

# Poly(ADP-ribose) polymerase-1 Overexpression as A Predictive Factor for Poor Chemotherapy Outcome in Triple Negative Breast Cancer

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**Abstract:** Triple-negative breast cancer (TNBC), is paradoxical breast cancer subtype. Some TNBC response well to chemotherapy but TNBC with remaining tumor mass after neoadjuvant chemotherapy have worse prognosis. Most TNBCs have defects in DNA repair pathway. To survive they activate alternative DNA repair pathway involving poly(ADP-ribose)polymerase-1 (PARP1), reflected by PARP1 overexpression. The aim of this study was to prove PARP1 overexpression as a predictive factor for poor chemotherapy outcome in TNBC. This study was conducted in retrospective case-control method. The PARP1 expressions immunohistochemically evaluated in 25 patients with positive chemotherapy clinical response and 25 patients with negative chemotherapy clinical response. The results were analyzed by Chi square and Odds ratio (OR) with significance at  $p < 0.05$ . The results showed that PARP1 overexpression significantly correlated with poor chemotherapy clinical response ( $\chi^2=6.522$ ,  $OR=4.57$ ,  $95\% IK=1.38-15.11$ ,  $p=0.011$ ). In conclusion TNBC with PARP1 overexpression have the possibility of showing poor chemotherapy clinical response 4.6 times higher than non-overexpression TNBC. Examination of PARP1 expression is important as predictive factors for chemotherapy outcome and consideration for utilization PARP1 inhibitor as targeted therapy in TNBC.

**Keywords:** triple negative breast cancer, chemotherapy clinical response, poly (ADP-ribose)polymerase-1.

## 1. Introduction

Triple-negative breast cancers (TNBC) is a clinicopathological term describing a subtype of breast cancer neither express hormone receptors, nor overexpress HER2. They are associated with poor prognosis [1], [2]. 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 preoperative chemotherapy [3]. Chemotherapy is the primary established systemic treatment for patients with triple-negative breast cancer in both the early and advanced-stages of the disease.

The lack of targeted therapies and the poor prognosis of TNBC patients have fostered a major effort to discover actionable molecular targets to treat patients with TNBC [4]. Most TNBCs have mutations in breast cancer susceptibility gene (BRCA) or BRCA-like mutation. Thus TNBC patients are unable to repair the double strand break (DSB) deoxyribonucleic acid (DNA). This condition lead cancer cells to be more sensitive to cytotoxic chemotherapy treatment [4], [5]. However, to survive some TNBCs will activate alternative DNA repair pathways through a base excision repair (BER) mechanism involving Poly(ADP-ribose)polymerase1 (PARP1) [5].

Poly(ADP-ribose)polymerase-1 belongs to a superfamily of enzymes that catalyzes the cleavage of  $NAD^+$  molecules resulting in the incorporation of ADP-ribose molecules to acceptor proteins. PARP1 have been involved in various cellular processes, especially in n DNA single strand break (SSB) repair through base excision repair (BER) [5], [7], [8].

In normal breast tissue there is no overexpression of PARP1[9].

Breast carcinoma with BRCA mutation is mostly TNBC. Cells with non-functional BRCA1 can not perform DNA repair via homologous recombination [5], to survive the cell will activate other pathways, such as through BER involving PARP1 activity hence PARP1 will be overexpressed [3], [8]. PARP1 overexpression can be used as a surrogate marker indicating mutations in BRCA as well as BRCA-like mutation. This is especially important because not all countries have the technology to diagnose BRCA mutations[10]. In a study conducted by Rojo et al in 2012, it was reported that PARP1 overexpression in early breast cancer was an independent predictive factor for poor survival rates [7]. If PARP1 activity is inhibited then tumor cells can not repair DNA and leading to cell death [6], [11].

The aim of this study is to prove PARP1 overexpression as a predictive factor for poor chemotherapy outcome in TNBC.

## 2. Material and Methods

### Specimens

Slides and paraffin embedded tissue blocks from 50 patients invasive breast carcinoma TNBC subtype were retrieved from the histopathology archives in Anatomic Pathology Laboratory of Sanglah Hospital, Bali in the year 2012-2017. Clinical data were from the medical report and cancer registry.

### Histopathologic evaluation

The slides from these cases were reviewed and histopathologic diagnoses in the histopathologic reports were

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confirmed independently by two pathologists and one resident.

### Chemotherapy Response Evaluation

Chemotherapy clinical response is tumor size assessment before and after neoadjuvant chemotherapy using 3 series of polychemotherapy (flourouracil, adriamycin, and cyclophosphamide [FAC]). The chemotherapy response was assessed by 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) [13], [14].

In this study the clinical response of chemotherapy is differentiated into positive response as control group, which consists of clinical complete response and clinical partial response. Negative response as the case group, consisting of clinical stable disease and clinical progressive disease [15]. The chemotherapy clinical responses were obtained from Sanglah Hospital medical records and oncologic surgeon cancer registration data.

### Immunohistochemistry and interpretation

Tissue section at 4 μm thickness from each case were prepared for immunostaining. After 30 minutes incubation in a 60°C oven, deparaffinization, and rehydration tissue sections were treated with 3% hydrogen peroxide for 10 minutes. Following incubation in blocking buffer for 30 minutes in room temperature, the slides were incubated with one of the following primary antibodies PARP1 *polyclonal rabbit*, 1:200 dilution. The colour was visualized by DAB as chromogen.

Immunostaining were interpreted independently by two pathologists and one resident. Immunohistochemistry results evaluated by a semiquantitative approach using Histo-score (H-score). PARP1 expression was assessed on the nuclear staining throughout the invasive area. The intensity is given score 0 (negative), 1(weak), 2(moderate) and 3(strong). The percentage of cells at each staining intensity level is assigned using the following formula:  $\{[1 \times (\% \text{ cells } 1+)] + [2 \times (\% \text{ cells } 2+)] + [3 \times (\% \text{ cells } 3+)]\}$ . From the calculation obtained H-score with a range of 0-300. The cut-off point for PARP1 expression based on median H-score, which is 200. Samples show PARP1 overexpression if H-score  $\geq$  200 and non-overexpression if H-score  $<$ 200 [5], [12].

### Statistical analysis

Descriptive statistics were calculated. Chi square test and Odds ratio was used to assess the association between PARP1 expression with chemotherapy clinical response. P value less than 0.05 was considered significant. All statistical analyses were performed using SPSS 16.0.

### 3. Result

In the study period (2012-2017) there were 50 patients met the study criteria, consisting of 25 patients with positive chemotherapy clinical response as control group and 25 TNBC patients with negative clinical chemotherapy response as case group.

**Table 1:** Clinicopathological characteristic and chemotherapy clinical response

Variable	Chemotherapy clinical response		Total	p value	Odds ratio
	Positive (Control) N=25	Negative (Case) N=25			
Age					
Youngest	31	31		0,573	NS
Eldest	69	67			
Mean	45.16	43.64			
Histology					
NST	25 (100%)	25 (100%)	50 (100%)	-	NS
Grade					
1	0 (0%)	0 (0%)	0 (0%)	0,120	S
2	10 (40%)	5 (20%)	15 (30%)		
3	15 (60%)	20 (80%)	35 (70%)		
Stage					
Early	9 (36%)	5 (20%)	14 (28%)	0,200	NS
Advanced	16 (64%)	20 (80%)	36 (72%)		
PARP1					
Over expression	7 (28%)	16 (64%)	23 (46%)	0,011	4.57
Non-over expression	18 (72%)	9 (36%)	27 (54%)		

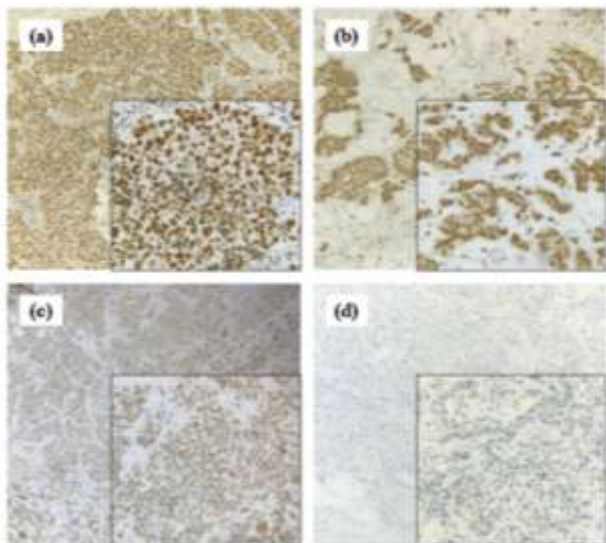
The youngest age is 31 years old and the eldest 69 years old. The mean age of the case group was  $43.64 \pm 8.9$  years, with an age range 31 to 67 years. The mean age of the control group was  $45.16 \pm 9.9$  years, with an age range 31 to 69 years. Based on the Shapiro-Wilk Normality test, the age data was normally distributed and on t-independent test p value was 0.573, which stated that there was no difference between the age of the control and the case group.

All histopathology diagnosis was invasive carcinoma of no special type (NST) in this study. Grading characteristics show no sample with grade 1. Grade 2 was 15 (30%) and grade 3 was 35 (70%). On statistic analysis p value was 0.120 and 95% confidence interval = 0.10-1.32 showed there is no relationship between grading and chemotherapy clinical response.

The clinical stage showed 14 (28%) samples were early breast cancer and 34 (72%) were advanced breast cancer. From the statistic analysis p value was 0.200 and 95% confidence interval was 0.12-1.59 which showed there is no relationship between stages and chemotherapy clinical response.

PARP1 immunohistochemical examination showed there was overexpression in 7 (28%) patients of control group and 16 (64%) patients of case group, while 18 (72%) patient of control group and 9 (36%) patients of case group showed no

overexpression. Base on statistic analysis p value was 0.011 and 95% confidence interval was 1.38-15.11, it showed that there is a relationship between PARP1 expression with chemotherapy clinical response. The Odds ratio was 4.57 and concluded that PARP1 overexpression is a predictive factor of negative chemotherapy clinical response.



**Figure 1:** PARP1 staining. (a) strong (3+), (b) moderate (2+), (c) weak (1+), (d) negative (0).

#### 4. Discussion

Triple Negative breast cancer is more common in younger women than Luminal or HER2 enriched subtype [2]-[4]. In some studies in Indonesia involving Bali, the average age of a woman diagnosed TNBC was in the fourth decade [17], [18]. In accordance with previous studies, in this study the average age of the sample was  $43.64 \pm 8.9$  years in the case group and  $45.16 \pm 9.9$  years in the control group. Shapiro-Wilk Normality test showed the age data was normally distributed and on t-independent test p value was 0.573, which stated that there was no difference between the age of the control and the case group.

The incidence of TNBC at this younger age group is related to the presence of a hereditary etiology involving genes that function in the repair of DNA damage. BRCA1 mutations are more common in TNBC than other subtype [19], [20]. In a study conducted by Purnomosari, et al (2007) found that the percentage of breast carcinoma patients with BRCA mutations was significantly higher in Denpasar (25%) than in Jakarta (7.2%) and Jogjakarta (0%). The incidence of TNBC at a young age is also associated with a BRCA-like breast carcinoma, a group that has a defect in DNA repair function in the absence of BRCA germline mutation [21], [22]. BRCA-like TNBC's behavior is not only a molecular characteristic but also provides clinical features such as breast carcinoma with BRCA mutations [23].

Most of TNBC is invasive carcinoma of no special type and more than 80% is high grade [8], [24]. In this study we found that all samples were diagnosed histopathologically as invasive carcinoma of no special type with 70% grade 3 and 30% grade 2. Other features of TNBC are more aggressive,

especially in developing countries more frequently diagnosed at the advanced stages [25]. In this study 72% of patients were clinically diagnosed as advanced breast cancer and 28% early breast cancer.

This study found no relationship between grading and stadium with clinical chemotherapy response ( $p = 0.120$  and  $p = 0.200$ ). This is in accordance with the results in several recent studies. Domagala et al (2011) conducted a study on women in Poland from 2006 to 2008 assessing the BRCA mutation relationship with PARP1 expression. There was no correlation between PARP1 expression and histology type ( $p = 0.65$ ), grade ( $p = 0.60$ ), lymph node status ( $p = 0.47$ ), and tumor size ( $p = 0.49$ ) [26]. Research conducted by Rojo et al in 330 cases of invasive breast carcinoma diagnosed between 1998 and 2000 through retrospective consecutive sampling, suggested that histology type, tumor size, metastasis to lymph nodes, and proliferation index were not significantly associated with over expression PARP1 [7]. Similarly, the results of the study conducted by Mazzotta et al (2016), found there was no relationship between age ( $p = 0.74$ ), histology type ( $p = 0.43$ ), tumor size ( $p = 0.14$ ) and tumor grade ( $p = 0.05$ ) [27].

Poly (ADP-ribose) polymerase-1 is not overexpressed in normal tissue, both in breast tissue and in other organs [28]. PARP1 is essential for single strand break DNA repair. PARP1 induces cell viability through DNA repair [5]. In the event of DNA damage, PARP1 will undergo activation, detect the location of DNA damage, then recruit BER multiprotein complex, thus allowing the polymer complex to repair the DNA [3], [5]. Triple Negative Breast Cancer generally has mutations in BRCA or BRCA-like mutation. Cells with non-functional BRCA's can not perform DNA repair via HR pathway [5]. In the non-functioning of HR for DNA repair, the cell will activate BER involving PARP1 activity [8], [29]. The dependence on PARP1 activity for DNA repair results in immunohistochemical PARP1 over expression. In the usual ductal hyperplasia, PARP1 expression is obtained similar to normal breast epithelium. In contrast, in 31.2% of invasive breast carcinoma cases have PARP1 over expression PARP1 [7]. In the overall sample (case and control group), 23 (46%) samples showed PARP1 overexpression.

In this study 16 (64%) samples in the group with a negative chemotherapy clinical response (case group) and 7 (28%) samples in the positive chemotherapy clinical response group (control group) showed PARP1 over expression. The results of statistical analysis comparing PARP1 expression in case and control group showed a significant difference wherein the expression in case was higher than control ( $p = 0.011$ ). This difference proves that PARP1 over expression is a predictive factor of negative chemotherapy clinical response of 4.6 times (OR = 4.57).

Until now writer have not found any other research in Bali that correlates PARP1 expression with clinical chemotherapy response. Park et al (2015) compared the expression of several DNA-damaged molecules including PARP1 in breast carcinoma. One of the results of this study was in patients receiving adjuvant chemotherapy with high PARP1



expression providing shorter overall survival (OS) and disease free survival (DFS) thus to give a worse prognosis. Overall survival is the time from the date of operation to the date of death of any cause. Disease free survival is the time from the date of surgery to the date of primary, regional or remote recurrence, as well as the appearance of a secondary tumor or DCIS [29].

Several other studies have also shown similar results. In a study conducted by Rojo et al (2012) it was reported that the hazard ratio (HR) for death in patients with PARP-1 overexpression was 7.24 (95% CI; 3.56-14.75). And in the multivariate analysis, PARP-1 overexpression was an independent prognostic factor for DFS (HR 10.05; 95% CI 5.42-10.66) and OS (HR 1.82; 95% CI 1.32-2.52) [7].

Mazzotta et al (2016) conducted a retrospective cohort study of 114 patients with primary operable breast carcinoma by comparing PARP1 expression, BRCA and clinicopathologic variables. The results of the multivariate analysis of the study showed that high PARP1 expression was associated with decreased DFS (P = 0.012) and OS (P = 0.026). Hazard ratio for DFS in tumors with PARP1 overexpression was 6.61 (95% confidence interval (CI): 1.52-28.80) and for OS 1.59 (95% CI: 1.40-181.19) Mazzotta et al concluded that PARP1 overexpression can be used as an independent prognostic factor in patients with breast carcinoma. In addition, PARP1 overexpression may represent a marker of poorer prognosis, for both patients with poor clinical and less aggressive clinical conditions [27].

In conclusion based on the results of this study, TNBC patients with PARP1 overexpression is 4.6 times more likely to have a poor chemotherapy clinical response than those who does not overexpressed PARP1. In subsequent developments it is expected that administration of PARP1-inhibitors as targeted therapy for TNBC patients with PARP1 overexpression may improve their prognosis.

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