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The relationship between poly ADP-ribose polymerase-1 gene expression and protein kinase β expression with neoadjuvant chemotherapy response in triple negative breast cancer subtype

Relación entre la expresión del gen de la poli ADP-ribosa polimerasa-1 y la expresión de la proteína cinasa β en la respuesta a la quimioterapia neoadyuvante en el subtipo de cáncer de mama triple negativo

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SUMMARY

Introduction: *Breast cancer is the most common and deadliest cancer among women worldwide. One of its subtypes with a particularly poor prognosis is triple-negative breast cancer (TNBC). Patients with TNBC often receive various therapeutic approaches, including neoadjuvant chemotherapy. However, the wide genetic heterogeneity of TNBC results in highly variable responses to chemotherapy. Poly(ADP-ribose) polymerase-1 (PARP1) expression, a defense mechanism against chemotherapy, and Protein Kinase β (AKT) activation, which increases tumor aggressiveness, are thought to influence chemotherapy responses in these patients.* **Methods:** *This is an analytical observational study with a Nested case-control design within a cohort, examining the relationship between PARP1 and AKT expression and the good or poor response to neoadjuvant chemotherapy in TNBC. The study was conducted at Wahidin Sudirohusodo Hospital, UNHAS Teaching Hospital, Hermina Hospital, and Pelamonia Hospital Makassar, with the study sample consisting of women aged 18 years and above diagnosed with TNBC based on histopathological and immunohistochemical examinations who were scheduled to undergo*

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neoadjuvant chemotherapy. **Results:** *Of the 56 TNBC patients studied, 30 had a poor chemotherapy response, and 26 had a good response. A difference in the mean PARP1 expression was observed between the good chemotherapy response group (7.380 ± 1.044) and the poor chemotherapy response group (9.693 ± 1.767; p < 0.001). High PARP1 gene expression was found to be a risk factor for poor chemotherapy response in TNBC (OR 121.8, 95 % CI 132.3-1 120.6, p <0.001; Adj. OR 132.3, 95 % CI 133.05 – 1 315.42, p <0.001). This study could not demonstrate an association between AKT and chemotherapy response in TNBC (OR 0.852, 95 % CI 0.157-4.63, P 1.0).* **Conclusion:** *These findings indicate that high PARP1 levels are a risk factor for poor neoadjuvant chemotherapy response in patients with TNBC.*

Keywords: *PARP1, Protein Kinase β/AKT, triple negative breast cancer, neoadjuvant chemotherapy*

RESUMEN

Introducción: *El cáncer de mama es el cáncer más común y mortal en mujeres a nivel mundial. Uno de sus subtipos, con un pronóstico particularmente desfavorable, es el cáncer de mama triple negativo (CMTN). Las pacientes con CMTN suelen recibir diversos enfoques terapéuticos, incluida la quimioterapia neoadyuvante. Sin embargo, la amplia heterogeneidad genética del CMTN da lugar a respuestas muy variables a la quimioterapia. Se cree que la expresión de la Poli (ADP-ribosa) polymerasa-1 (PARP1) como mecanismo de defensa frente a los efectos de la quimioterapia, así como la activación de la proteína quinasa β (AKT), que aumenta la agresividad tumoral, influyen en las respuestas a la quimioterapia en estas pacientes.* **Método:** *Este es un estudio observacional analítico con un diseño de casos y controles anidados en cohorte, que examina la relación entre la expresión de PARP1 y AKT y la buena o mala respuesta a la quimioterapia neoadyuvante en el TNBC. El estudio se realizó en el Hospital Wahidin Sudirohusodo, el Hospital Docente UNHAS, el Hospital Hermina y el Hospital Pelamonia Makassar, con una muestra del estudio compuesta por mujeres de 18 años o más, diagnosticadas con CMTN según exámenes histopatológicos e inmunohistoquímicos, que se someterán a quimioterapia neoadyuvante.* **Resultados:** *De los 56 pacientes con CMTN estudiados, 30 presentaron una respuesta deficiente a la quimioterapia y 26, una buena. Se encontró una diferencia en la expresión media del gen PARP1 entre el grupo de buena respuesta a la quimioterapia (7,380 ± 1,044) y el grupo de respuesta deficiente (9,693 ± 1,767), con p < 0,001. Se observó que una alta expresión del gen PARP1 era un factor de riesgo para una respuesta deficiente a la quimioterapia en el cáncer de mama triple negativo (OR: 121,8; IC del 95 %: 132,3-1 120,6; p < 0,001; OR ajustado: 132,3; IC del 95 %: 133,05-1 315,42; p < 0,001). Este estudio no demostró una relación entre AKT y la respuesta a la quimioterapia en el cáncer de mama triple negativo (OR: 0,852; IC del 95 %: 0.157-4,63; p < 1,0).* **Conclusión:** *Estos hallazgos demuestran que una alta expresión de PARP1 es un factor de riesgo de una respuesta deficiente a la quimioterapia neoadyuvante en pacientes con cáncer de mama triple negativo.*

Palabras clave: *PARP1, proteína quinasa β/AKT, cáncer de mama triple negativo, quimioterapia neoadyuvante.*

INTRODUCTION

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Breast cancer (BCC) is the most common malignancy and the leading cause of cancer death in women worldwide. According to GLOBOCAN data in 2020, there were 2.089 million new cases of BCC, with an incidence rate of 11.6 % and a mortality rate of 521 900. Although the highest incidence rates are still recorded in developed countries, rapid increases are occurring in Southeast Asian countries, including Indonesia, making BCC a significant public health problem in the region (1-3).

BCC has several intrinsic subtypes defined by hormone receptor expression and molecular features, including the Triple-Negative Breast Cancer (TNBC) subtype, which accounts for 10 %-20 % of cases. TNBC has aggressive clinical characteristics, low survival rates, but a relatively better response to chemotherapy compared to other subtypes—a phenomenon known as the "TNBC Paradox." However, the molecular heterogeneity of TNBC leads to variability in therapeutic responses, necessitating a deeper understanding of the molecular factors that influence the efficacy of chemotherapy (4-7).

One important factor influencing TNBC chemotherapy response is the DNA repair mechanism. Mutations in the BRCA1/BRCA2 genes, which are involved in homologous recombination repair, are frequently found in TNBC and are associated with increased sensitivity to platinum agents. Furthermore, Poly (ADP-ribose) polymerase-1 (PARP1) plays a

crucial role in DNA single-strand break repair and angiogenesis, making it a potential therapeutic target. PARP1 inhibitors have been shown to provide a good therapeutic response in patients with BRCA1/2 mutations (8-10).

In addition to PARP1, the PI3K/AKT signaling pathway also plays a significant role in the development and progression of TNBC. Activation of this pathway—whether through mutations or loss of key regulators such as PTEN (Phosphatase and Tensin Homolog)—is associated with increased cancer cell proliferation, enhanced angiogenesis, and the development of resistance to chemotherapy. Mutations in the PIK3CA gene are found in 20 %–40 % of PROM cases and are associated with treatment resistance. Protein Kinase β/AKT, a key component of this pathway, contributes to tumor cell survival, proliferation, and angiogenesis (11,12).

Given the important roles of PARP1 and Protein Kinase β/AKT in TNBC molecular mechanisms and the limited research in this area, further studies are needed to understand the relationship between their expression and chemotherapy response. This research is expected to contribute to the development of personalized medicine for TNBC patients, thereby increasing treatment effectiveness and improving patient prognosis (13,14).

METHODS

This study was an observational analytic study with a nested case-control design within cohorts of Triple-negative breast cancer (TNBC) patients undergoing neoadjuvant chemotherapy at Dr. Wahidin Sudirohusodo General Hospital, Hasanuddin University Teaching Hospital, Pelamonia Hospital Makassar, and Hermina Hospital Makassar (March 2023–March 2024). Samples were selected consecutively from TNBC patients who met the inclusion criteria. The case group comprised patients with a poor response to neoadjuvant chemotherapy, whereas the control group had a good response, as defined by RECIST Version 1.1 criteria. The minimum sample size was 52 patients (26 per group), calculated using a two-proportion comparison (15).

PARP1 gene expression was assessed at the Molecular Biology and Immunology Laboratory, while Protein Kinase B (AKT) expression was analyzed by immunohistochemistry at the Anatomical Pathology Laboratory of Hasanuddin University. Inclusion criteria included female TNBC patients aged ≥18 years with complete clinical and histopathological data. In contrast, exclusion criteria included male patients, immunodeficiency, severe comorbidities, severe chemotherapy-related side effects, and damaged paraffin blocks. Therapeutic response was evaluated based on changes in tumor size and lymph node enlargement (16).

Instrument

The research instrument consists of primary and secondary data collection instruments and laboratory examinations to analyze molecular and histopathological expressions in triple-negative breast cancer (TNBC) patients. Primary data were obtained by examining PARP1 gene mRNA expression using real-time polymerase chain reaction (RT-PCR) techniques from fresh tumor biopsy tissue. The analysis was conducted in the Molecular Biology and Immunology Laboratory of the Faculty of Medicine, Hasanuddin University, using the Rotor-Gene 3000 (Corbett Research) and iQ5 iCycler (Bio-Rad) instruments. Immunohistochemistry (IHC) was performed to detect Protein Kinase B (AKT) protein expression in paraffin-embedded tissue, using anti-AKT primary antibodies and Streptavidin–horseradish peroxidase (SA-HRP) secondary antibodies (17).

The research materials included PARP1 gene primers, fresh TNBC biopsy tissue, paraffin-embedded breast cancer tissue blocks, anti-AKT primary antibody, SA-HRP secondary antibody, graded alcohol solutions, distilled water, phosphate-buffered saline (PBS), 10 % formalin, and 3 % hydrogen peroxide. The tools included medical records, data collection sheets, microscopes, microtomes, objective lenses, coverslips, micropipettes, and incubators. Interpretation of IHC results was performed semi-quantitatively using the H-score method (range 0–300), with <1 % stained cells considered negative. A competent Anatomical Pathology specialist carried out the examination (18).

Secondary data were collected from the medical records of TNBC patients at partner hospitals, including clinical variables such as histopathological results, age, menopausal status, parity, tumor size, lymph node enlargement, tumor-infiltrating lymphocytes (TIL), lymphovascular invasion (LVI), and the type, schedule, and dose of chemotherapy. This combination of molecular, histopathological, and clinical data enables analysis of the relationships among PARP1 and AKT expression, clinical characteristics, and therapeutic response in TNBC patients (19,20).

The research was initiated after obtaining approval from the Ethics Committee of the Faculty of Medicine, Hasanuddin University, and research permits from Wahidin Sudirohusodo Hospital, UNHAS Teaching Hospital, Hermina Hospital, and Pelamonia Hospital, Makassar. Primary data collection involved examining PARP1 mRNA expression in fresh biopsy tissue and immunohistochemical analysis of paraffin biopsy blocks from TNBC patients. PARP1 gene mRNA expression was assessed by Real-Time Polymerase Chain Reaction (RT-PCR) using nucleic acid extracted from fresh tumor tissue of TNBC patients during diagnostic testing in the Microbiology Department of the Molecular Biology and Immunology Laboratory, Faculty of Medicine, Hasanuddin University, Makassar. Pathological examination was performed by immunohistochemistry (IHC) using a primary antibody against Protein Kinase β/AKT on paraffin blocks from primary tumor biopsy specimens. Semi-quantitative calculations were performed, which would be visible in the cell nucleus in a large, microscopic field of view. Interpretation of IHC staining was performed semi-quantitatively using the H-score method = (1x% weakly stained cells) + (2x% moderately stained cells) + (3x% strongly stained cells). The H-score ranges from 0 to 300; values <1 % stained cells are considered negative. This examination was performed by a specialist in Anatomical Pathology at the Department of Anatomical Pathology, Hasanuddin University Teaching Hospital, Makassar (10).

The research module was designed to analyze the relationship between molecular expression and clinical characteristics in patients with triple-negative breast cancer (TNBC). The module includes three main components: examination of target gene and protein expression, clinical data collection, and supporting laboratory analysis (13). This module uses a set of standardized research materials and tools, including PARP1 gene primers, an anti-AKT primary antibody, Streptavidin–horseradish peroxidase (SA-HRP) secondary antibody, fixative and buffer solutions, a light microscope, a microtome, and Rotor-Gene 3000 and iQ5 iCycler devices for RT-PCR analysis. IHC staining results are interpreted semi-quantitatively using the H-score method, which provides a value range of 0–300 to assess the intensity and proportion of stained cells (6). In addition to molecular and histopathological data, this module uses secondary data from patient medical records. The combination of components in this module enables a comprehensive analysis the roles of PARP1 and AKT expression in relation to chemotherapy response in TNBC patients (6).

Statistical analysis

Data analysis was conducted in stages, employing descriptive and inferential statistics. Descriptive statistics were used to compare the characteristics of the subjects and research variables between the case and control groups. Data were presented in a cross-tabulation with relative frequencies to assess the comparability of characteristics between groups. Next, bivariate analysis was performed using the Chi-Square test or Fisher's Exact test, depending on whether the Chi-Square test assumptions were met. The results were presented in the form of Odds Ratio (OR) values along with 95 % confidence intervals and p-values. Variables with p-values < 0.25 in the bivariate analysis were included in the multivariate analysis to control for potential confounders. Multivariate analysis was performed using Multiple logistic regression to obtain Adjusted Odds ratios (aOR) and controlled p-values. The results of the analysis were summarized using 95 % confidence intervals and a significance level of $\alpha = 0.05$. The entire data analysis was performed using IBM SPSS Statistics version 25.0 (21,22).

RESULTS

Table 1. Frequency distribution of research subject characteristics based on chemotherapy response.

Characteristics	N	Chemotherapy Response				P
		Good Amount	%	Bad Amount	%	
1. Age						0.012**
- < 40 years	7	7	100.0	0	0	
- ≥ 40 years	49	23	46.9	26	53.1	
2. Menopausal Status						0.012**
- Menopause	49	23	46.9	26	53.1	
- No menopause	7	7	100.0	0	0	
3. Parity Amount						0.712*
- 0 - 3	23	13	56.5	10	43.5	
- ≥ 4	33	17	51.5	16	48.5	
4. Enlarged Lymph Nodes						0.186*
- There is	27	12	44.4	15	55.6	
- There isn't any	29	18	62.1	11	37.9	
5. Tumor Size						0.002*
- T2	4	4	100.0	0	0	
- T3	18	14	77.8	4	22.2	
- T4	34	12	35.3	22	64.7	
6. Pathological Picture						0.455*
- Ductal	45	23	51.1	22	48.9	
- Non-Ductal	11	7	63.6	4	36.4	
7. LVI						0.277**
- Positive	18	8	44.4	10	55.6	
- Negative	38	22	57.9	16	42.1	
8. TIL						0.803*
- Positive	29	16	55.1	13	44.9	
- Negative	27	14	51.8	13	48.2	
9. Chemotherapy Dosage						0.277**
- In accordance	47	27	57.4	20	42.6	
- It is not in accordance with	9	3	33.3	6	66.7	
10. Types of Chemotherapy						1.00**
- First Line Chemo	55	29	52.7	26	47.3	
- Not First Line Chemo	1	1	100.0	0	0	
11. Chemotherapy Schedule						0.070*
- On time	38	24	63.2	14	36.8	
- Late	18	6	33.3	12	66.7	

*Chi-Square Test, *Fisher's Exact Test.

This study involved 56 Triple Negative Breast Cancer (TNBC) patients, consisting of 30 patients with poor chemotherapy responses and 26 patients with good chemotherapy responses. Based on patient characteristics (Table 1), most patients were older than 40 years, and all premenopausal patients responded well to chemotherapy. Age and menopausal status were significantly associated with chemotherapy response (p=0.012), whereas parity was not (p=0.712). In terms of tumor

characteristics, most patients had large tumors (T4) with significant differences between the good and poor response groups. Lymph node enlargement, histopathological type, lymphovascular invasion (LVI) status, and tumor-infiltrating lymphocytes (TIL) did not differ significantly between groups.

Based on treatment characteristics, almost all patients received first-line chemotherapy at standard doses, whereas one patient received second-line chemotherapy. There was no significant difference in the appropriateness of chemotherapy doses (p=0.277). However, delays in chemotherapy schedules were more frequent in the poor-response group (46 %) than in the good-response group (2.0 %), although this difference was not statistically significant (p=0.07). Overall, the results of this study indicate that age, menopausal status, and tumor size are important determinants of chemotherapy response in TNBC patients. In contrast, other variables, such as histopathological type, LVI, and TIL, do not appear to influence treatment outcomes significantly (Table 1).

The relationship between PARP1 gene expression and neoadjuvant chemotherapy response in TNBC patients

The relationship between PARP1 gene expression and chemotherapy response was assessed by comparing mean PARP1 expression between poor- and good-response groups using a parametric test (Figure 1). Data distribution was first tested for normality using a boxplot and the Kolmogorov-Smirnov test. PARP1 gene expression data were obtained as normally distributed (p=0.200). The unpaired T-test showed a significant difference (mean difference = 2.312, $p < 0.001$) in PARP1 gene expression between the poor chemotherapy response group (mean = 9.693, SD = 1.767) and the good chemotherapy response group (mean = 7.380, SD = 1.044) (Table 2).

The analysis then determined the cutoff value to classify low and high PARP1 gene expression levels. The ROC curve was used to determine the cut-off point based on the Yoden Index (Figure 2). The optimal cutoff value was 8.682. After the cutoff value was determined, PARP1 gene expression was classified as high (>8.682) or low (<8.682) (Table 3).

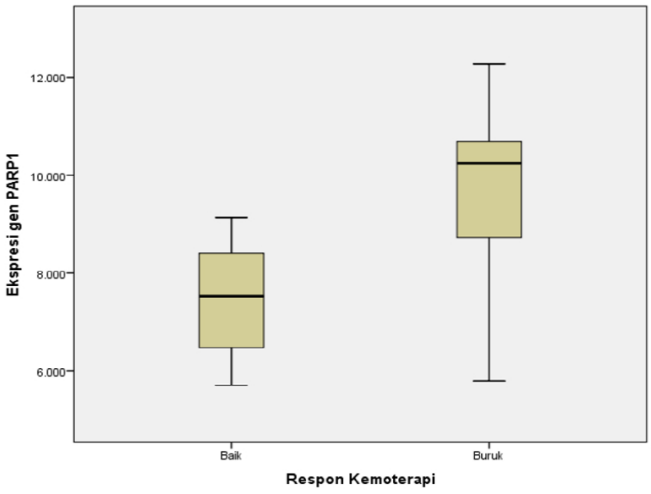

Figure 1. Distribution of PARP1 gene expression data in patients with good and poor chemotherapy responses.

Table 2. Parametric Test of Differences in Mean PARP1 gene expression values in patients with poor chemotherapy response compared to those with good chemotherapy response.

	Total (n=56)	Chemotherapy Response		Average Difference	p-value
		Bad (n=26)	Good (n=30)		
PARP 1 Gene Expression (Mean (±SD))	8.454 (± 1.83)	9.693 (± 1.767)	7.380 (± 1.044)	2.312	<0.001*

*Unpaired T-test

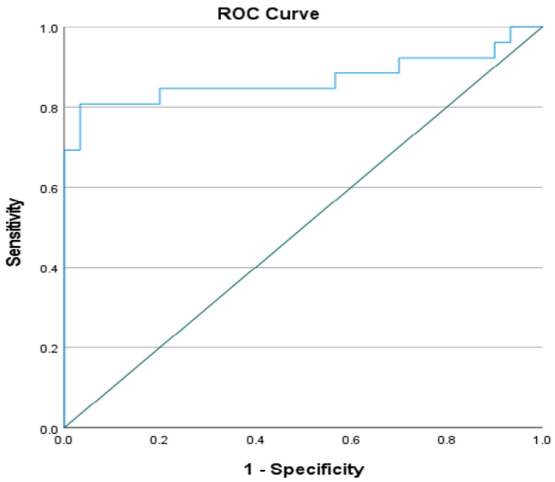

Figure 2. Receiver Operating Characteristics (ROC) curve.

Table 3. Cut-off Parameters based on Youden Index

Parameter	Cut-off	AUC	p-value	IK95 %
PARP 1 Gene Expression	8.682	0.757	<0.001	0.757-0.981

Bivariate analysis with categorical comparison using the Chi-Square test showed an association between high PARP1 gene expression and poor chemotherapy response. High PARP1 gene expression was a risk factor for poor chemotherapy response in TNBC patients (OR = 121.8; 95 % CI, 132.38 –1,120.6; p < 0.001) (Table 4).

Table 4. Relationship of PARP1 gene expression groups to neoadjuvant chemotherapy response.

	Total (n=56)	Chemotherapy Response		OR (95 % CI)	p-value
		Bad (n=26)	Good (n=30)		
PARP 1 Gene Expression					
- Tall	22 (39.3 %)	21 (80.8 %)	1 (3.3 %)	121.8	<0.001*
- Low	34 (60.7 %)	5 (19.2 %)	29 (96.7 %)	(132.38 – 1,120.6)	

*Chi-Square Test.

The results of the Protein Kinase β/AKT CPI examination showed that 50 patients had intense staining, and 6 had weak staining. A comparative analysis using the Chi-Square test showed no difference in the Protein Kinase β/AKT CPI between the poor and good chemotherapy response groups (OR = 0.852; 95 % CI, 0.157–4.636) (Table 5).

Table 5.The Relationship Between PARP1 Gene Expression and Protein Kinase β/AKT Expression and Neoadjuvant.

	Total (n=56)	Chemotherapy Response		OR (95 % CI)	p-value
		Bad (n=26)	Good (n=30)		
CPI Protein Kinase β/AKT					
- Strong	50 (89.3 %)	23 (88.5 %)	27 (90.0 %)	0.852	1,000*
- Weak	6 (10.7 %)	3 (11.5 %)	3 (10.0 %)	(0.157 – 4.636)	

*Chi-Square test.

Multivariate analysis using binary logistic regression was conducted to examine the relationship between characteristics with p < 0.25 (potential confounding variables) and the independent variables, PARP1 Gene Expression and Protein Kinase β/AKT, and chemotherapy response. In the bivariate analysis, the independent variable Protein Kinase β/AKT was not significantly associated (p > 0.25); therefore, it was excluded from the multivariate analysis (Table 6 and 7).

Table 6.The Relationship Between the PARP1 Gene and Protein Kinase β/AKT Expression and Neoadjuvant Chemotherapy Response

Variables	B	Adjusted OR	IK 95 %	Adjusted p-value
CPI Protein Kinase β/AKT	0.735	2.086	0.086 – 50.403	0.651*
PARP 1 Gene Expression	4.885	132.295	133.05 – 1315.42	< 0.001*
Constant	-3.178	0.042		

*Binary logistic regression test

Table 7.Model 1 Relationship between Characteristics and Expression of the PARP1 Gene on Neoadjuvant Chemotherapy Response.

Variables	B	Adjusted OR	IK 95 %	Adjusted p-value
Age (numerical data)	-.079	0.924	0.786 - 1.086	0.336
Tumor size (T)	-21,972	0.000	0.000 - ~	0.997
Lymph Nodes (N)	-2,992	0.050	0.002 – 1.119	0.059
Chemotherapy Schedule	2.155	8.632	0.335 – 222.305	0.193
PARP1 mRNA	24,132	30226222344.769	0.000 - ~	0.997
Constant	24,380	38718233854.244		0.997

*Binary logistic regression test.

Based on the correlation matrix, it was found that there was a strong correlation between tumor size and PARP1, so the researchers created an alternative model by eliminating the tumor size variable (T) and the age variable based on the established operational definition (age ≥40 and <40) which did not meet the requirements for multivariate analysis due to the frequency of 0 in the cross-table with chemotherapy response (Model 2). Model 2 showed that PARP1 Gene Expression was significantly related to chemotherapy response (adj. P < 0.001; adj. OR 122.673; 95 % CI 12.249 – 1 228.583). Model 2 had a good Hosmer-Lemeshow test value (0.302) and Nagelkerke R-squared, which showed high predictive power, although slightly decreased (0.695) (Table 8).

Table 8. Model 2 Relationship between Characteristics and PARP1 Gene on Neoadjuvant Chemotherapy Response.

Variables	B	Adjusted OR	IK 95%	Adjusted p-value
Lymph Nodes (N)	-0.336	0.715	0.110 – 4.635	0.725
Chemotherapy Schedule	0.515	1.674	0.230 – 12.200	0.611
PARP1 mRNA	4.810	122.673	12,249 – 1228,583	<0.001
Constant	-3,233	0.039		0.008

The probability of a good chemotherapy response can be calculated based on the following equation:

Probabilitas respon NAC good (P)

$$P = \frac{1}{1 + e^{y}}$$

With the value of y obtained from:

y =constant+b1(Var 1)+b2(Var 2)+...+bn(Var n)

= -3,233-0.336(N)+0.515(Jadwal kemoterapi)+4,81(mRNA PARP1)

From this equation, a test was conducted to assess the model's discrimination ability. The ROC curve showed an AUC of 91..0 % (p < 0.001) (Figure 3, Table 9).

DISCUSSION

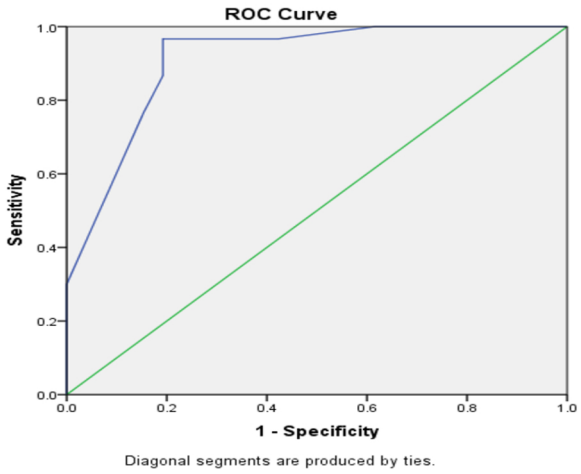

Figure 3. ROC Curve of Binary Logistic Regression Equation Model.

Table 9. Area under the curve of the NAC response probability equation

Variables	AUC	SE	P	IK 95 %
Logistic Regression Equation Model	0.910	0.040	<0.001	0.832-0.988

This study examined the clinical characteristics and chemotherapy response in patients with Triple Negative Breast Cancer (TNBC), a subtype of breast cancer that does not express estrogen, progesterone, or HER2 receptors. Of the 56 samples analyzed, most patients were older than 40 years and postmenopausal. These results differ from most international studies, which report TNBC is more common in young, premenopausal women. This difference is thought to be due to the habit of patients in Indonesia who seek treatment at an advanced stage, resulting in a diagnosis at an older age. These findings align with those of Yeh et al. (23) and Qiu et al. (24), who highlight the influence of early detection factors and the healthcare system on age at TNBC diagnosis. In terms of tumor characteristics, the majority of patients had large tumors (T3–T4), and some had demonstrated lymphovascular invasion (positive LVI). These results reinforce the fact that TNBC tends to be more aggressive with a high rate of metastasis compared to other breast cancer subtypes. Although positive LVI was found in one-third of cases, there was no significant difference between the groups with good and poor chemotherapy responses. Similarly, positive Tumor Infiltrating Lymphocytes (TIL) were observed in nearly half of cases, suggesting that the local immune response may influence therapeutic outcomes but was not the primary determinant in this study (25,26).

Regarding chemotherapy, almost all patients received first-line therapy at standard doses. However, 18 patients experienced delays in chemotherapy schedules, with delays occurring more frequently in the group with a poor response to therapy (46 %) than in the group with a good response (20 %). Although not yet statistically significant, these results suggest that adherence to the chemotherapy schedule may affect treatment effectiveness. Overall, this study confirms that TNBC in Indonesia has a more aggressive clinical profile, tends to be diagnosed at an older age and in the postmenopausal phase, and exhibits variations in chemotherapy response that may be influenced by biological and systemic factors (27,28).

The study results show that high PARP1 gene expression plays a significant role in poor chemotherapy response in triple-negative breast cancer (TNBC) patients. This suggests that PARP1 activation is associated with tumor cell resistance to cytotoxic therapy, potentially reducing the effectiveness of neoadjuvant chemotherapy. These findings align with several previous studies reporting that increased PARP1 activity can enhance DNA repair capacity in cancer cells, thereby reducing sensitivity to DNA-damaging chemotherapeutic agents (29,30). In contrast, high levels of Kinase β/AKT protein were not significantly associated with chemotherapy response in TNBC patients. These results suggest that although the AKT pathway contributes to cancer cell proliferation and survival, its role in chemotherapy resistance in TNBC may be influenced by other factors, including genetic variation, the tumor microenvironment, and interactions with other signaling pathways. Thus, PARP1 gene expression can be used as a potential predictor of neoadjuvant chemotherapy response in TNBC patients, whereas the role of AKT warrants further study using a prospective cohort design and more stringent control of confounding variables (31).

Poly(ADP-ribose) polymerase 1 (PARP1) is a key enzyme in DNA repair mechanisms that maintains cell survival by activating proteins such as histones, p53, and transcription factors. Activation of PARP1 in cancer cells is thought to increase DNA repair capacity and prevent apoptosis, thereby making cancer cells resistant to the cytotoxic effects of chemotherapy. In addition, PARP1 contributes to angiogenesis by increasing the expression of genes associated with hypoxia-inducible factor-1α (HIF-1α), Pecam-1, and osteopontin (OPN), which support tumor growth and metastasis (32).

The results of this study indicate that high PARP1 gene expression is significantly associated with poor chemotherapy response in TNBC patients (OR 121.8; 95 % CI 13 238-1 120.6; p<0.001), and remains an independent predictor after multivariate analysis (Adjusted OR 132.295; 95 % CI 13 305–1 315.42). This finding is consistent with Siraj et al. (33), who reported that nuclear PARP1 overexpression is an independent prognostic factor for poor clinical outcomes, including large tumor size, distant metastasis, and increased Ki-67 expression. Biologically, increased PARP1 expression reflects heightened DNA repair activity, which, in turn, mitigates the cytotoxic effects of chemotherapy. However, Rashed (34) reported that high PARP1 expression correlated with better chemotherapy response in TNBC patients in Egypt. These differences are likely due to population genetic variation, assessment methods, and the type of chemotherapy regimen used. Overall, these results strengthen PARP1's role as a potential biomarker and independent predictor of chemotherapy resistance in patients with TNBC.

Protein Kinase β/AKT is a crucial component of the PI3K signaling pathway, which regulates the proliferation, growth, and survival of cancer cells. AKT activation can increase angiogenesis by regulating hypoxia-inducible factor-1α (HIF-1α) and vascular endothelial growth factor (VEGF), and suppress apoptosis by activating antiapoptotic transcription factors such as NF-κB and CREB (cAMP Response Element-Binding protein). AKT activation also inactivates the p53 tumor suppressor gene, which typically induces apoptosis by increasing proapoptotic factors such as Bax, PUMA, and NOXA. This mechanism makes cancer cells more resistant to the cytotoxic effects of chemotherapy (35). However, this study showed that Protein Kinase β/AKT expression was not significantly associated with neoadjuvant chemotherapy response in TNBC patients (OR 0.852; 95 % CI 0.157-4.636). Although 50 patients showed strong expression and only six patients showed weak expression, the difference was not statistically significant.

This may be due to the limited sample size in the low-expression group or to the presence of other molecular factors that interact with the PI3K/AKT pathway, such as MAPK, Notch, and Wnt/β-catenin. Thus, although AKT is known to contribute to chemotherapy resistance, this study cannot yet demonstrate a significant association between Protein Kinase β/AKT expression and poor chemotherapy response in TNBC. Further studies with a prospective cohort design and a larger sample size are needed to clarify this relationship (36).

The results of multivariate analysis showed that PARP1 gene expression was the most consistent and significant predictor of neoadjuvant chemotherapy response in patients with Triple Negative Breast Cancer (TNBC). After model simplification, PARP1 expression remained significantly associated with poor chemotherapy response, with a high adjusted OR (122.67; 95 % CI, 12.25–1 228.58; $p < 0.001$). This confirms that patients with high PARP1 expression have a greater risk of experiencing resistance to chemotherapy compared to those with low expression. Other clinical factors, such as lymph node status and adherence to the therapy schedule, were not significantly associated, suggesting that PARP1 plays a dominant biological role in the mechanism of chemotherapy response. The resulting prediction model also demonstrated excellent discrimination (AUC 91 %; $p < 0.001$), further supporting the potential of PARP1 as a strong predictive biomarker in TNBC clinical practice (37).

Although these results support a key role for PARP1, interpretation should be cautious due to the limited sample size, which yields wide confidence intervals. Overall, these findings align with those of Rojo et al. (38) and Domagala et al. (39), who reported an association between high PARP1 expression and tumor aggressiveness and resistance to anthracycline- and taxane-based chemotherapy. However, differing results from Oplustilova et al. (2012) showed that high PARP1 expression may increase sensitivity to PARP inhibitor therapy (Olaparib) (31). Therefore, these studies not only confirm PARP1's role as a marker of resistance to conventional chemotherapy but also strengthen its potential as a basis for developing more specific targeted therapies for TNBC patients, supporting the direction toward personalized treatment in the future (40).

Limitations

This study has several important limitations. The use of secondary data can introduce bias due to limited objectivity and reliability in the assessment of study variables. Furthermore, technical constraints affected data completeness, including difficulties obtaining paraffin blocks due to discrepancies between the biopsy facility location and the patient's medical record. Furthermore, not all physicians responsible for the patients (DPJP) proceeded with histopathology examination with immunohistochemistry (IHK), so breast cancer subtypes could not always be definitively identified. This limited sample size could, in turn, affect the robustness of the analysis and the generalizability of the study results.

CONCLUSION

This study indicates that PARP1 gene expression plays a crucial role in determining chemotherapy response in patients with triple-negative breast cancer (TNBC). Increased PARP1 gene expression was associated with poor chemotherapy response, whereas high levels of the kinase β-AKT protein were not significantly associated with this response. Therefore, PARP1 gene expression may be a predictor of poor chemotherapy response in TNBC patients, providing a scientific basis for its use as a molecular biomarker in clinical practice.

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