 program.

### Ethics Statement

This study was conducted after obtaining Research Ethics Approval No. 308/KEP/FK/2020 obtained from the Research Ethics Committee of Medical Faculty, Universitas Andalas. The results of ethical approval are used as the ethical basis for this study.

## RESULTS AND DISCUSSION

The 56 data on breast cancer patients, most of the patients (87.5%) were diagnosed at the age of 40 years or more. There were more cases of breast cancer diagnosed for the first time in old age (≥ 40 years) than at young age. The youngest age when breast cancer was first diagnosed was 28 years old and the oldest at 70 years old with an average patient age of 52.6 years. For the largest stage is IIIB, which is valued at 42.9%. There were 25 positive HER2 (+3) expressions and 38 strong CDH1 (+2 and +3) expressions. The characteristics of the samples examined are shown in Table 1.

**Table 1.** Sample Characteristics

| Characteristics | N | % |
|---|---|---|
| Age at diagnosis | | |
| < 40 years old | 7 | 12.5 |
| ≥ 40 years old | 49 | 87.5 |
| Stage | | |
| I | 0 | 0.0 |
| IIA | 5 | 8.9 |
| IIB | 23 | 41.1 |
| IIIA | 3 | 5.4 |
| IIIB | 24 | 42.9 |
| IV | 1 | 1.8 |
| HER2 | | |
| 0 | 20 | 35.7 |
| +1 | 1 | 1.8 |
| +2 | 10 | 17.9 |
| +3 | 25 | 44.6 |
| CDH1 | | |
| 0 | 10 | 17.9 |
| +1 | 8 | 14.3 |
| +2 | 29 | 51.8 |
| +3 | 9 | 16.1 |

HER2 expression was assessed by a scoring system according to ASCO guidelines (Wolff et al., 2018), namely 0, +1, +2, and +3. Where a score of +3 is categorized as positive; 0 and +1 are categorized as negative; whereas for a score of +2 it is recommended to examine in situ hybridization (ISH). Due to the high cost of ISH screening, very few patients are able to do it. So for therapeutic purposes, a score of +2 is categorized as negative. In this study, scores of 0, +1, and +2 were categorized as negative HER2 and scores of +3 were categorized as positive HER2.

There were 25 patients (44.6%) of positive HER2 expression and 31 patients (55.4%) negative HER2. Figure 1 shows the results of negative (a) and positive (b) HER2 IHC staining. CDH1 expression was classified based on the intensity of staining which consisted of 4 categories 0 (negative), +1 (weak), +2 (moderate), and +3 (strong). For practical and statistical purposes, the researcher categorized the cases as weak (0, +1) and strong (+2, +3) (ElMoneim & Zaghloul, 2011). The number of strong CDH1 expression was 38 patients. Meanwhile, 18 patients had weak CDH1 expression. Figure 2 shows the results of IHC staining on weak (a) and strong (b) CDH1. In this study, it was found that breast cancer patients with positive HER2 expression had the strongest CDH1 group, which was 80.0% (20 patients), as shown in Table 2. From this data there was a tendency that the higher the HER2 score, the stronger the CDH1. However, the statistical test results of this correlation are not very significant, the value of $p = 0.083$.

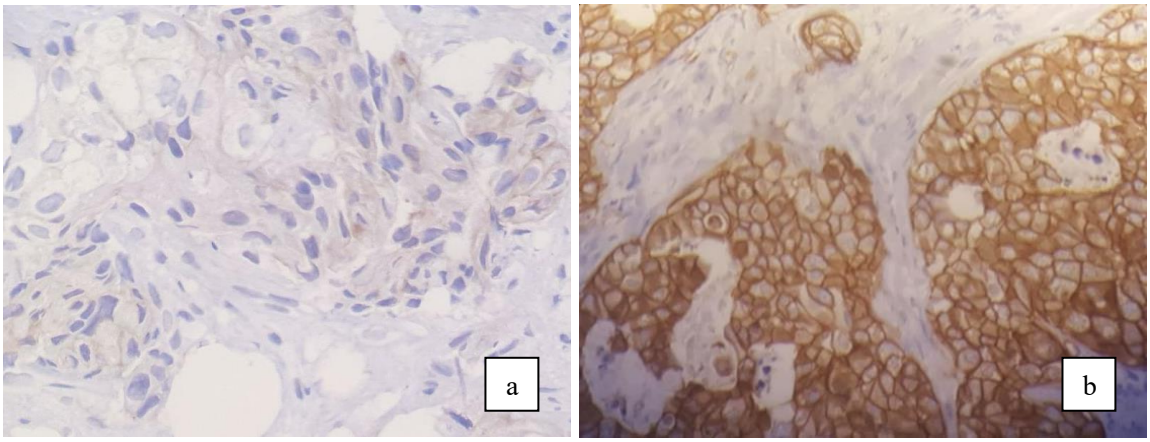


**Figure 1**. IHC staining results (a) HER2 +1/Negative, (b) HER2 +3/Postive

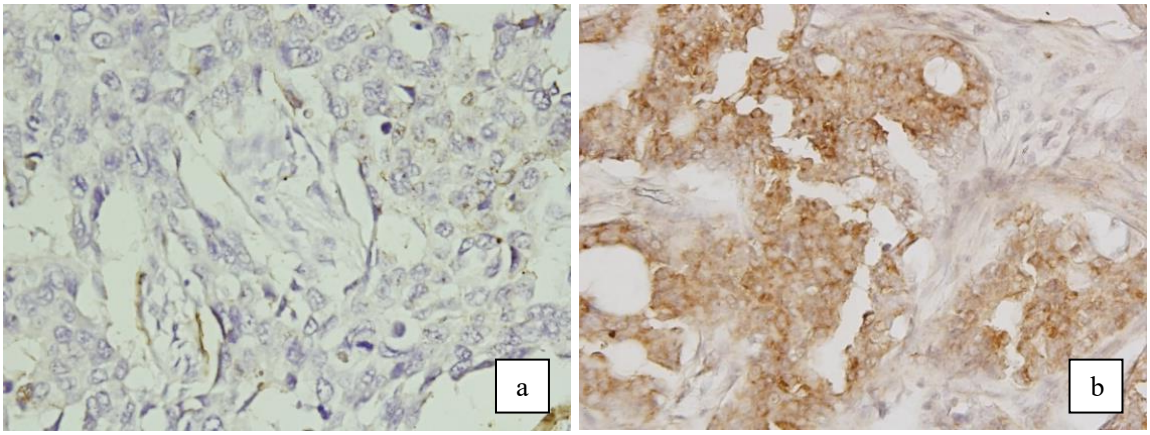


**Figure 2**. IHC staining results (a) CDH1+1/Weak, (b) CDH1 +3/Strong

**Table 2.** Relations of HER2 and CDH1 Expression

| Variable | HER2 | | P value |
|---|---|---|---|
| | Negative (0, +1, +2) | Positive (+3) | |
| **CDH1** | | | |
| **Weak (0, +1)** | 13 (41.9%) | 5 (20.0%) | 0.083 |
| **Strong (+2, +3)** | 18 (58.1%) | 20 (80.0%) | |

Similar results were found by Younis *et al.*, (2007), that positive HER2 expression has a strong CDH1 which is as much as 70.0% of the study sample. However, this study concluded that there was no significant relationship between CDH1 expression and HER2 expression (p = 0.69). The same conclusion is also drawn by Singhai *et al.* (2011) which found 87% of the patient group with positive HER2 expression had strong CDH1. Not much different, Horne *et al.* (2018) found that 744 (86.3%) breast cancer patients had positive HER2 scores with strong CDH1 scores.

Research on the relationship between HER expression and CDH1 expression has not been widely conducted in Indonesia. Until now, there has only been one study conducted by Januardi *et al.*, (2019). They concluded that there was no significant correlation (p = 0.753) between HER2 expression and CDH1 expression in breast cancer patients. It was also found that patients with positive HER2 had more weak CDH1 expression (34.8%) than strong CDH1 expression (30.4%). The same conclusion is reached by Pang *et al.*, (2013) in China, there was no statistical correlation

between HER2 expression and CDH1 expression ($p = 0.92$). In addition, this study also found that the higher the HER2 expression, the weaker the CDH1 expression.

From these reports, it is known that the expression of E-Cadherin is still inconsistent in the field of oncology. Many reports mention that E-Cadherin plays a role in carcinogenesis and metastasis. However, there are also many reports that conclude that there is no role for E-Cadherin in carcinogenesis and metastasis. In theory, it is known that the functional loss of E-cadherin is the most important feature of tumor cell formation and spread through the epithelial-to-mesenchymal transition (EMT) (Li, Yin, Zhang, Liu, & Chen, 2017). The possible role of EMT as a mechanism for carcinogenesis, especially in invasive, metastatic cell formation and drug resistance, has been the subject of intensive study over the past few years and provides tremendous advances in clinicians' understanding of this phenomenon (Kalluri & Weinberg, 2009). The loss of E-cadherin expression is widely seen as one of the important and defining events in the development of EMT. Since the EMT process is characterized by loss of adhesion cells, it is intuitive to assume that regulation of E-cadherin expression is necessary for EMT to occur (Baranwal & Alahari, 2009).

The theory of E-cadherin re-expression at an advanced stage was strengthened by data from this study where the most stage was IIIB. In the literature, it is stated that stage IIIB means the cancer has spread to the chest wall and has metastasis to several nearby nodes/lymph nodes (Hammer, Fanning, & Crowe, 2008). This is also supported by the fact in the field that some of the paraffin blocks examined were the result of biopsy from nodes/lymph nodes of breast cancer patients. So, in this study it can be concluded that there has been re-expression of E-cadherin in HER2-positive patients.

From this explanation, it can be concluded that there is a relationship between HER2 expression and E-cadherin expression in breast cancer patients. However, in this study the relationship was not statistically significant which might be due to several limitations in this study, including (1) the proportion of samples with known HER2 scores and E-cadherin examination was not balanced due to the limited number of samples; (2) the results of biopsy of breast cancer patients. those who were examined for IHC were not uniform, some were primary tumors, the rest were nodes/KGB; and (3) some of the paraffin blocks borrowed from the hospital had immature conditions so that when they were examined the IHC did not give good staining results.

## CONCLUSION

80.0% of HER2 positive patients were in the strong CDH1 group. There was a correlation between HER2 expression and CDH1 expression in breast cancer patients, but this correlation was not significant ($p>0.05$). The existence of this correlation can be a basis for consideration of carrying out a CDH1 examination to help diagnose and predict prognosis in breast cancer patients with positive HER2. Further researches are suggested to use different examination techniques such as FIS and other sampling methods to provide better results.

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## CONFLICT OF INTEREST

We have no conflict of interest related to this work.

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