ORIGINAL ARTICLE

Indonesia Journal of Biomedical Science (*IJBS*) 2020, Volume 14, Number 2: 78-81
P-ISSN.2085-4773, E-ISSN.2302-2906

Published by DiscoverSys

PTEN and PARP1 expression as predictors of neoadjuvant chemotherapy response in breast cancer: A case-control study

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ABSTRACT

Background: Most breast cancer patients in developing countries present at an advanced stage and require neoadjuvant chemotherapy (NACT) before mastectomy. Chemotherapy in breast cancer generally kills cancer cells via apoptotic mechanisms, and chemotherapy response could be predicted by assessing biological markers associated with apoptosis, including phosphatase and tensin homolog deleted on chromosome ten (PTEN) and Poly-ADP-ribose polymerase 1 (PARP1).

Objective: This study investigated the correlation between PTEN and PARP1 expression and NACT response in breast carcinoma

Methods: Breast carcinoma patients who received NACT in 2017-2018 were consecutively selected, consist of 22 patients with positive NACT response and 22 patients with negative NACT response. Immunohistochemical examination of PTEN and PARP1 was carried out, and their expression was categorized with high and low PTEN expression and high and low PARP1 expression. Statistical analysis using chi-square test was performed to assess the relationship between clinicopathological characteristics, PTEN expression, PARP1 expression, and response to NACT. The significance test was determined at $p < 0.05$.

Results: There was a significant relationship between tumor size ($p=0.030$) and PTEN expression ($p=0.035$) with the response to NACT in breast carcinoma. Low PTEN expression had a risk of negative clinical response to NACT by 3.754 times compared to breast cancer with high PTEN expression. Meanwhile, there was no significant relationship between PARP1 expression and response to NACT in breast carcinoma.

Keywords: breast cancer, PTEN, PARP1, NACT, predictive factors

Cite this Article: Sriwidyani, N.P., Saputra, H . 2020. PTEN and PARP1 expression as predictors of neoadjuvant chemotherapy response in breast cancer: A case-control study. *IJBS* 14(2): 78-81. DOI: 10.15562/ijbs.v14i2.261

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Received: 2020-09-02
Accepted: 2020-09-25
Published: 2020-09-30

INTRODUCTION

Breast cancer is the most common malignancy in women around the world, with an increasing incidence and a high mortality rate. In developing countries, many breast cancer patients come at an advanced stage of the disease, where the patient management options are NACT before definitive surgical therapy. Several clinicopathological and biological markers were associated with chemotherapy response.

The susceptibility of cancer cells to chemotherapy-induced apoptosis depends on the balance between pro-apoptotic signals and survival (anti-apoptosis) signals. There are two pathways to the chemoresistance mechanism. First is the blockade or down-regulate of the pro-apoptotic pathway, and second is the upregulate of the anti-apoptotic pathway. The important of the anti-apoptotic pathway involved in the chemoresistance process is the phosphatidyl inositol-3-kinase (PI3K)/protein kinase B (AKT) signaling pathway (and over-activation of the PI3K/AKT signaling pathway is caused by the loss of phosphatase and tensin homolog deleted on chromosome ten (PTEN) which function as negative regulator of this pathway.[1-3]

Nevertheless, some cancer cells will activate alternative DNA repair pathways through the base excision repair (BER) mechanism involving Poly-ADP-ribose polymerase 1 (PARP1).[4,5] Activation of these alternative DNA repair pathways allows cancer cells to survive chemotherapy.

The objective of this study was to evaluate the role of PTEN and PARP1 expression in predicting response to NACT in breast carcinoma.

MATERIAL AND METHODS

Patients and tissue samples

The sample of this research was invasive breast carcinoma patients who received NACT. The clinical response to NACT is divided into the positive response (complete clinical response or partial clinical response) and negative response (stable clinical disease or progressive clinical disease). Data were obtained from the patient's medical record. Paraffin blocks from biopsy materials before NACT were examined histopathologically at the Anatomical Pathology Laboratory, Sanglah General

Hospital Denpasar, from 2 January 2017 to 31 December 2018. We obtained 22 cases with positive response to NACT and categorized as case group, and we selected 22 cases with negative response to NACT consecutively from 2 January 2017 and categorized as control group. This research protocol was approved by the ethical committee Faculty of Medicine, Universitas Udayana.

Immunohistochemistry

PTEN and PAP1 expression were determined by immunohistochemistry. The selected paraffin blocks were sliced into 4 mm thickness to performed immunostaining. The section was stained with rabbit anti-human PTEN monoclonal antibody (SP170, Abcam, USA) and with rabbit anti-human PARP1 polyclonal antibody (N2C1, GeneTex, USA). The endogenous peroxidase enzyme was blocked with 0.3% hydrogen peroxide for 20 minutes. Heat-induced epitope, retrieved in the citric buffer for 20 minutes as antigen retrieving. Immunoreactivity was detected with Starr Trek Universal HRP Detection (Lab Vision, USA).[6]

Immunostaining results of PTEN and PARP1 were determined by counting the nuclear staining cancer cells. PTEN and PARP1 expression was assessed on brown stained nuclei of the entire tumor tissue with a semi-quantitative approach using a modified histo-score (H-score). In PTEN immunostaining, subject with H-score <90 was categorized as low PTEN expression, and H-score ≥90 classified as high PTEN expression.[7] Meanwhile, in PARP1 immunostaining, subject with H-score <200 was categorized as low PARP1 expression and H-score ≥200 classified as high PARP1 expression.[8]

Statistical analysis

Correlation between clinicopathological variables, PTEN expression, PARP1 expression, and clinical response to NACT in breast cancer patients were determined by chi-square test with $p<0.05$ significance.

RESULTS

From the years of 2017-2018, consecutive sampling was carried out—Twenty-two patients with positive NACT response and 22 patients with negative NACT response. The patient age range was 32-72 years, with a mean of 50.23 years. The characteristics of the research subjects are presented in Table 1.

This study found 25 (56.8%) patients was in the >50 year group. More than half samples (63%) were found in high grade. In pathological staging, we found 27 (61.4%) patients were in T1-2, 32 (72.7%) patients had positive nodal status, and 5 (11.4%) patients were with positive metastasis. The result of PTEN and PAP1 expression by immunohistochemistry were in Figure 1 and 2, respectively. More than half of the samples (52.3%) were high PTEN expression and almost of the samples (95.5%) were high PARP1 expression.

Chi-square analysis was performed to examine the relationship between the clinicopathology and the expression of PTEN and PARP 1 and the clinical response to NACT in breast cancer. The results of the statistical analysis are presented in Table 2.

From the results of statistical analysis, it was found that the variables which proved to be significantly related were primary tumor variables with clinical response to NACT ($p=0.030$) and PTEN expression with clinical response to NACT ($p=0.035$). The bivariate test showed that breast cancer with low PTEN expression had a risk of negative clinical response to NACT chemotherapy of 3.754 times compared to breast cancer with high PTEN. Other clinicopathological characteristics and PARP1 expression showed no significant correlation.

Table 1. Clinicopathological characteristics of patients (n=44)

Characteristics	Patients (no)	Percentage (%)
Age		
≤50 year	19	43.2
>50 year	25	56.8
Grade		
Low (grade 1-2)	16	36.4
High (grade 3)	18	63.6
Tumor size		
T1-2	27	61.4
T3-4	17	38.6
Nodal status		
Negative	12	27.3
Positive	32	72.7
Metastasis		
Negative	39	88.6
Positive	5	11.4
NACT response		
Negative	22	50
Positive	22	50
PTEN		
Low	21	47.7
High	23	52.3
PARP1		
Low	2	4.5
High	42	95.5

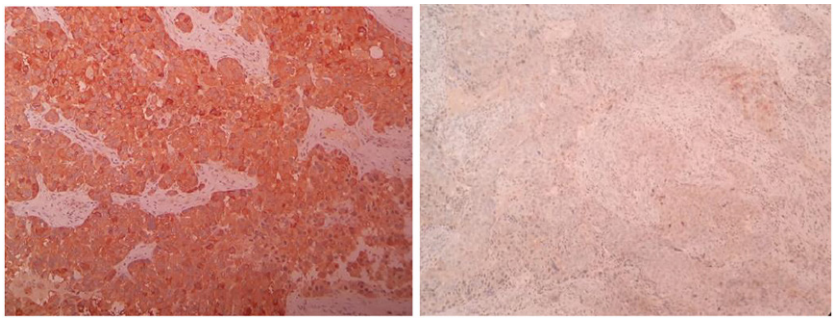

Figura 1 Breast carcinoma with high PTEN expression (A) and low expression (B). (PTEN immunohistochemistry, 400X)

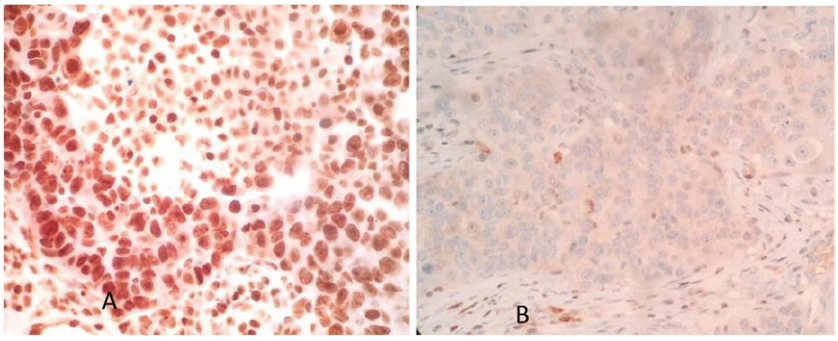

Figura 2 Breast carcinoma with high PARP expression (A) and low expression (B). (PARP1 immunohistochemistry, 400X)

Table 2. The relationship between clinicopathological characteristics and expression of PTEN and PARP1 with the clinical response of NACT in breast cancer

Characteristics	NACT Response		P	OR	95% CI
	Negative (n=22)	Positive (n=22)			
Age					
≤50 year	9	10	0.763		
>50 year	13	12			
Grade					
Low	8	8	1.000		
High	14	14			
Tumor size					
T1-2	10	17	0.030	0.245	0.067-0.902
T3-4	12	5			
Nodal status					
Negative	6	17	1.000		
Positive	16	5			
Metastasis					
Negative	18	21	0.154		
Positive	4	1			
Ekspresi PTEN					
Rendah	14	7	0.035	3.754	1.076-13.073
Tinggi	8	15			
Ekspresi PARP1					
Rendah	2	0	0.148		
Tinggi	20	22			

DISCUSSION

PI3K/AKT/PTEN signaling pathways are essential in various mechanisms of tumorigenesis and tumor progression. It involves in malignant cell transformation, tumor growth, invasion, migration, and angiogenesis.[3] Nuclear PTEN over-expression is associated with poor overall survival in early breast carcinoma.[9] PTEN hypermethylation is a marker early tumorigenesis in breast cancer patients.[10] PTEN loss has a significant correlation with larger tumor size, lymph node metastasis, higher stage, and triple-negative subtype tumor. PTEN loss significantly associated with worse disease-free survival and overall survival in breast cancer patients.[11]

PI3K/AKT/PTEN signaling pathways also involves mechanisms of resistance to chemotherapy.[12] The susceptibility of cancer cells to chemotherapy-induced apoptosis depends on the balance between pro-apoptotic signals and survival (anti-apoptosis). Blockade or down-regulation of the pro-apoptotic pathway or up-regulation of the anti-apoptotic pathway are chemoresistance mechanisms.[13] Down-regulation of PTEN expression results in loss of inhibition of the survival pathway (anti-apoptosis) PI3K/AKT resulting in over-activation of this pathway leading to inhibition of chemotherapy-induced apoptotic regression. Activation of AKT will protect breast cancer cells from apoptosis due to chemotherapy by inactivating pro-apoptotic factors such as Bad and caspase 9.[1] Conversely, high PTEN expression will increase the response of breast carcinoma to chemotherapy.[14] Based on this, the reduction or loss of PTEN expression can be a predictive factor for negative NACT response in breast carcinoma.

High PARP1 expression did not significantly have a risk of negative NACT response in breast cancer (p=0.148). Breast carcinoma with BRCA mutation, non-functional BRCA1 cannot perform DNA repair through the homologous recombination (HR) pathway. In a state of malfunctioning HR for DNA repair, cells will activate other pathways, one of which is through base excision repair (BER) mechanisms that involving PARP1 activity.[5,15,16] PTEN is also essential in maintaining genomic stability.[4,5] PTEN deficiency causes HR defects in tumor cells. HR defects, either due to BRCA or PTEN deficiency, can be potential targets for PARP inhibitor therapy.[17] In 31.2% of cases of invasive breast carcinoma also showed over-expression of PARP1.[9] It seems that the role of PARP1 is mainly in breast cancer with BRCA mutation triple-negative breast cancer.[10,18,19] In contrast, in sporadic breast cancer, it is not dominant, especially in DNA repair in chemotherapy-induced cell damage.

Published by DiscoverSys | *IJBS* 2020; 14(2): 78-81 | doi: 10.15562/ijbs.v14i2.261

CONCLUSION

Based on the results of this study, it can be concluded that there is a significant relationship between PTEN expression and response to NACT in breast carcinoma. Low PTEN expression had a risk of negative clinical response to NACT by more than three times compared to breast cancer with high PTEN expression. But there is no significant relationship between PARP1 expression and response to NACT in breast carcinoma. Further study of PARP1 expression and its association with the NACT response, specifically in breast cancer patients with BRCA mutation, is needed.

AUTHOR CONTRIBUTION

All authors have contributed to all processes in this research, including preparation, data gathering, and analysis, drafting, and approval for publication of this manuscript.

CONFLICT OF INTEREST

The authors declare no conflict of interest regarding the publication of this article.

FUNDING

This research was supported by grant from DIPA PNBP Universitas Udayana 2018 (No: 61/UN14.2.2/UPPM/2018)

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