

Aldehyde dehydrogenase-1 expression and prognosis in triple-negative breast cancer

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Abstract

Objective: Understanding of other biological characteristics of Triple-negative breast cancer, the development of new therapeutical approaches and identification of new markers is necessary due to the lack of biological markers like ER, PR and HER2. The purpose of this study was to investigate the prognostic effect of the expression of aldehyde dehydrogenase-1 (ALDH1) in TNBC and its relationship with the clinico-pathological features

Methods: In this study 87 patient files were searched for clinico-pathological data obtained from the files and paraffin blocks. The prognostic value of these clinical data and ALDH1 positivity were evaluated by determining disease-free survival.

Results: TNM stage I vs III ($p=0.03$), vascular invasion ($p=0.05$), chemotherapy indication ($p=0.02$) were significantly associated with DFS. Multivariable analyses didn't demonstrate any statistically significant relationship between ALDH1 ($p=0.61$) and DFS.

Conclusions: We didn't find any statistically significant relationship between ALDH1 positivity and DFS. There was no correlation between ALDH1 expression and tumor's pathological features.

Keywords: aldehyde dehydrogenase 1, immunohistochemistry, triple-negative breast cancer

Introduction

Triple-negative breast cancer is defined by absent expression of the estrogen receptor [ER], progesterone receptor [PR], and the human epidermal growth factor receptor 2 [HER2] negative status [1]. Given the lack of these biologic markers endocrine therapy and HER2-directed therapies are not recommended in the treatment of TNBC [18-20]. Although the same general principles used in the diagnosis and treatment of non-triple-negative breast cancer generally apply to TNBC, it has a lot of differences in risk factors, molecular and histologic features, clinical behavior and chemotherapy sensitivity [2-6]. TNBC defines a heterogeneous mix of breast tumors [7]. TNBC generally has a poorer

prognosis among patients with breast cancer [21]. ER, PR and HER2 are the important biologic markers used in targeted treatment of breast cancer [22]. Understanding of other biological characteristics of TNBC, the development of new therapeutical approaches and identification of new markers is necessary due to the lack of biological markers like ER, PR and HER2. CD44+, CD24-/low and ALDH1+ are the most consistently used biomarkers to identify the breast cancer stem cell groups [16-17]. The clinical importance of ALDH1 in TNBC is a less investigated issue. The purpose of this study was to investigate the prognostic effect of the expression of aldehyde dehydrogenase-1 (ALDH1) in TNBC and its relationship with the clinico-pathological features

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Materials and Methods

In this study we included 87 patients followed between 2000 and 2012 in Cerrahpaşa Faculty of Medicine, Department of Medical Oncology Clinic diagnosed with TNBC. Formalin-fixed, paraffin-embedded tissues of these patients were retrieved from the Department of Pathology, in the same hospital.

Number of patients	87 total
Age	52.7years (28-80)
Family history	67 total
Yes	28 (41.8%)
No	39 (58.2%)
Height	159cm (134-177)
Body weight	73.5kg (51-159)
BSA	1.7m ² (1.42-2)
Chemotherapy treatment	
Neoadjuvant	14 (16.1%)
Adjuvant	59 (67.8%)
Metastatic	13 (14.9%)
No treatment	1 (1.1%)

The patient files were searched retrospectively for age, sex, comorbidities, the presence of family history of cancer, breast cancer detection methods, pregnancy/number of children, height, weight, BSA, ECOG, biopsy date, whether or not operated, type of surgery, tumor size / TNM stage and localization, axillary nodal status, the presence of the tumor in the border of surgery, pathologic evaluation, the first chemotherapy option: adjuvant, neoadjuvant or metastatic, chemotherapy regimen series, number of cycles, the start/end dates and all were recorded.

The surveillance after treatment for early stage breast cancer was performed each 3 months for the first two years, every 6 months for the next three years and then every year after five years. Patients with metastatic disease were followed with a surveillance routine after receiving 3 and 6 cycles of chemotherapy.

Formalin-fixed, paraffin-embedded tissues was cut into 3 µm thick sections, deparaffinized, stained with hematoxylin-eosin and

Tumor size (cm)	3.1 cm (0.7-10)
Primary Tumor (T)	87 patients
T1	17 (19.5%)
T2	45 (51.7%)
T3	10 (11.4%)
T4	15 (17.2%)
Regional Lymph Nodes (N)	87 patients
N0	38 (43.7%)
N1	35 (40.2%)
N2	12 (13.8%)
N3	2 (2.3%)
Anatomic Stage	87 patients
IA	11 (12.6%)
IIA	28 (32.2%)
IIB	19 (21.8%)
IIIA	8 (9.2%)
IIIB	6 (6.9%)
IIIC	2 (2.3%)
IV	13 (14.9%)
Dissected axillary lymph node	12.6 (1-38) (83 patients)
Positive axillary lymph node	2.7 (0-23) (83 patients)
Nuclear grade	78 patients
grade 1	9 (11.5%)
grade 2	30 (38.5%)
grade 3	39 (50%)
Histological type	86 patients
Invasive ductal carcinoma	73 (84.9%)
other histological subtypes	13 (15.1%)
Lymphatic invasion	70 patients
Yes	32 (45.7%)
No	38 (54.3%)
Perineural invasion	69 patients
Yes	6 (8.7%)
No	63 (91.3%)
Vascular invasion	68 patients
Yes	7 (10.3%)
No	61 (89.7%)
Multicentric	76 patients
Yes	8 (10.5%)
No	68 (89.5%)
Histological grade	86 patients
Grade 1	2 (2.3%)
Grade 2	23 (26.7%)
Grade 3	61 (70.1%)
Tumor localization	87 patients (129 localizations)
upper outer quadrant	69 (53.5%)
upper inner quadrant	21 (16.3%)
lower outer quadrant	23 (17.8%)
lower inner quadrant	14 (10.8%)
Periareolar	2 (1.6%)

immunohistochemically with ALDH1 (ALDH1a1: Biocare assay kit) then examined by light microscopy.

The prognostic value of these clinical data and ALDH1 positivity were evaluated by determining disease-free survival and tumor characteristics from the clinicopathological data obtained from the files and paraffin blocks.

Immunohistochemical staining of ALDH1 was classified as positive when tumor cells showed cytoplasmic positivity and negative when tumor cells showed cytoplasmic negativity.

The prognostic value of these clinical data and ALDH1 positivity were evaluated by determining disease-free survival and tumor characteristics from the clinicopathological data obtained from the files and paraffin blocks. Among 87 files included in the present study, the metastatic ones and those who received neoadjuvant therapy but found to be metastatic before receiving adjuvant therapy were excluded. Statistical analyses include 71 patients.

The DFS (disease-free survival) was defined as the time from the diagnosis to the date of breast-cancer-derived relapse/metastasis.

DFS for patients who received adjuvant therapy (month): (the date of relapse or last visit date for those without relapse – operation date)/30, for those who received neoadjuvant therapy (month): (the date of relapse or last visit date for those without relapse – operation date after neoadjuvant therapy)/30.

Statistical Analysis

Statistical analysis was performed using the SPSS (SPSS 16.0, SPSS Inc. Chicago, Illinois). DFS was calculated with the Kaplan–Meier technique, analyses of the prognostic factors and DFS were performed with the log-rank test. Multivariable analyses were conducted for the factors statistically significant in the Cox Proportional Hazards model.

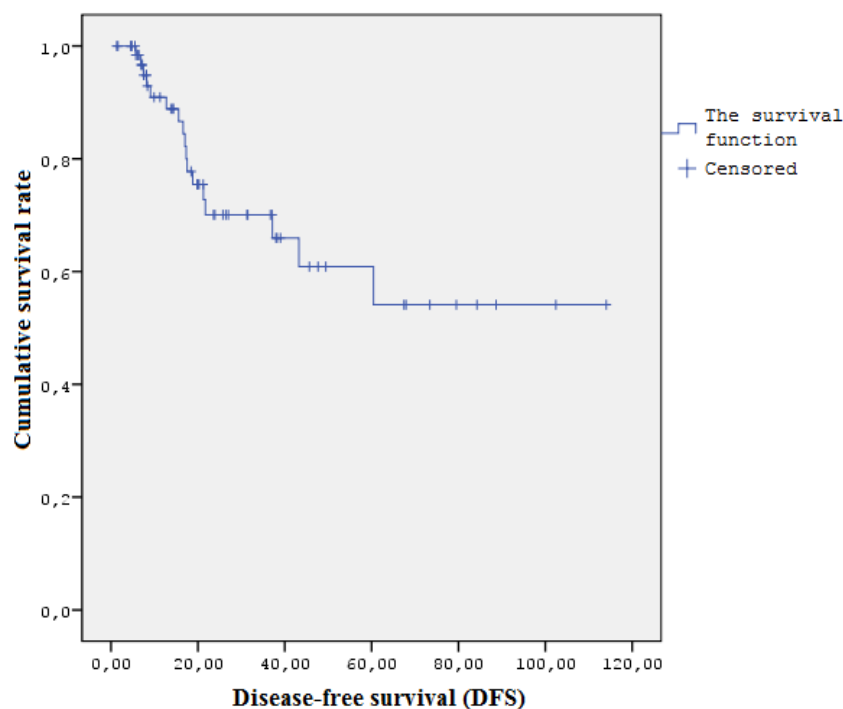


Figure 1: DFS for non-metastatic group

Results

Study population: The age on the patient files included in the present study ranged from 28 to 80 years old (mean of 52.7 years). 62 patients had no comorbidity, 5 had diabetes mellitus, 17 had essential hypertension, and 2 had hypothyroidism. The family history was not noted in 20 percent of the patient files. 39% of the patients had no family history, 28% of them had cancer diagnosed in their family. Based on the diagnostic evaluation of suspected breast cancer one of the patients was diagnosed after physical examination, one during routine screening and all others (n: 85) were suspected by their self-examination. Height ranged from 134 to 177cm (mean of 159 cm), body weight 51 to 159 kg (mean of 73.5kg), Body Surface Area (BSA) ranged from 1.42 to 2m²(mean of 1.7 m²).

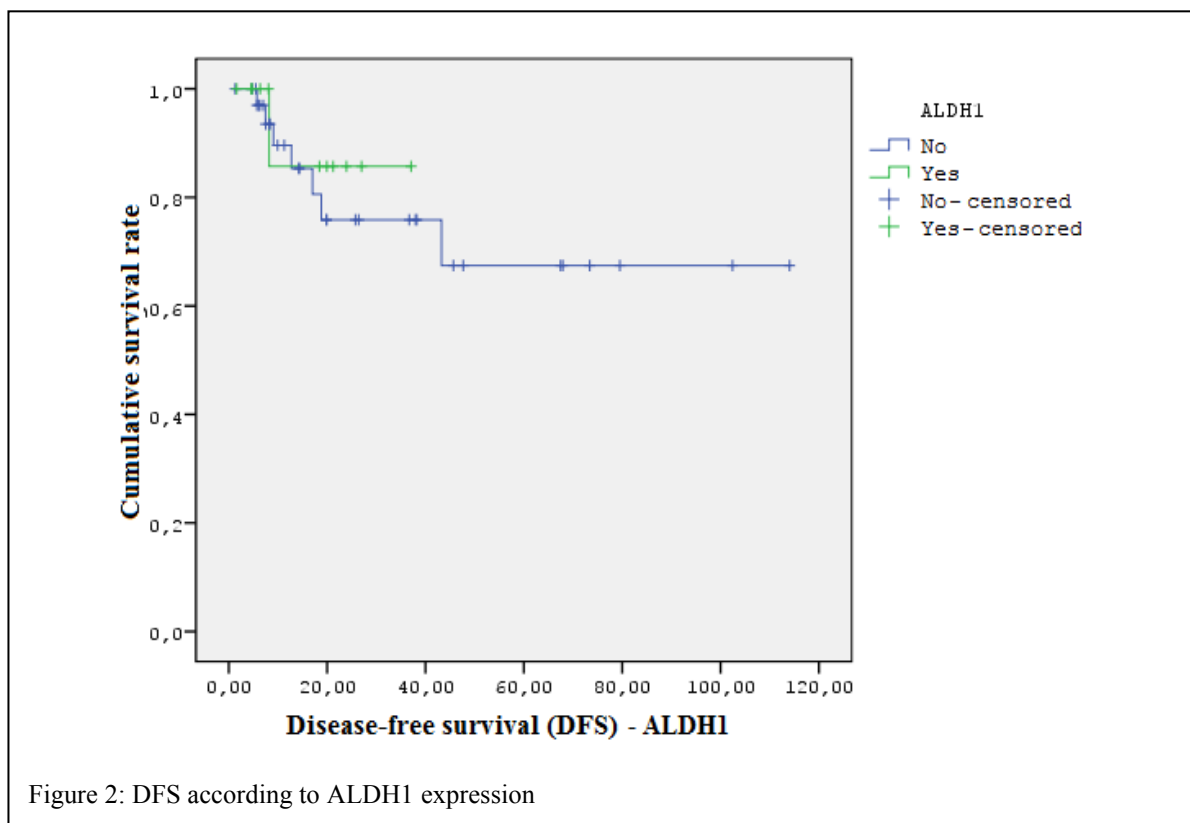
Tumor characteristics: Among all the patients 13 (14.9%) were metastatic at the beginning, 14 (16.1%) received neoadjuvant treatment, 59

received adjuvant treatment, 1 (1.1%) didn't receive any treatment. Tumor size was mean of 3.1 cm (0.7-10 cm). 17 of the cases (19.5%) were T1, 45(51.7%) T2, 10 (11.4%) T3, 15 (17.2%) T4. 38 of the cases were N0, 35 N1, 12 N2, 2 N3.

Of the 78 patients with known nuclear grade 9 were (11.5%) grade 1, 30 (38.5%) grade 2 and 39 (50%) grade 3. According to the histological grade 2.3% grade 1, 26.7% grade 2 and 70.1% grade 3. According to the anatomic stage 11 IA, 28 IIA, 19 IIB, 8 IIIA, 6 IIIB, 2 IIIC, 13 IV.

Of all patients included in the present study, 84.9% of cases were invasive ductal carcinoma, 15.1% the other histological types.

45.7% of the tumors showed lymphatic invasion whereas 54.3% showed no invasion. 8.7% of the tumors showed perineural invasion whereas 91.3% showed no invasion. 10.3% of the tumors showed vascular invasion whereas 89.7% showed no invasion. 10.5% of the tumors were multicentric whereas 89.5% were unicentric. Among 87 patients,



51 had tumor on their right breast, 36 on their left breast. Of the 129 localizations seen (knowing than some of the tumors locate in more than one quadrant) 69 (53.5%) were located upper outer, 21 (16.3%) upper inner, 23 (17.8%) lower outer, 14 (10.8%) lower inner, 2 (1.6%) periareolar.

Surgical treatment: Among 70 patients undergoing surgery, 6 Mastectomy and Sentinel lymph node biopsy (SLNB), 23 Breast-conserving surgery (BCS) and Axillary lymph node dissection (AD), 11 Breast-conserving surgery (BCS) and Sentinel lymph node biopsy (SLNB), 27 Modified radical mastectomy (MRM), 2 Roll excision, 1 Total mastectomy was performed.

Medical treatment: 87 patients included in this study received chemotherapy, 59 of them adjuvant (8 AC, 27 FEC/FAC, 6 FAC+Taxane, 11 AC+Taxane, 5 CMF, 1 Cyclophosphamide + Taxane), 14 neoadjuvant (10 FAC+Taxane, 1 FEC/FAC, 1 AC+Taxane, 1 Cyclophosphamide + Taxane, 1 Taxane), 13 metastatic disease chemotherapy regimens, 1 patient didn't accept the treatment.

Disease-free survival (DFS) results: A total of 18 patients (25.4%) had relapsed during the follow-up. The median follow-up period until relapse was 18.64 months (0.4 - 46.7). The median follow-up period for all patients was 714 days (31-2645). DFS of non-metastatic group is shown on Figure 1. A total of 11 patients were found to be ALDH1-positive. Analysis of the DFS showed no difference among ALDH1-positive and ALDH1-negative tumors ($p=0.61$) (Figure 2)

Multivariable analyses were conducted for the factors that were associated with DFS in the univariable analyses. Multivariable analyses didn't demonstrate any statistically significant relationship between ALDH1 ($p=0.930$), other prognostic factors and DFS. There was no correlation between

ALDH1 expression and tumor's pathological features.

Discussion

Breast cancer is a heterogeneous disease with a lot of histological special types showing different molecular, histologic and clinic features [15]. We achieved a significant reduction in breast cancer mortality with the screening programs and treatment facilities in adjuvant treatment of early stage breast cancer. The mortality rates are being reduced even more by the use of targeted therapy. TNBC accounts for 15% to 20% of breast cancers [3]. Endocrine therapy and HER2-directed therapies are not used in the treatment of TNBC since it is characterized by the lack of biological markers like ER, PR and HER2 [23]. TNBC generally has a poorer prognosis compared to patients with other breast cancer subtypes. Considerable effort has been made to develop new therapeutical approaches in TNBC [8].

The classical pathological variables such as tumor grade, lymph node status, and tumor size, are the most important prognostic factors in breast cancer. Ki67 status, tumor grade, lymphatic, perineural, vascular invasion are also independent prognostic factors that can affect the therapy choice [9, 11].

Based on the diagnostic evaluation of suspected breast cancer one of the patients was diagnosed after physical examination, one during routine screening and all others ($n:85$) were suspected by their self-examination. These findings support the fact that TNBC will more likely present as an 'interval cancer' between two screenings. These findings also show that TNBC screening and diagnosis need to be improved [12-14].

In our study 14 patients (16.1%) received neoadjuvant therapy because of locally advanced disease, 59 patients (67.8%) received adjuvant

therapy. DFS of patients receiving neoadjuvant therapy was statistically significant short than others ($p=0.02$, 24 months DFS; %31.2 vs %80.5). This finding maybe reflective of locally advanced disease, but also specially in TNBC may suggest that neoadjuvant chemotherapy may have limited effect and delaying of the surgical treatment may affect the prognosis.

A total of 11 patients were found to be ALDH1-positive and this positivity doesn't seem to be statistically significant alone. ALDH1 as a biomarker to identify the breast cancer stem cell groups that can lead to targeted therapies in breast cancer was not found to be a prognostic value alone. Further research combining with other biomarkers and with a greater number of patients is necessary to confirm the role of ALDH1 in TNBC.

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Conflict of Interest: The authors declared that they had no conflicts of interest.

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