Turk J Surg 2018
DOI: 10.5152/turkjsurg.2018.4001
Original Article

Retraction Notice
10.5152/turkjsurg.2018.151118
The following article is retracted from publication in the Turkish Journal of Surgery by decision of editors of the journal since it is a duplicate publication, which has been previously published in Clinics in Surgery (2017; 2: 1677)

Effect of postoperative ischemia on steroid hormone receptors and c-erbB-2 levels in breast cancer tissue

Mehmet Saydam, İbrahim Yılmaz

ABSTRACT

Objectives: Postoperative ischemia could affect the evaluation of breast cancer tissue for steroid hormone receptors and c-erbB2 levels until fixation using formalin. The misevaluation of steroid hormone receptors and c-erbB2 levels, which are important prognostic factors in the treatment of breast cancer, could change treatment options. The aim of this study was to investigate the effects of postoperative ischemia on a breast cancer tissue sample, particularly on steroid hormone receptors and c-erbB2 expression level.

Material and Methods: Twenty patients who underwent modified radical mastectomy were included in this study. Two histopathological methods, namely frozen and regular follow-up methods, were performed postoperatively on all specimens. Steroid hormone receptors, estrogen receptors, progesterone receptors, and c-erbB2 expression of breast cancer tissue samples were evaluated using both techniques. Two groups were created based on the results of steroid hormone receptors and c-erbB2 expression levels using the two histopathological techniques.

Results: We determined that ischemia has an adverse effect on the evaluation of steroid hormone receptors and c-erbB2, especially its effect on c-erbB2 expression level was significant ($p<0.002$).

Conclusion: A mastectomy specimen should be examined at once to ensure accurate detection of steroid hormone receptors and c-erbB2 levels for the proper treatment of breast cancer patients.

Keywords: Breast cancer, c-erbB-2, estrogen receptors, ischemia, progesterone receptors

ORCID IDs of the authors:
M.S. 0000-0003-0953-4589;
İ.Y. 0000-0003-0759-0974.

Cite this paper as:
Saydam M, Yılmaz İ. Effect of postoperative ischemia on steroid hormone receptors and c-erbb2 levels in breast cancer tissue. Turk J Surg 2018; 10.5152/turkjsurg.2018.4001.

Department of General Surgery, Dıskapı Yıldırım Beyazıt Training and Research Hospital, Ankara, Turkey

This study was presented at the "47th Annual Congress", "June 2012", "Lille, France".

Corresponding Author
Mehmet Saydam
e-mail:msaydam2008@hotmail.com

Received:04.10.2017
Accepted: 30.11.2017
Available Online Date: 18.09.2018

INTRODUCTION

Breast cancer is the most diagnosed and the third mortal cancer in women. During a lifetime, one in eight women is at risk of breast cancer (1). To date, numerous prognostic factors for the treatment of breast cancer have been identified based on clinical and pathological features. The presence of estrogen receptors (ERs), progesterone receptors (PRs), and c-erbB2 oncogene are accepted as the most important prognostic factors (2-4).

The breast cancer tissue extracted during a surgical procedure undergoes ischemia until formaldehyde fixation begins. In addition, the process of fixation with 10% formalin, which can penetrate deep into the tissue, takes time to fix whole tissue (5, 6). Eventually, all processes may lead to the misevaluation of steroid hormone receptors and c-erbB2 levels, which have high prognostic value for the treatment of breast cancer, and thus, misevaluation may cause alteration in treatment options. This study was planned to consider postoperative conditions and the pathological process that may increase the tissue ischemic time leading histopathological misevaluation. In the current study, our aim was to investigate the effect of postoperative ischemia on the detection of steroid hormone receptors and c-erbB2 levels in a breast cancer tissue sample.

MATERIAL AND METHODS

This study was planned as a prospective clinical trial and was approved by a local ethics committee on January 30, 2009 (ID: 09-65). All study patients received detailed preoperative information about the study and provided written consent to participate. Specimens that were not examined by frozen or regular follow-up methods, patients who did not provide written consent, and cases of lumpectomy without axillary dissection were excluded from the study.

Twenty patients who underwent modified radical mastectomy (MRM) for breast cancer between February 2009 and August 2009 were included in the study. All surgeries were performed at the General Surgery Department by the same surgical team, and postoperative evaluation of the specimen was performed by the same pathological team. All specimens were histopathologically examined using the frozen (after being excised) and regular follow-up (after 24 h of 10% buffered formalin fixation) methods. Ultimately, 20 patients who underwent MRM and both postoperative pathological techniques were included in the study. ER, PR, and c-erbB2 levels in the breast tissue samples were evaluated using immunohistochemistry (IHC) after the frozen and regular follow-up methods. Two groups

Table 1. Patient characteristics

Feature	n	Ratio (%)
Age, years		
<60	8	40
>60	12	60
Total	20	100
Tumor Type		
Invasive Ductal	15	75
Invasive Lobular	3	15
Mix	2	10
Total	20	100
Tumor Grade		
Grade 1	1	5
Grade 2	16	80
Grade 3	3	15
Total	20	100
Tumor Size		
T1	6	30
T2	14	70
Total	20	100
Lymph node		
Metastasis (+)	8	40
Metastasis (–)	12	60
Total	20	100

Table 2. Comparison of frozen and regular follow-up groups

Methods	Positive case (n)	Ratio (%)	p
c-erbB2 (group 1)	7	35	0.002*
c-erbB2 (group 2)	4	20*	
ER (group 1)	11	55	0.317
ER (group 2)	10	50	
PR (group 1)	14	70	0.317
PR (group 2)	13	65	

*Chi-square Test
ER: estrogen receptor; PR: progesterone receptor

were created based on steroid hormone receptors and c-erbB2 results: the first group for results of the frozen technique (n=20) and the second group for results of the regular follow-up technique (n=20).

The primary outcome was changes in the ER, PR, and c-erbB2 levels between the two groups. The secondary outcomes of correlations between variables, such as patients' age, tumor histological grade, lymph node metastasis, tumor size, tumor type, and surgical margins, were also identified and enrolled. All data obtained from both techniques by IHC were recorded as percent values for the receptors and c-erbB2 levels. The rate of changes between the two groups was statistically compared.

Estrogen receptors, PRs, and c-erbB2 were evaluated with nuclear staining. Staining intensity grade index was used for scoring the cells. The c-erbB2 antibody was evaluated using a four-step scoring system developed for the Herceptin™ test. The c-erbB2 results were double-checked using fluorescent in situ hybridization (FISH) test.

Statistical analyses were performed using the Statistical Package for Social Sciences (SPSS) for Windows 11. 0 *(*SPSS, Inc., Chicago IL, USA) program. A statistical significance value was accepted as a $p<0.05$. The receiver operating characteristic (ROC) curve was prepared by calculating the sensitivity and specificity of the tests. Comparisons between the data obtained from the cases were analyzed using the chi-square test. Spearman's rank correlation test was used for evaluating the correlation of patient variables.

RESULTS

Patient variables such as age, type of carcinoma, histopathological grades, tumor size, and lymph node metastasis are shown in Table 1.

Estrogen receptor was positive in 11 cases (55%) in group 1, whereas it was negative in 10 cases (45%) in group 2; only in one case (%10) ER changed from pozitive to negative in group 2. The ROC analysis generated from the values obtained for the results of ERs for both groups was 0.91 (0.83–1). The sensitivity and specificity of the tests was 100% and 90%, respectively, and differences between the two groups in terms of ERs were not significant (p=0.31; Table 2).

Progesterone receptor was positive in 14 cases (70%) in group 1 and in 13 cases (65%) in group 2. In one case PR changed from positive to negative (%10) in the regular follow up group 2 The ROC analysis, which was generated using the values obtained for the PR results for both groups, was 0.93 (0.77–1). The sensitivity and specificity of the frozen and regular follow-up methods for PRs was 100% and 86%, respectively. The differences between the groups in terms of PRs were not significant (p=0.31; Table 2).

The c-erbB2 results were negative for 13 patients (65%) in group 1 and 16 patients (80%) in group 2. The c-erbB2 turned to negative for 3 cases (%30) after the regular follow up group 2. The sensitivity and specificity was determined as 0.57 (57%) and 1 (100%), respectively, in the ROC analysis. The difference between the two pathological tests was significant (p=0.002; Table 2).

There was a significant correlation between the groups in the terms of PR positivity (p=0.0001) and ER positivity (p=0.0001). The relationship between the groups in terms of c-erbB2 positivity (p=0.001) was significant. The negative relationship between cancer grade–ER positivity (p=0.03), PR positivity–lymph node metastasis (p=0.04), and c-erbB2 positivity–ER positivity (p=0.01) was moderately significant. Statistically moderate significant relationships were noted between tumor size and lymph node metastasis (p=0.023) and between age and ER positivity (p=0.027; Table 3).

DISCUSSION

Breast cancer mortality has been decreasing because of improved screening programs, which lead to early diagnosis of breast cancer depending on the progress of targeted therapy (7). Targeted hormonal therapy, which was reported as the

Table 3. Correlation analysis between the variables

Correlations	Age	Cell type	Grade	Tumor size	Lymph Node	ERs	PRs	c-erbB2	ERs	PRs	c-erbB2	
	p	0.600										
Grade	p	0.230	0.078									
Tumor Size	p	0.574	0.130	0.5n 27								
Lymph Node	p	0.731	0.923	0.923	0.023*							
ERs	p	0.027*	0.923	0.031*	0.518	0.369						
PRs	p	0.709	0.173	0.130	0.421	0.105	0.223					
c-erbB2	p	0.274	0.324	0.762	0.384	0.450	0.450	0.924				
ERs	p	0.074	0.749	0.042	1.000	0.673	0.0001*	0.054	0.660			
PRs	p	0.471	0.291	0.180	0.285	0.045*	0.450	0.0001*	0.678	0.177		
c-erbB2	p	0.122	0.749	0.468	0.819	0.833	0.011	0.355	0.001*	0.025	0.508	
FISH	p	0.122	0.749	0.468	0.819	0.833	0.011	0.355	0.001*	0.025	0.508	-

*Spearman's Rank Correlation
ER: estrogen receptor; PR: progesterone receptor; FISH: fluorescence in situ hybridization

most effective treatment option for hormone-positive patients, is related to hormone receptor levels, tyrosine kinase inhibitors, and presence of c-erbB2 (8, 9). Immunohistochemistry, FISH, and Slot Blots are some of the current techniques used for determining ER, PR, and c-erbB2 levels in breast cancer tissue (10, 11).

Immunohistochemistry has some advantages, such as cost effectivity, ability to work with conventional microscopes, and easy storage of stained preparations. Although c-erbB2 levels detected using the FISH method is considered the gold standard, compatible results between FISH and IHC techniques (98%) were obtained once the standard applications were performed by IHC (12). All the c-erbB2 results performed by IHC in advance were double-checked using FISH in our study.

Estrogen receptors, PRs, and c-erbB2 receptors remain stable in a medium at pH 7.4, but ischemia shifts pH value to acidosis, which could affect the expression of the receptors. Therefore, factors such as environmental temperature, pH, osmolarity, quality of the fixing solution, ratio of the fixing solution to the specimen volume, the specimen's residence phase in the fixing solution, tissue thickness, and size seem to be the most important factors for accurate postoperative evaluation of cancer tissue (13).

Hormone receptor positivity is typically determined in approximately 75%–80% of breast cancer patients (14). In our study, the ratio was 55% in group 1 and 50% in group 2 in the terms of ERs and 70% in group 1 and 65% in group 2 in terms of PRs. These values are consistent with those in the literature. ER positivity and age have a positive correlation, and hormone receptor positivity has a higher rate in postmenopausal patients than in premenopausal patients (15). In addition, PR levels are mostly associated with menopause (16), and many studies have shown that prognosis and steroid hormone receptor positivity improve with age (17). In our study, nine patients aged >60 years had 75% ER positivity and two patients aged <60 years had 25% ER positivity.

Estrogen receptor was positive in 11 cases in group 1 and in 10 cases in group 2, and the sensitivity and specificity of these techniques by ROC was 1 (100%) and 0.9 (90%), respectively. The statistical data indicated that ER-positive cases of group 2 definitely have ERs. However, over 10% of ER-negative cases of group 2 could be false-negative.

Progesterone receptor was positive in 14 cases in group 1 and in 13 cases in group 2. The sensitivity and specificity analysis of these methods by ROC was 100% and 86%, respectively. It indicated that Progesterone receptor-positive cases in group 2 definitely carried PRs, but 14% of PR-negative cases in group 2 may be false-negative. As a result, according to the treatment strategy based on ERs and PRs of regular follow-up results, 10% of ER-negative cases and 14% of PR-negative cases could have received targeted hormonal therapy.

The c-erbB2 overexpression was reported as positive for 10%–34% of the invasive breast cancer cases, and more than half of them were also hormone receptor-positive (18, 19). We determined that c-erbB2 was positive in seven patients (35%) in group 1 and in four patients (20%) in group 2; 70% of them had hormone receptor positivity. The ROC analysis showed that positive results in group 2 and negative results in group 1 were the same. As a result, c-erbB2 positivity in group 2 shows 100% sensitivity. In contrast, if c-erbB2 was negative at the end of the regular follow-up technique, there might have been a 43% false negativity rate according to our study. Due to the regular follow-up results in our study, the trastuzumab therapy would not have been administered to the three patients who had only positive c-erbB2 results in the frozen technique. We concluded that c-erbB2 activity could be easily affected by ischemia compared with steroid hormone receptors; thus, to achieve efficient results, it is crucial to transport the samples in appropriate conditions and perform the test immediately.

Estrogen receptor-, PR-, and c-erbB2-negative cases also known as "triple negative" breast cancer, which has poor prognosis, accounts for approximately 10%–15% of breast cancer cases (20). Compared with hormone receptor-positive breast cancer, this group has less treatment options and has more potential to spread aggressively. Two patients (10%) in our study had "triple negative" breast cancer with axillary lymph node metastasis and grade III invasive lobular breast cancer. There were no steroid hormone receptors

or c-erbB2 changes detected for triple negative cases in the current study.

We performed a correlation analysis between variables such as cell type, grade, tumor size, lymph node, ERs, PRs, c-erbB2 levels, frozen and regular follow-up results, and FISH results. In our study, there were positive relations between lymph node metastasis and tumor size, primary tumor size, and incidence of nodal metastasis (Table 3) as reported in the literature (21).

In our study, ER was found negative for all three Grade III breast cancer cases. We had 6 Grade I and II cases and %35 of the cases were ER negative. In addition, our results were consistent with the literature (14), and we determined a negative correlation between ER and tumor grade in our study (p=0.03). The absence of ERs in tumor tissue seemed to be directly related to increased cellular replication, tumor de-differentiation, and eventually with tumor anaplasty, which are the main determinants of tumor grade.

There was a negative relationship between PR and lymph node metastasis in the correlation analysis (p=0.04). Ten PR-positive patients had no lymph node metastasis (72%). Four patients who had negative PRs had lymph node metastasis (67%), which was moderately significant in our study (22). Kumar et al. (23) reported that the presence of c-erbB2 was usually with the absence of ERs and PRs in breast cancer tissue, similar to our study where four of the seven patients had positive c-erbB2 and negative ERs (57%).

A limitation of the present study was the small sample size. Nevertheless, with no doubt, further prospective randomized clinical studies with more number of patients will be useful. Our study showed that steroid hormone receptors and c-erbB2 levels may interfere with tissue ischemia. Evaluation of mastectomy specimens with the shortest ischemic time and most suitable condition is considered extremely crucial.

CONCLUSION

Ischemic time seems to have a negative effect on the evaluation of steroid hormone receptors and c-erbB2 levels in our study. Based on these findings, transporting mastectomy material with most suitable conditions and evaluating the material as soon as possible should lead to decreased ischemic time, thereby detecting accurate steroid hormone receptors and c-erbB2 levels. This would ensure increase in the number of patients who could benefit from the targeted therapy. Numerous randomized controlled studies, which have a large number of patients and with miscellaneous parameters, are needed.

Ethics Committee Approval: Ethics committee approval was received for this study from the Ethics Committee of Haydarpaşa Training and Research Hospital (3.01.2009/09-65).

Informed Consent: Written informed consent was obtained from patients who participated in this study.

Peer-review: Externally peer-reviewed.

Author Contributions: Concept - M.S.; Design - M.S.; Supervision - İ.Y.; Resource - M.S.; Materials -İ.Y.; Data Collection and/or Processing - M.S., İ.Y.; Analysis and/or Interpretation - M.S., İ.Y.; Literature Search - İ.Y.; Writing Manuscript - M.S., İ.Y.; Critical Reviews - İ.Y.; Other - İ.Y.

Conflict of Interest: The authors have no conflicts of interest to declare.

Financial Disclosure: The authors declared that this study has received no financial support.

REFERENCES

1. Siegel RL, Miller KD, Jemal A. Cancer Statistics, 2015. CA Cancer J Clin 2015; 65: 5-29. [CrossRef]
2. Haskell CM, Casciato DA. Breast Cancer, 4th ed. Philadelphia PA, USA: Lippincott Williams Wilkins; 2000.
3. Ravdin PM, Cronin KA, Howlader N, Berg CD, Chlebowski RT, Feuer EJ, et al. The decrease in breast-cancer incidence in 2003 in the United States. N Engl J Med 2007; 356: 1670-1674. [CrossRef]
4. Cinieri S, Orlando L, Fedele P, Cusmai A, D'Amico M, Rizzo P, et al. Adjuvant strategies in breast cancer, new prospectives, questions and reflections at the end of 2007 St Gailen International Expert Consensus Conference. Ann Oncol 2007; 18: 63-65. [CrossRef]
5. Arima N, Nishimura R, Osako T, Nishiyama Y, Fujisue M, Okumura Y, et al. The importance of tissue handling of surgically removed breast cancer for an accurate assessment of the Ki-67 index. J Clin Pathol 2016; 69: 255-259. [CrossRef]
6. Kalkman S, Barentsz MW, van Diest PJ. The effects of under 6 hours of formalin fixation on hormone receptor and HER2 expression in invasive breast cancer: a systematic review. Am J Clin Pathol 2014; 142: 16-22. [CrossRef]
7. Hurtado-de-Mendoza A, Cabling ML, Lobo T, Dash C, Sheppard VB. Behavioral Interventions to Enhance Adherence to Hormone Therapy in Breast Cancer Survivors: A Systematic Literature Review. Clin Breast Cancer 2016; 16: 247-255. [CrossRef]
8. Li SG, Li L. Targeted therapy in HER2-positive breast cancer. Biomed Rep 2013; 1: 499-505. [CrossRef]
9. Blanchette PS, Pritchard KI. Adjuvant endocrine therapy for breast cancer: longer therapy and the need for personalized treatment-should we treat beyond the data?. Oncology (Williston Park) 2013; 27: 1230-1231.
10. Michailidou K, Beesley J, Lindstrom S, Canisius S, Dennis J, Lush MJ, et al. Genome-wide association analysis of more than 120,000 individuals identifies 15 new susceptibility loci for breast cancer. Nat Genet 2015; 47: 373-380. [CrossRef]
11. Berghoff AS, Bartsch R, Preusser M, Ricken G, Steger GG, Bago-Horvath Z, et al. G3Co-overexpression of HER2/HER3 is a predictor of impaired survival in breast cancer patients. Breast 2014; 23: 637-643. [CrossRef]
12. Ross JS, Fletcher JA, Linette GP, Stec J, Clark E, Ayers M, et al. The HER2/neu gene and protein in breast cancer 2003: Biomarker and target of therapy. Oncologist 2003; 8: 307-325. [CrossRef]
13. Hewitt SM, Lewis FA, Cao Y, Conrad RC, Cronin M, Danenberg KD, et al. Tissue handling and specimen preparation in surgical pathology. Arch Pathol Lab Med 2008; 132: 1929-1935.
14. Furmanski P, Dwight E, Saunders BS, Rich MA. The prognostic Value of Estrogen Receptor Determinations in Patients With Primary Breast Cancer. Cancer 1980; 46: 2794-2796. [CrossRef]
15. Charles RB, Frank WL, William NP. Section 4 Gynecology- Disorders of the Breast In: Beck W. Obstetrics and Gynaecology. Baltimore: Williams&Wilkins; 1998.
16. Cianfrocca M, Goldstein LJ. Prognostic and predictive factors in early-stage breast cancer. Oncologist 2004; 9: 606-616. [CrossRef]
17. Romieu I, Berlin JA, Coldits G. Oral contraceptives and breast cancer. Cancer 1990; 1: 2253-2263. [CrossRef]
18. Kumar V, Tewari M, Singh U, Shukla HS. Significance of HER-2/neu protein over expression in Indian breast cancer patients. Indian J Surg 2007; 69: 122-128. [CrossRef]
19. Ross JS, Fletcher JA, Linette GP, Stec J, Clark E, Ayers M, et al. The HER2/neu gene and protein in breast cancer 2003: Biomarker and target of therapy. The Oncologist 2003; 8: 307-325. [CrossRef]

20. Cleator S, Heller W, Coombes RC. Triple-negative breast cancer: therapeutic options. Lancet Oncol 2007; 8: 235-244. [CrossRef]
21. Seidman JD, Schnaper LA, Aisner SC. Relationship of the size of the invasive component of the primary breast carcinoma to axillary lymph node metastasis. Cancer 1995; 75: 65-71. [CrossRef]
22. He ZY, Wu SG, Yang Q, Sun JY, Li FY, Lin Q, et al. Breast Cancer Subtype is Associated With Axillary Lymph Node Metastasis. Medicine (Baltimore) 2015; 94: e2213. [CrossRef]
23. Kumar B, Lyman GH, Allen K, Cox CE, Schapira DV, Timing of weight gain and breast cancer risk. Cancer 1995; 76: 243-248. [CrossRef]