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Loss of PTEN Expression as a Predictive Factor for Poor Clinical Response of Neoadjuvant Chemotherapy in Triple Negative Breast Cancer

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Abstract: ***Triple-negative breast cancer (TNBC), is a paradoxical breast cancer subtype. Some TNBC have a good response to neoadjuvantchemo therapy however TNCB with remaining tumor mass after neoadjuvant chemotherapy have worse prognosis. One of the causes of chemotherapy resistance on TNBC is the over activation of the PI3K / Akt signaling pathway which caused by loss of phosphatase and tensin homologue deleted on chromosome ten (PTEN) as the negative regulator in this pathway. The aim of this study was to prove that loss of PTEN expression as a predictive factor for poor clinical response of neoadjuvant chemotherapy in TNBC. This study was conducted in retrospective case-control method. The PTEN expressions immunohistochemically evaluated in 23 patients with negative neoadjuvantchemo therapy clinical response as a case group and 23 patients with positive neoadjuvant chemotherapy clinical response as a control group.The results showed that there was significant mean difference of PTEN expression H-score between case group (mean=53.04±37.23) and control group (mean= 139.57±75.23); (MD 86.52±17.50, t=4.94, p=0.000). TNBC patients with loss of PTEN expression had a risk of 6.75 times higher showing a negative neoadjuvant chemotherapy clinical response than TNBC patients with no loss of PTEN expression (χ^2=7.165, OR=6.75, 95%CI 1.82-25.03, p=0.007). The examination of PTEN expression is important to predict the clinical response of neoadjuvant chemotherapy in TNBC.***

Keywords: triple negative breast cancer, clinical response of neadjuvant chemotherapy, PTEN, predictive factor

1. Introduction

Triple-negative breast cancers (TNBC) is a clinicopathological term describing a subtipe of breast cancer neither express hormone receptors, nor overexpress HER2. They are associated with poor prognosis. Previous studies have reported that patients with TNBC who receive neoadjuvant chemotherapy have a higher rate of pathological complete response than patients with other subtypes of breast cancer. At the same time, outcomes are extremely poor in patients who have residual disease after neoadjuvant chemotherapy. Chemotherapy is the primary established systemic treatment for patients with triple-negative breastcancer in both early and advanced-stages of the disease [1].

Previous study by Khokher et al (2011) have demonstrated thatpositive chemotherapy clinical response rate in TNBC is 67.3% while negative chemotherapy clinical response is 31%. This indicates that the positive chemotherapy clinical response rate of TNBC is quite high, but there are still about one in three cases of TNBC resistant to chemotherapy [2].

The susceptibility of cancer cells to chemotherapy-induced apoptosis depends on the balance between pro-apoptotic and anti-apoptotic signals. Blockade or downregulation of pro-apoptotic pathways or upregulation of anti-apoptotic pathways is one of the mechanisms of chemotherapy resistance. The anti-apoptotic pathway involved in the chemotherapy resistance in TNBC is the PI3K / AKT signaling pathway[3].

The PI3K/AKT signaling pathway isimportant for tumorigenesis. This pathway is associated with almost all aspects of tumor biology including cell transformation, growth, proliferation, migration, apoptosis evasion, genomic instability, angiogenesis, metastasis and cancer stem cells maintenance [4],[5]. Over activation in this pathway is caused by loss of PTEN. PTEN is a tumor suppressor gene that antagonizes the PI3K/AKT signaling pathway and suppresses cell survival as well as cell proliferation[6].In breast carcinoma, loss of PTEN expression was found in approximately 50% of cases and most commonly found in the molecular subtype of TNBC in 66% of cases [7].

PTEN might act as a regulator of the PI3K/AKT signaling activity by dephosphorylatingphosphatidylinositol (3,4,5)-trisphosphate(PIP3) to phosphatidylinositol 4,5-diphosphate (PIP2). PTEN keepingbasal levels of PIP3 below threshold for the signaling pathway activation so that cell development can be controlled [5]. Genetic and epigenetic mechanisms include mutation, deletion, silencing transciption or protein instability are thought to be involved in inactivation regulation of PTEN [4],[5].

PTEN inactivation lead to excessive accumulation of PIP3, which responsible in Akt translocation to the cell membrane through bonding with pleckstrin homology (PH) domain. This process followed by conformational change to Akt which lead to Akt phosphorylation by phosphoinositide-dependent kinase 1 (PDK1) and activate the Akt. This Akt activity lead to apoptosis inhibition [4],[5]. This is one of the cause of chemotherapy resistance in TNBC.

The aim of this study is to prove loss of PTEN expression as a predictive factor for poor clinical response of neoadjuvant chemotherapy in TNBC.

2. Material and Methods

2.1 Specimens

Slides and paraffin embedded tissue blocks from 46 patients invasive breast carcinoma TNBC subtype were retrieved from the histopathology archives in Anatomic Pathology Laboratory of Sanglah Hospital and Prima MedikaHospital in Bali from 2015 to 2017. Clinical data were gathered from the medical reports and cancer registries.

2.2 Histopathologic evaluation

The slides from these cases were reviewed andhistopathologic diagnoses in the histopathologic reports were confirmed independently by two pathologists and one resident.

2.3 NeoadjuvantChemotherapy Clinical Response Evaluation

The clinical response to neoadjuvantchemotherapy is measured by thetumor size assessment before and after neoadjuvant chemotherapy using 3 series of polychemotherapy (flourouracil, adriamycin, and cyclophosphamide [FAC]). The neoadjuvantchemotherapy clinical response was assessedby oncologic surgeon according to the World Health Organization (WHO) and Union for International Cancer Control (UICC) criteria divided into clinical complete response (no clinically detectable tumor mass, determined by two assessments at intervals of no less than 4 weeks), clinical partial response (reduced tumor size equal or more than 50% determined by 2 assessments at intervals of no less than 4 weeks, and no new tumor growth), clinically stable disease (a reduction in tumor mass less than 50% or an increase in tumor mass less than 25%), clinical progressive disease (tumor size increased more than 25% or new lesions growth) [1], [9].

In this study, theclinical response of neoadjuvantchemotherapy is differentiated into negative response as case groupandpositive response as control group. Negative response consisting of clinical stable diseaseand clinical progressive disease.Positive response consisting of clinical complete response and clinical partial response[10]. The clinical responses of neoadjuvant chemotherapy were obtained from Sanglah Hospital's medical records, Prima Medika hospital's medical recordsand onkologic surgeon's cancer registration data.

2.4 Immunohistochemistry and interpretation

The tissue section at 4 µm thickness from each cases were prepared for immunostaining. After 30 minutes incubation in a 600^{o}C oven, deparaffinizationand rehydration, the tissue sections were treated with 3% hydrogen peroxide for 10 minutes. This process is followed by incubation of the sections in blocking buffer for 30 minutes in room temperature. The slides then incubated with one of the following primary antibodies PTEN *rabbit anti-human monoclonal (clone SP170)*. The colour was visualized by DAB as chromogen. Immunostaining were interpreted independently by two pathologists and one resident. Immunohistochemistry resultswere evaluated by a semiquantitative approach using Histo-score (H-score). ThePTEN expression was assessed on the nuclear and/or cytoplasmic staining throughout the invasive area. The intensityscoreis givenby 0 (negative), 1(weak), 2(moderate) and 3(strong) (Figure 1). The percentage of cells at each staining intensity level is assigned using the following formula: {[1 × (% cells 1+)] + [2 × (% cells 2+)] + [3 × (% cells 3+)]}. The H-score was obtained from the calculation with a range of 0-300. The samples shownegative (loss) PTEN expression if H-score <90 and positive(no loss) PTEN expression if H-score ≥ 90[12].

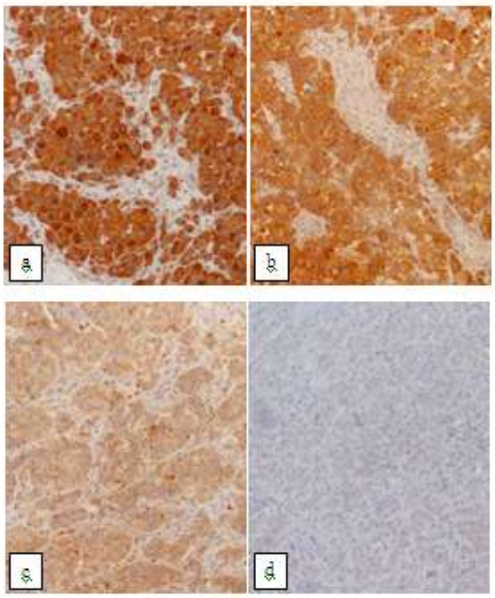

Figure 1: PTEN immunohistochemistrystaining intensity (a) strong (3+), (b) moderate (2+), (c) weak (1+), (d) negative (0) (x100)

2.5 Statistical analysis

The descriptive statistics then calculated and the t-independent test was used to assess mean difference ofPTEN expression H-score between case group and control group. Furthermore, Chi square test and Odds ratio were used to assess the association betweenPTEN expression and neoadjuvant chemotherapy clinical response. Thep-value of<0.05 was considered significant. All statistical analyses were performed using SPSS 20.0.

3. Result

In thisstudy period (2015-2017) there were 46 patients met the study criteria, consisting of 23 patients with positive neoadjuvantchemotherapy clinical response as control group and 23 TNBC patients with negative neoadjuvantchemotherapy clinical response as case group.

The youngest age is 31 years old and the eldestis62 years old. The mean age of the case group was 49.91 ± 7.03 years, with an age rangefrom38 to 62 years. The mean age of the control group was 48.09 ± 7,69 years, with an age rangefrom 31 to 58 years.

All of the histopathology diagnosis was invasive carcinoma of no special type (NST). There is no sample with grade 1 based on grading characteristics. Grade 2 was 9 (19.6%) and grade 3 was 37 (80.4%). The clinical stage showedthatall of samples was advanced breast cancer (Table 1).Based on t-independent testshown in table 2, there was a significant mean difference of PTEN expression H-score between case group and control group (p-value=0.000). In table 3, theChi square analysis result (p-value=0.007), it showed that there is anassociation between PTEN expression andneoadjuvant chemotherapyclinical response. The Odds ratio of6,75 (Table 3) meansthat TNBC patients with loss of PTEN expression had a 6.75 times higher risk of showing poorclinical response of neoadjuvant chemotherapy than TNBC patients with no loss of PTEN expression.

Table 1: Clinicopathological characteristic of samples

Variable	*Neoadjuvant chemotherapy clinical response*		*Total*
	Negative (case) n=23	*Positive (control) n=23*	
Youngest Eldest Mean	38 62 49.91±7.03	31 58 48.09±7.69	-
Invasive carcinoma of NST	23 (100%)	23 (100%)	46 (100%)
1 2 3	0 (0%) 3 (13%) 20 (87%)	0 (0%) 6 (26.1%) 17 (73.9%)	0(0%) 9 (19.6%) 37 (80.4%)
Early Advance	0 (0%) 23 (100%)	0 (0%) 23 (100%)	0 (0%) 46 (100%)

Table 2: Mean difference of PTEN expressionH-score in case group and control group

	Mean of PTEN H-score	*Mean difference*	*95%CI*	*p-value*
Case	53.04±37.23	86.52±17.50	50.88-122.16	0.000
Control	139.57±75.23			

Table 3: The assosiasion between PTENexpression and neoadjuvant chemotherapy clinical response

PTEN	*Chemotherapy clinical response*		*OR*	*95%CI*	*p-value*
	Negative (Case)	*Positive (Control)*			
Negative	18 (69.2%)	8 (30.8%)	6.75	1.82-25.035	0.007
Positive	5 (25%)	15 (75%)			

4. Discussion

Triple negative breast cancer is more common in younger women than Luminal or HER2 enriched subtype [1],[12],[13]. In some studies in Indonesia involving Bali, showed thatthe average age ofwomendiagnosedwithTNBC was in the fourth decadeof theirs live[14],[15]. In accordance with previous studies,this study's average age of the sample was 49.91±7.03 years in the case group and 48.09±7.69 years in the control group. Shapiro-Wilk Normality test showed the age data was normally distributed (p>0.05). The incidence of TNBC in this younger age group is related to the presence of a hereditary etiology involving genes that function in the repair of DNA damage, such as BRCA1 which more common mutation in TNBC [16].

Most of TNBC is invasive carcinoma of no special type and 60%-90% TNBC is high grade [1],[17],[18]. In this study, we found that all samples were diagnosed histopathologically as invasive carcinoma of no special type with 80.4% grade 3 and 19.6% grade 2. While all of patients were clinically diagnosed as advanced breast cancer. Other features of TNBC are more aggressive, especially in developing countrieswhich is more frequently diagnosed at the advanced stages [19],[20].

In this study, we found significant mean difference of PTEN expression H-score between case group and control group based on t-independent test (p = 0,000). In addition,theH-score in case group was found lower than H-score in control group (53.04 ± 37.23 and 139.57 ± 75.23, respectively). The difference in PTEN expression between the two groups provesthat the loss of PTEN leads to overactivation of the PI3K / AKT signaling pathway. The PI3K / AKT signaling pathway is an anti-apoptotic pathway involved in the chemotherapy resistance mechanism on TNBC [3].

PTEN might act as a regulator of PI3K/AKT signaling activity by dephosphorylating phosphatidylinositol (3,4,5)-trisphosphate (PIP3) to phosphatidylinositol 4,5-diphosphate (PIP2) by specifically releasing D3 phosphate from the inositol ring thereby reducing PIP3 levels in the membrane. PTEN keeping basal levels of PIP3 below a threshold for the signaling pathway activation so that cell development can be controlled [5]. Downregulation of PTEN expression results in the loss of anti-apoptotic PI3K / AKT pathway inhibition resulting in overactivation of this pathway leading to inhibition of apoptotic regression from chemotherapy. Akt activation will protect breast cancer cells from apoptosis due to chemotherapy by inactivating pro-apoptotic factors such as Bad and caspase 9 [21]. Otherwise, the high expression of PTEN will increase the response of breast carcinoma to chemotherapy [22].

PTEN inactivation lead to excessive accumulation of PIP3, which responsible in Akt translocation to the cell membrane through binding with pleckstrin homology (PH) domain. This process followed by confirmation change to Akt which lead to Akt phosphorylation by phosphoinositide-dependent kinase 1 (PDK1) and activate the Akt. Akt activity lead to apoptosis inhibition. This is one of the cause of chemotherapy resistance in TNBC [5],[22].

Until now author have not found any otherstudyin Bali that correlate PTEN expression with clinical response of neoadjuvantchemotherapy in TNBC. Previous studies of breast carcinoma correlate loss of PTEN expression to clinicopathological features. One of the results of a meta-analysis study conducted by Xu, et al (2017) analyzed 17 studies involving 4343 patients with breast carcinoma. The study found that the decrease in PTEN expression was significantly associated with OS (HR = 1.83, 95%CI 1.32-2.53) and DFS (HR = 2.43, 95%CI 1.31-4.53). This meta-analysis study showed that low PTEN expression can predict a shorter OS and DSF, thus providing a worse prognosis [23].

Several other studies have also shown similar results. In a study conducted by Li, et al (2017) reported that breast carcinoma patients with loss of PTEN expression showed significantly poor OS and DSF (HR = 1.63, 95%CI 1.04-2.22, p<0.00001 and HR = 1.41, IK 95% 1.08-1.73, p <0.000, respectively). Thus it is concluded that the loss of PTEN can predict a poor prognosis in breast carcinoma [24]. This study is in line with study conducted by Beg, et al (2015) who reported the loss of PTEN in TNBC is associated with rapid cell proliferation and poor prognosis (p = 0.0408) [11].Negative neoadjuvantchemotherapy clinical response due to loss / decrease of PTEN leads to overactivation of the PI3K / Akt signaling pathway giving a poor prognosis in TNBC patients because residual cancer cells which resistant to chemotherapy are able to growth, proliferate, survive and migrate so that the recurrence rate and metastatic in TNBC become higher than non-TNBC [8].

In conclusion based on the results of this study, TNBC patients with loss of PTEN expression had a 6.75 times higher risk of showing poor clinical response of neoadjuvant chemotherapy than TNBC patients with no loss of PTEN expression.

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