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Association between expression of ALDH1 and TGFb2 genes, histological and clinical findings in breast cancer



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Yasan Sadeghian¹, Esmail Samizadeh², Mohammad Ali Mohammadi-vajari³,
Mohammad Hossein Lashkari^{4*}, Ehsan Sadeghian⁵, Seyedeh Nasim Hosseini⁶,
Mehdi Jafari⁷, Tahereh Sorbi⁸

¹General Physician, School of Medicine, Shahid Beheshti University of Medical Sciences and Medical Researcher, AJA University of Medical Sciences, Tehran, Iran

²Pathologist, Department of Pathology, School of Medicine, Imam Reza Hospital, AJA University of Medical Sciences, Tehran, Iran

³General physician, School of Medicine, Tehran University of Medical Sciences, Tehran, Iran

⁴Professor of Surgery, School of Medicine, AJA University of Medical Sciences, Tehran, Iran

⁵Resident, Department of General Surgery, School of Medicine, Tehran University of Medical Sciences, Tehran, Iran

⁶Biotechnologist, National institute of genetic engineering and biotechnology (NIGEB), Tehran, Iran

⁷Epidemiologist, Digestive Disease Research Institute, Tehran University of Medical Sciences, Tehran, Iran

⁸Anesthesiologist, Department of Anesthesiology, School of Medicine, Shariati Hospital, Tehran University of Medical Sciences, Tehran, Iran

*Corresponding to:
Mohammad Hossein Lashkari;
Department of General Surgery,
AJA University of Medical Sciences,
Tehran, Iran;
mhlashkari8@gmail.com

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ABSTRACT

Background: Breast cancer is one of the most common malignancies. The relationship between the pathogenesis of breast tumours and their invasive behaviour is an important issue that has been discussed recently. ALDH1 and TGFb2 genes can be mentioned among the most crucial signalling and molecular pathways that play an active role in tumour invasive behaviour. The aim of this study was to determine the expression level of ALDH1 and TGFb2 genes in breast cancer and its relationship with histology in breast cancer patients.

Method and material: This cross-sectional study was done on 65 breast cancer patients who had been referred to the Army 501 hospital during the years of 1992-1994. Data was collected using the patient records, and the results of immunohistochemically staining were obtained in the laboratory. Data were analyzed by SPSS software using t-test, Chi-square and Fisher tests. The level of

significance was considered to be 0.05.

Results: In this study, we assessed the expression of these genes in tissue samples of 65 patients with breast cancer and its relation with the histology and clinical progress. The expression of ALDH1 in the malignant breast tissue was 83.5% (55 out of 65 samples) and the expression of TGFb2 gene showed 54 samples (83.1%) were positive. There was a significant relationship between lymphatic drainage and ALDH1 expression.

Conclusion: There was a significant relationship between ALDH1 expression, tumour size, neuronal invasion, tumour grade, metastasis, and lymph node involvement. There was also a significant relationship between TGFb2 expression and tumour size, metastasis, tumour grade, and lymph node involvement. There was no significant relationship between the expression of ALDH1, TGFb2, and the type of pathology and history of chemotherapy.

Keywords: breast cancer, pathology, ALDH1, TGFb2

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INTRODUCTION

Breast cancer is the most common malignant disease, most fatal and emotionally and psychologically most effective cancer in women. It is also the second leading cause of cancer death after lung cancer among women in the world.^{1,3,4} Currently, this type of cancer is the most prevalent cancer in developed and developing countries.⁴ In Iran, 37,000 women in Iran suffer from breast cancer each year.^{2,3,5} Several risk factors such as benign breast diseases, history of previous breast cancer, especially at lower levels, familial history and genes, increase the probability of this cancer by 2 to 3 times.²⁻⁹

Various factors such as tumour size, the involvement of lymph nodes, malignancy, metastasis to distant areas, and age are considered as factors in the long-term prognosis of patients.

There is a theory that tumours like breast tumours have cancer stem cells that can reproduce cancer cells after a long period of recovery from the disease, which leads to relapse, metastasis and ultimately death. Unlike previous studies that required the presence of several superficial surface markers for the recognition of stem cells in breast cancer, recent studies have suggested the first isoform of aldehyde dehydrogenase (ALDH1) as a simple method for the identification and isolation of stem cells. These studies indicated that ALDH1 is a marker of breast cancer in the stem cells and is associated with a worse tumour prognosis and a shorter patient's life expectancy.¹⁰

Another important route known as breast cancer malignancies is Transforming Growth Factor Beta 2 (TGFb2). In normal cells of epithelial breast and the early stages of cancer progression, TGFb2 acts as a growth inhibitor with its repressive effect on tumour

Table 1. Frequency distribution of clinical findings by age groups

Diagnosis	Overall mean	Age		Total
		<40	>40	
DCIS	17.75	1	7	8
IDC	14.51	12	38	50
ILC	10.80	1	4	5
PC	15.50	1	1	2
Total		15	50	65

Table 2. Frequency distribution of clinical findings by grade of malignancy

Diagnosis	Grade of malignancy			Total
	1	2	3	
DCIS	0	5	3	8
IDC	6	20	24	50
ILC	0	5	0	5
PC	0	2	0	2
Total	6	32	27	65

Table 3. Final diagnosis and vascular and lymph node involvement

Diagnosis	Vascular involvement		Lymph node involvement		Total
	Yes	No	Yes	No	
DCIS	2	6	2	6	8
IDC	23	27	32	18	50
ILC	0	5	2	3	5
PC	0	2	0	2	2
Total	25	40	36	29	65

Table 4. Relationship between the grade of malignancy and vascular and lymph node involvement

Grade of malignancy	Vascular involvement		Lymph node involvement		Total
	Yes	No	Yes	No	
1	3	3	5	1	6
2	8	24	13	19	32
3	14	13	18	9	27
Total	25	40	36	29	65

growth through inhibition of cell proliferation. But eventually, with the advancement of cancer, TGF β 2 finds oncogenic function and tumour induction and increases the potential for invasion and metastasis.¹¹⁻¹³ A previous study by Marcato, *et al.* showed a significant statistical relationship between neurovascular invasion, tumour grade, lymphatic involvement and ALDH1 expression among 19 cases.¹⁴ Another study by Robertson *et al.* in 2012 performed TGF β 2 signalling on seven pre-clinical models, suggesting that the genes involved in TGF β 2 signalling are not expressed or expressed less in patients with breast cancer.¹⁵ Therefore, the aim of this study was to determine the level of expression of ALDH1 and TGF β 2 genes in breast cancer and its relationship with the histology and clinical findings in breast cancer patients.

METHOD

This cross-sectional study was done on a total of 65 breast cancer patients who had been referred to the Army 501 hospital during the years of 92-94. The sampling method was preparatory and non-probable, and all eligible patients were enrolled in the study. In this study, data was collected using the patient records, and the results of immunohistochemical staining were obtained in the laboratory. Information about each patient was recorded in the data collection sheets that were already prepared for this task. The results of all sample data including sample number, diagnosis, grading and tumour characteristics, and age of the patient were extracted from the patient records in the hospital archives. The slides of patients with various types of breast cancer like patients with fibro-adenoma were removed from the arch of the lam section among all types of breast tissue pathologies in this centre. H & E slides of blocks were reviewed by a cancer pathologist who did not know the patient's history in pathology section and the suitable paraffin block with minimum bleeding and necrosis and appropriate fixation was selected for the study. The IHC test was done on paraffin blocks in Professor Kamalian's laboratory. Samples were incubated for one hour with primary antibodies: Concentrated monoclonal rabbit, clone: ALDH1 EP1933 (Biocare Medical) and TGF β RII (L-21): sc-400 (Santa Cruz Biotechnology). Data were analyzed by SPSS software using t-test, Chi-square and Fisher tests. The level of significance was considered to be 0.05.

RESULTS

This study was performed on pathological specimens of 65 patients. The mean age of the

Table 5. Relationship of gene expression (ALDH1 and TGFb2 expression) and clinical findings

ALDH1 expression	Diagnosis				Total
	DCIS	IDC	ILC	PC	
Positive	5	45	4	1	55
Negative	3	5	1	1	10
Total	8	50	5	2	65
TGFb2 expression	DCIS	IDC	ILC	PC	Total
Positive	5	44	4	1	54
Negative	3	6	1	1	11
Total	8	50	5	2	65

Table 6. Relationship of ALDH1 expression and pathological characteristic

ALDH1 expression	History of chemotherapy		Neurovascular invasion		Lymphatic involvement		Metastasis	
	Yes	No	Yes	No	Yes	No	Yes	No
Positive	50	5	25	30	35	20	20	35
Negative	9	1	0	10	1	9	2	8
Total	16	2	25	40	36	29	22	43

subjects was 51.6 ± 13.5 . The mean age in Ductal Carcinoma in Situ (DCIS) group was 55, Papillary Carcinoma (PC) group was 59, Invasive Ductal Carcinoma (IDC) group was 50.42, and Invasive Lobular Carcinoma (ILC) group was 54.8 years old. Fifteen patients (23.1%) were less than 40 years old, and 50 (76.9%) were over 40 years old. All of the patients were women. Thirty-one samples of breast cancer (47.8%) found on the left side and 34 (52.2%) were on the right side. Among the total samples, 8 cases (12.3%) were reported by DCIS, 50 cases (76.9%) IDC, 5 cases (7.7%) ILC and 2 cases (3.1%) PC as seen in Table 1.

According to the tumour grade of malignancy, 6 samples (9.3%) were grade 1, 32 samples (49.2%) were grade 2, and 27 samples (41.5%) were grade 3. The relationship between the grade of malignancy and the final diagnosis is shown in Table 2.

In the Nottingham Score, 1 sample (1.5%) had a score of 3, 5 samples (7.7%) had score 5, 25 samples (38.5%) had score 6, 8 samples (12.3%) had score 7, 20 samples (30.8%) had score 8 and 6 samples

(9.2%) had score 9. Overall, 25 (38.5%) cases had vascular and nervous involvement (Table 3 and 4).

According to the expression of ALDH1 gene, 55 samples (83.5%) were ALDH1 positive, and 10 (16.5%) were negative. There was significant relationship between positive expression and metastasis ($p=0.03$), lymph node involvement ($p=0.02$), tumour grade ($p=0.04$), vascular invasion ($p=0.04$) and tumour size ($p=0.03$). Whereas, there was no relationship between gene expression and age, pathology and history of chemotherapy ($p>0.05$) (Table 5 and 6).

The expression of TGFb2 gene showed 54 samples (83.1%) were positive, and 11 samples (16.9%) were negative. In the group with positive TGFb2 gene expression, 21 had metastasis, and in the group with negative gene expression, only 1 metastasis was present. ($p=0.03$) Also, 34 cases with positive expression had lymph node involvement, and in the group with negative gene expression, only 2 people were involved ($p=0.04$). There was a significant difference between positive gene expression and neuronal invasion ($p = 0.01$), and tumor size ($p = 0.02$). But there was no relationship between gene expression and age (0.6), pathology ($p=0.51$) and history of chemotherapy ($p=0.7$) (Table 7 and 8).

DISCUSSION

Breast cancer is one of the most common malignancies. The relationship between the pathogenesis of breast tumours and invasive tumour behaviour is an important issue that has recently been mentioned. Among the most important signalling and molecular pathways that play an active role in the invasive behaviour of the tumour, aldehyde dehydrogenase 1 (ALDH1) and Transforming Growth Factor Beta 2 (TGFb2) can be mentioned.⁹ The first isoform of aldehyde dehydrogenase, ALDH1, is a marker of breast cancer in stem cells, and its occurrence is associated with worse tumour prognosis and shorter patient longevity.¹¹ Another important pathway known in breast cancer malignancies is TGFb2, which is one of the regulators of important biological processes, including cell proliferation, differentiation, migration and apoptosis, it has an intricate role in the development of cancer due to its suppressive functions.¹¹ In this study, we assessed the expression of ALDH1 and TGFb2 genes in tissue samples of 65 patients with breast cancer and its relationship with the histology and clinical progress.

The positive expression of ALDH1 in malignant breast tissue was 83.5% (55 out of 65 samples). The rate of expression of ALDH1 found vary in different studies. Ohi, *et al.* study showed 60% of 106 samples

Table 7. Relationship of TGFb2 expression and pathological characteristic

TGFb2 expression	History of chemotherapy		Neurovascular invasion		Lymphatic involvement		Metastasis	
	Yes	No	Yes	No	Yes	No	Yes	No
Positive	49	5	22	32	34	20	21	33
Negative	11	0	3	8	2	9	1	10
Total	60	5	25	40	36	29	22	43

Table 8. Relationship of gene expression (ALDH1 & TGFb2 expression) and grade of tumour

ALDH1 expression	Tumour grade			Total
	1	2	3	
Positive	6	23	26	55
Negative	0	9	1	10
Total	6	32	27	65
TGFb2 expression	1	2	3	Total
Positive	6	22	26	54
Negative	0	10	1	11
Total	6	32	27	65

expressed ALDH1²², whereas a study by Zheng, *et al.* showed 64% of 75 samples.^{20,22} The expression of ALDH1 was reported from 4% to 13.4% in another study.¹⁶ In Nalwoga study, on 192 breast tumours, ALDH1 expression was positive in 48% of the samples. Morimoto also showed that among 203 breast cancer samples, only 10.3% of cases expressed ALDH1. Charafe-Jauffret reported an increase of 34% in ALDH1 expression in inflammatory breast cancer. Resetkova also reported this by 18%. In our study, there was a significant relationship between lymphatic involvement, tumour size, neurovascular invasion, tumour grade, metastasis, and expression of ALDH1. This result is similar with the results of the study by Ghorbani A and Tahamtani F.^{16,17} Due to these findings can ALDH1 as a prognostic factor to be used for determination of patient's situation. In our study, there is a statistically significant relationship between TGFb2 expression and tumour size, metastasis, tumour grade, and lymph node involvement. These findings support the result of the previous study by Zheng and Gaos studies.²⁰⁻²³ From this study, authors have realized that these findings still have some limitations. Therefore, further explorations are needed to provide novel insights into this kind of study.

CONCLUSION

There was a significant relationship between tumour size, neuronal invasion, tumour grade, metastasis, lymph node involvement, and expression of ALDH1 dan TGFb2. There was no significant relationship between the expression of ALDH1, TGFb2, and the type of pathology and history of chemotherapy, which can be due to 1- Different sample size 2- Difference in agreed percentage to approve the positive markers 3- different techniques.

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ETHICAL CLEARANCE

This study has obtained ethics approval from the Ethics Committee of AJA University of Medical Sciences, Tehran University of Medical Sciences, and Army 501 hospital prior to the study conducted.

CONFLICT OF INTEREST

We declare that there were no conflicts of interest in this study.

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AUTHOR CONTRIBUTION

All of authors are equally contributed to the study from the study framework, data gathering, data analysis, until reporting the result of study.

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