Breast Disease **40** (2021) S33–S37
DOI 10.3233/BD-219005
IOS Press

Increased aldehyde dehydrogenase 1 (ALDH1) levels are associated with chemo-responsiveness in breast cancer patients treated with taxane–adriamycin–cyclophosphamide regimen

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Abstract.
BACKGROUND: Increased plasma aldehyde dehydrogenase 1 (ALDH1) levels have been proposed to predict cancer chemoresistance. However, studies have reported inconsistent results, depending on the type of cancer cells used.
OBJECTIVE: This study aimed to investigate the correlation between plasma levels of ALDH1 and chemotherapy responses to the taxane–adriamycin–cyclophosphamide (TAC) regimen in breast cancer patients.
METHODS: Thirty breast cancer patients who underwent chemotherapy using the TAC regimen were included in this study. Blood sampling was performed before chemotherapy was initiated and after the first and third cycles of chemotherapy administration. After 3 cycles of chemotherapy, patients were categorized as non-responsive if the tumor size was reduced <30%, if the tumor size remained the same or increased, or if any new tumors were discovered. Patients were defined as responsive after 3 cycles of chemotherapy if the tumor mass disappeared, if the tumor size was reduced by at least 30% of the initial size and if no new tumors were found.
RESULTS: Among the 30 patients, only five were responsive to the TAC regimen. The clinical response to TAC was not correlated with the patient's age, cancer grading, or tumor stage. A change in the ALDH1 levels was observed after the third cycle of TAC administration, with significantly higher ALDH1 levels observed in responsive compared with non-responsive patients ($p < 0.05$).
CONCLUSION: The results of this study may indicate a role for ALDH1 in chemoresponsiveness, rather than chemoresistance, for the TAC regimen in breast cancer patients. Further research remains necessary to confirm this result.

Keywords: Aldehyde dehydrogenase 1, ALDH1, breast cancer, taxane–adriamycin–cyclophosphamide, chemo-responsive

1. Introduction

Breast cancer is the most common malignancy identified in women worldwide. In the United States, breast cancer was reported in 231,840 women in 2016, and the survival rates ranged from 25% to 99% [1]. Breast cancer treatment, particularly at advanced stages, typically relies on systemic chemotherapy that may precede (neo-adjuvant) or follow (adjuvant) surgical procedures [1]. Taxane–adriamycin–cyclophosphamide (TAC) is a neo-adjuvant regimen associated with longer survival than other regimens [2]. TAC has been reported to be more effective than fluorouracil–adriamycin–cyclophosphamide (FAC) for breast cancer treatment, and TAC is more cost-effective than other regimens [3].

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However, the clinical response to chemotherapy may differ between one patient and another. A recent study has shown that chemotherapy treatment may fail due to the presence of cancer stem cells (CSCs), which are responsible for chemoresistance and the initiation of new cancer cell growth. CSCs have self-renewal characteristics similar to those observed in normal stem cells [4], and CSCs have been hypothesized to contribute significantly to breast cancer heterogeneity and the risk of recurrence [5].

Due to the important roles played by CSCs in determining chemotherapy outcomes, many studies are currently focused on identifying specific CSC biomarkers. In addition to CD44 and CD24, increasing evidence has indicated that aldehyde dehydrogenase 1 (ALDH1) may serve as a new biomarker for CSCs [6]. ALDH is an enzyme that converts aldehydes into their corresponding acids and is characterized by broad localization to multiple subcellular areas, including the cytosol, nucleus, and cellular organelles, endoplasmic reticulum, and mitochondria [7]. ALDH1 is a specific ALDH subtype found in the cytosol that plays a role in detoxification and is responsible for the oxidation of intracellular aldehydes [8]. ALDH1 expression has been detected in many stem cells, including in normal stem cells, cancer stem cells, and drug-resistant cancers [9].

The role played by ALDH1 in the prognostic prediction of cancer patient outcomes remains controversial. Several studies have claimed that ALDH1 expression is not correlated with the clinicopathological features of breast carcinoma [10,11] or ovarian cancer [8], whereas other studies have demonstrated that ALDH1 expression is associated with worse responses and reduced survival in breast cancer patients [12,13]. Therefore, this study aimed to investigate the correlation between plasma ALDH1 levels and the clinical responses to TAC chemotherapy in breast cancer patients.

2. Methods

2.1. Population and samples

Thirty breast cancer patients were included in this study. All patients were diagnosed with advanced breast cancer and received TAC chemotherapy at Dr. Wahidin Sudirohusodo Hospital and Hasanuddin University Teaching Hospital. All protocols in this study complied with the institutional ethical committee for clinical research under the Faculty of Medicine, Hasanuddin University (UH19040192).

2.2. Evaluation of chemotherapy response

The clinical response to chemotherapy was measured according to the following formula:

$$\%\text{clinical response} = \frac{\text{initial tumor size} - \text{final tumor size}}{\text{initial tumor size}} \times 100.$$

The calculated clinical response for each patient was categorized as either responsive or non-responsive based on RECIST (response evaluation criteria in solid tumor) criteria [14]. Patients were classified as responsive after receiving 3 cycles of chemotherapy if the tumor mass disappeared, if the tumor mass reduced in size by at least 30% relative to the initial size and if no new tumors were identified. Patients were classified as non-responsive after 3 cycles of chemotherapy if the tumor size was reduced by <30% relative to the initial size, if the tumor size remained the same or increased relative to the initial size, or if any new tumors were discovered.

2.3. ALDH1 plasma analysis

Blood samples were obtained before chemotherapy was initiated and after the first and third cycles of chemotherapy administration. According to the manufacturer's instructions, plasma levels of ALDH1 were analyzed using an ALDH1 kit (BT-Lab) and measured using enzyme-linked immunosorbent assay (ELISA, Thermo Scientific®).

2.4. Statistical analysis

All data are presented as the mean ± standard deviation (SD). All data were tested for normal distribution using the Kolmogorov–Smirnov test. Normally distributed data were analyzed using an independent *t*-test to identify significant differences between the responsive and non-responsive groups. Non-normally distributed data were further analyzed with the Wilcoxon signed-rank test. Correlations between variables were analyzed using Pearson Chi-square or Spearman correlation coefficient analyses. Significant differences were defined at $P < 0.05$.

Table 1
The number of patients who were responsive and nonresponsive to TAC chemotherapy

Category of response	Patient number (*n*, %)	Clinical response ± SD (%)	*P*-value
Responsive	5 (16.70)	+36.5 ± 5.37	0.000
Non-responsive	25 (83.30)	−27.7 ± 3.77	

(+) indicates a reduction, and (−) indicates an increase in tumor size, measured after the third cycle of chemotherapy and presented relative to the initial tumor size. The *P*-value was obtained from a *t*-test analysis comparing the responsive and non-responsive groups.

Table 2
Correlation between patients' age and their clinical response to the TAC regimen

Age	Category of response	*N* (%)	*P*-value*
≥50 years	Responsive	2 (6.7)	0.869
	Non-responsive	11 (36.7)	
<50 years	Responsive	3 (16.7)	
	Non-responsive	14 (46.7)	

*Pearson chi-square analysis.

3. Results

3.1. The clinical response of patients to the TAC regimen

The percentage clinical response was calculated for each patient based on RECIST criteria. In this study, only 5 patients (16.70%) were categorized as responsive, defined as those patients who demonstrated at least a 30% reduction in tumor size. However, 25 patients (83.30%) were categorized as non-responsive, among whom the tumor size increased by 27% ± 3.77% on average (see Table 1).

3.2. Correlation between patients' demographic variables and their clinical responses to TAC regimens

The patients involved in this study were 34–65 years old. As shown in Table 2, the chemotherapy response was not correlated with age, as assessed by Pearson's Chi-square analysis ($p = 0.869$).

This study also attempted to determine whether the clinical response to TAC was affected by the histopathology grade of the tumor mass. Among the patients examined, 6.7% were classified as low-grade, 63.3% as moderate-grade, and 30% were high-grade (Table 3). However, no significant relationship was identified between tumor severity (based on histological grade) and clinical response in this study ($p = 0.314$).

Table 3
Correlation between tumor histology grading and patient's clinical response to the TAC regimen

Histology grading	Category of response	*N* (%)	*P*-value*
Low-grade	Responsive	1 (3.3)	0.314
	Non-responsive	1 (3.3)	
Moderate-grade	Responsive	2 (6.7)	
	Non-responsive	17 (56.7)	
High-grade	Responsive	2 (6.7)	
	Non-responsive	7 (23.3)	

*Spearman's correlation coefficient analysis.

Table 4
Correlation between breast cancer stage and patient's clinical response to the TAC regimen

Breast cancer stage	Category of response	*N* (%)	*P*-value*
Stage II	Responsive	2 (6.7)	0.401
	Non-responsive	7 (23.3)	
Stage III	Responsive	1 (3.3)	
	Non-responsive	13 (43.3)	
Stage IV	Responsive	2 (6.7)	
	Non-responsive	5 (16.7)	

*Spearman correlation coefficient analysis.

In addition to histological grading, the contributions of breast cancer stage to each patient's clinical response to TAC chemotherapy were also analyzed (Table 4). Among patients with Stage II breast cancer, 2 (6.70%) cases were responsive, but most Stage II patients (23.30%) were non-responsive. Among patients with Stage III breast cancer, only 1 was responsive (3.30%), whereas a high percentage of patients were non-responsive (43.30%). Among patients with Stage IV breast cancer, 6.7% were responsive, and 16.7% were non-responsive. Based on Spearman's correlation coefficient analysis, no correlation was identified between breast cancer stage and clinical response ($p = 0.401$).

Table 5
Comparison between ALDH1 levels in breast cancer patients according to whether they were responsive or non-responsive to TAC chemotherapy

Chemotherapy cycle	Clinical response	*N*	ALDH1 level ± SD (ng/ml)	*P*-value*
Pre-chemo	Responsive	5	0.94 ± 0.52	0.933
	Non-responsive	25	1.00 ± 0.70	
1st cycle	Responsive	5	1.33 ± 1.00	0.522
	Non-responsive	25	0.97 ± 0.76	
3rd cycle	Responsive	5	1.79 ± 1.18	0.011
	Non-responsive	25	0.62 ± 0.40	

*Independent-samples *t*-test analysis.

3.3. *Correlation between ALDH1 plasma level and patient's clinical response*

Plasma levels of ALDH1 were examined to investigate whether the ALDH1 plasma level in breast cancer patients could predict the patients' clinical responses to the TAC regimen (Table 5). The differences in ALDH1 levels between responsive and non-responsive patients were analyzed using the independent-samples *t*-test. The ALDH1 levels before the initiation of TAC chemotherapy were not significantly different between the responsive and non-responsive patients. After the first cycle of chemotherapy, the changes in ALDH1 levels were minimal in both groups and were not significantly different ($p = 0.522$).

In contrast, after the third cycle of chemotherapy, a significant increase was observed in the ALHD1 levels of responsive patients, whereas the non-responsive patients presented a reduction in the ALDH1 plasma levels, from 1.00 ± 0.70 ng/dl before treatment to 0.62 ± 0.40 ng/dl after the third cycle of chemotherapy. Consequently, a significant difference in plasma ALDH1 levels was identified between the responsive and non-responsive patients after the third cycle of chemotherapy.

4. Discussion

The detection of CSCs has been associated with cancer recurrence and metastases, and the contribution of CSCs to poor prognosis among patients has been demonstrated in many studies [15]. One biomarker for CSC is ALDH1, which is an intracellular enzyme responsible for cellular detoxification. However, the role played by ALDH1 for the prediction of prognosis among breast cancer patients remains controversial, with contradictory reports described by different studies.

This study categorized patients' clinical responses based on the RECIST criteria. Among the 30 included patients receiving the TAC regimen, 25 patients were classified as non-responsive (83.30%). Instead of tumor size reduction, the majority of these non-responsive patients experienced an increase in tumor size. This clinical response to the TAC regimen was not associated with the patients' age, histological tumor grade, or breast cancer stage, which agrees with the result reported by previous research, in which no association between age, tumor size, histology, chemotherapy response, or survival was reported [16].

In this study, changes in ALDH1 levels in breast cancer patients were observed after chemotherapy, especially after the third cycle, relative to the levels observed before chemotherapy. In our cohort, 22 patients experienced decreased plasma ALDH1 levels, and only 8 patients presented increased plasma ALDH1 levels. Interestingly, most of the patients who presented with increased plasma ALDH1 levels were responsive to the TAC treatment. This finding suggested that higher ALDH1 plasma levels may serve as a good predictor for chemoresponsiveness, rather than chemoresistance, among breast cancer patients who received the TAC regimen. This finding contradicts the findings reported by Ajani et al. (2013), which found that ALDH1 expression was an excellent predictor of chemoresistance to therapy in esophageal/gastroesophageal junction carcinoma [17].

However, the results of our study were supported by the study conducted by Chang et al. (2009), which showed that increased ALDH1 expression was correlated with a favorable clinical response to chemotherapy [8]. Even among breast cancer patients, the role

played by ALDH1 as a predictor of chemotherapy resistance remains debatable. Different types of cancer cells at different clinical stages may also contribute to the contradictory relationships between ALDH1 levels and breast cancer prognosis [13].

5. Conclusion

The clinical responses to TAC chemotherapy varied and were not influenced by patients' ages, histological grades, or breast cancer stages. A significant difference was observed in the plasma ALDH1 levels between responsive patients and those who were non-responsive to the TAC chemotherapy regimen. After the third cycle, the responsive patients presented significantly higher levels of plasma ALDH1 than the non-responsive patients, which may indicate a potential role for ALDH1 in chemoresponsiveness, rather than chemoresistance, to the TAC regimen in breast cancer patients. Further research that includes a larger number of research subjects remains necessary to confirm this result.

Acknowledgements

The authors would like to thank all of the staff members at Dr. Wahidin Sudirohusodo Hospital and Hasanuddin University Teaching Hospital in Makassar for their support during this research.

Conflict of interest

None.

Funding

None.

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