



## The Effect of Postoperative Ischemia on Steroid Receptors and C-ErbB-2 Levels in Breast Cancer Tissue

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### Abstract

**Background:** Postoperative ischemia could affect the evaluation of steroid receptors and c-erbB-2 levels in breast cancer tissue until being fixed by formalin. Misevaluation of steroid hormone receptors and c-erbB-2 levels that have high prognostic factor for the treatment of breast cancer may change the treatment options. The aim of this study is to investigate the postoperative ischemia effects to the breast cancer specimen, especially on steroid receptors and c-erbB-2 expression.

**Methods:** Twenty patients who underwent modified radical mastectomy were included in this study. Two histopathological methods which are frozen and regular follow-up methods were performed postoperatively to all of the specimens. Steroid receptors; Estrogen Receptors (ERs), Progesterone Receptors (PRs), and c-erbB-2 expression of breast tissue samples were evaluated in both techniques. Two groups were created based on two histopathological technique results of steroid receptors and c-erbB-2.

**Results:** We determined that ischemia has a negative effect on the evaluation of steroid receptors and c-erbB-2 and especially the alteration at c-erbB-2 expression was statistically significant ( $p < 0.002$ ).

**Conclusion:** Mastectomy specimen should be examined in the shortest ischemia time in order to determine the accurate detection of steroid receptors and c-erbB-2 levels.

**Keywords:** Breast cancer; C-erbB-2; Estrogen receptors; Ischemia, Progesterone receptors

### Introduction

Postoperative ischemia could affect the evaluation of steroid receptors and c-erbB-2 levels in breast cancer tissue until being fixed by formalin. Misevaluation of steroid hormone receptors and c-erbB-2 levels that have high prognostic factor for the treatment of breast cancer may change the treatment options. The aim of this study is to investigate the postoperative ischemia effects to the breast cancer specimen, especially on steroid receptors and c-erbB-2 expression. Two histopathological methods which are frozen and regular follow-up methods were performed postoperatively. Steroid receptors; Estrogen Receptors (ERs), Progesterone Receptors (PRs), and c-erbB-2 expression of breast tissue samples were evaluated in both techniques. We determined that ischemia has a negative effect on the evaluation of steroid receptors and c-erbB-2 and especially the alteration at c-erbB-2 expression was statistically significant ( $p < 0.002$ ). Mastectomy specimen should be examined in the shortest ischemia time. Breast cancer is the most common and the third mortal cancer cancer in women. During lifetime, one of eight women is at risk of breast cancer [1]. To date, numerous prognostic factors have been identified based on the clinical and pathological features for the treatment of breast cancer. Presence of Estrogen Receptors (ERs), Progesterone Receptors (PRs) and c-erbB-2 oncogene are accepted as among the most important prognostic factors [2-4]. Breast cancer tissue extracted by the surgical procedure can be in a ischemic period until formaldehyde fixation process begins. In addition, the process of 10% formalin fixation which is able to penetrate deep into the tissue, moves on within a fixed time [5,6]. Eventually, all of these processes may cause to misevaluation of steroid hormone receptors and c-erbB-2 levels, which have high prognostic value for the treatment of breast cancer, and thus it may cause to change the treatment options. This study was planned for considering of the postoperative conditions and also pathological process may increase the ischemic process. In current study, our aim is to investigate the impacts of postoperative ischemia to the detection of hormone receptors and c-erbB-2 levels in the breast cancer tissue.

### Patients and Methods

This study was planned as a prospective clinical trial and was approved local ethics committee,

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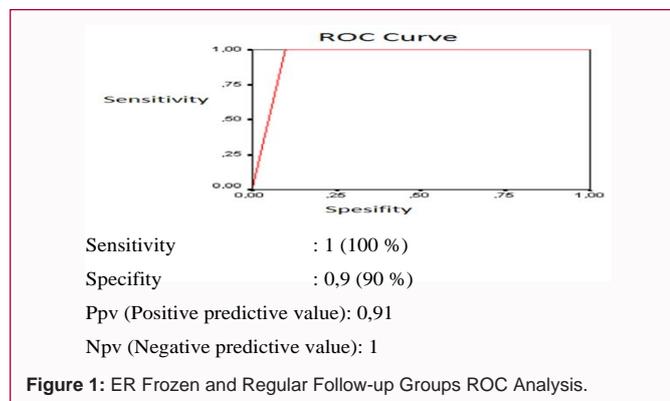
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**Table 1:** Patients' Features.

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

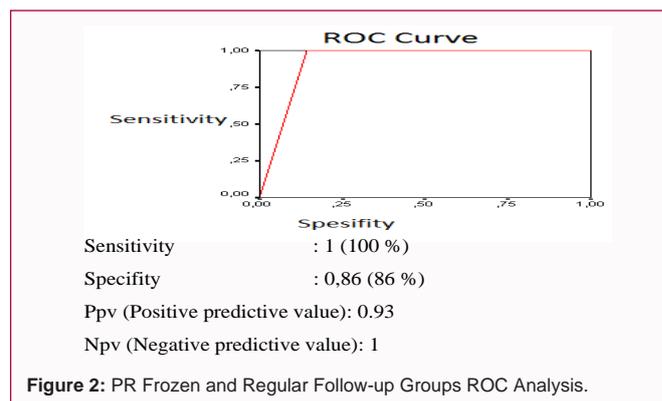


dated 30.01.2009 and with 09-65 ID number. All patients in the study received detailed preoperative information about the study, and confirmed their willingness to participate with the written consent form. Exclusion criteria included: lumpectomy without axillary dissection, not being performed frozen or regular follow-up examination, and not having written consent form. Twenty patients who underwent Modified Radical Mastectomy (MRM) operation for breast cancer on February 2009 to August 2009 period were included the study. All patients underwent surgery in the General Surgery Department by the same surgical team, and performed postoperative evaluation of the specimen by the same pathological team. Two histopathological methods which are frozen technique after being excised, and 24 hours 10% buffered formalin fixation-regular follow up technique were performed to all of the specimens. Ultimately, 20 cases that have MRM operation, both postoperative pathological techniques, and diagnosed of carcinoma were included

**Table 2:** The significance analysis between the groups.

Methods	Positive Case (n)	Ratio (%)	p
c-erbB-2 (group 1)	7	35	<b>0.002*</b>
c-erbB-2 (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



into the study. ERs, PRs and c-erbB-2 levels of breast cancer tissue samples were evaluated by immunohistochemically after the frozen and regular follow-up techniques. Two groups were created based on steroid receptors and c-erbB-2 results; first group for results of frozen technique (n=20), and second group for results of after 24 hours 10% buffered formalin fixation-regular follow up technique (n=20). The primary outcome of the study was ERs, PRs and c-erbB-2 levels alterations between the groups. The secondary outcome of the study was the correlations between the variables such as patient's age, tumor histological grade, lymph node metastasis, tumor size, tumor type, and surgical margins were also identified and enrolled. Positive and negative values that obtained from both techniques by immunohistochemically were recorded as percent values for each receptor and the c-erbB-2 levels. The rate of changes between the two groups was compared statistically. ERs, PRs and c-erbB-2 nuclear staining were evaluated. Staining intensity grade index was used for scoring the cells. The c-erbB-2 antibody was evaluated by four-step scoring system developed for the Herceptin™ test. The c-erbB-2 results were double checked by Fluorescent in Situ Hybridization (FISH) test.

All of the statistical applications were performed via "SPSS for Windows 11. 0" (Statistical Package for the Social Sciences, USA) program. Statistical significance value was accepted as  $p < 0.05$ . Receiver Operating Curve (ROC) was prepared by calculating the sensitivity and specificity of the tests. Comparisons between the data obtained from the cases were analyzed by chi-square test. Spearman's rank correlation test was used for the evaluation of patient variables correlation.

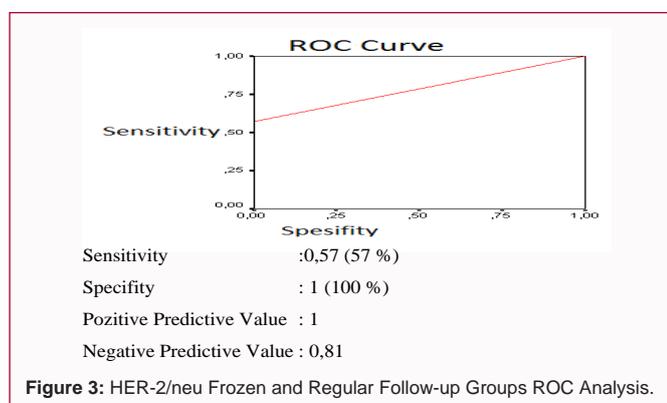
## Results

All of the patients' variables such as age, type of carcinoma, histopathological grades, tumor size, and lymph node metastasis were showed in (Table 1). ERs were positive in 11 cases (55%) in group 1, whereas ERs were negative in 10 cases (45%) in group 2 and cumulative change percentage for ERs were 10% between the groups.

**Table 3:** The correlation analysis between the variables.

Correlations		age	Cell type	Grade	Tumor size	Lymph Node	ERs (Gr-1)	PRs (Gr-1)	c-erbB-2 (Gr-1)	ERs (Gr-2)	PRs (Grp-2)	c-erbB-2 (Grp-2)
Cell type	<i>p</i>	0,600										
Grade	<i>p</i>	0,230	0,078									
Tumor Size	<i>p</i>	0,574	0,130	0,527								
Lymph Node	<i>p</i>	0,731	0,923	0,923	<b>0,023*</b>							
ERs (Gr-1)	<i>p</i>	<b>0,027*</b>	0,923	<b>0,031*</b>	0,518	0,369						
PRs (Gr-1)	<i>p</i>	0,709	0,173	0,130	0,421	0,105	0,223					
c-erbB-2 (Gr-1)	<i>p</i>	0,274	0,324	0,762	0,384	0,450	0,450	0,924				
ERs (Gr-2)	<i>p</i>	0,074	0,749	0,042	1,000	0,673	<b>0,0001*</b>	0,054	0,660			
PRs (Grp-2)	<i>p</i>	0,471	0,291	0,180	0,285	<b>0,045*</b>	0,450	<b>0,0001*</b>	0,678	0,177		
c-erbB-2 (Grp-2)	<i>p</i>	0,122	0,749	0,468	0,819	0,833	0,011	0,355	<b>0,001*</b>	0,025	0,508	
FISH	<i>p</i>	0,122	0,749	0,468	0,819	0,833	0,011	0,355	<b>0,001*</b>	0,025	0,508	-

\* Spearman's rank correlation



The ROC analysis that is generated from the values obtained for ERs results for both groups were found as 0.91 [0.83-1] (Figure 1). Sensitivity and specificity of the tests were 100% and 90% respectively and differences between the two groups in terms of ERs were not statistically significant ( $p = 0.31$ ) (Table 2).

PRs were positive in 14 cases (70%) in group 1, and positive in 13 cases (65%) in group 2. Percentage cumulative change for PRs was 10% between the groups. The ROC analysis (Figure 2) which was generated with the values obtained for the PRs results for both groups were found as 0.93 [0.77-1]. Sensitivity and specificity of the tests were 100% and 86%, respectively (Figure 2). Differences between the groups in terms of PRs were not statistically significant ( $p = 0.31$ ), (Table 2).

The c-erbB-2 results of 13 patients (65%) were negative in group 1 and c-erbB-2 results of 16 patients (80%) were negative in group 2. The cumulative percentage change for c-erbB-2 was 30% between the groups. The sensitivity and specificity were determined as 0, 57 (57%), 1 (100%), respectively, in ROC analysis (Figure 3). The difference between the two pathological tests were statistically significant ( $p=0.002$ ) (Table 2). There was a statistically significant correlation between groups in the terms of PRs positivity ( $p=0.0001$ ) and ERs positivity ( $p=0.0001$ ), respectively. Relationship between groups in the term of c-erbB-2 positivity ( $p=0.001$ ) was statistically significant. Negative relationship between cancer grade-ERs positivity ( $p=0.03$ ), PRs positivity-lymph node positivity ( $p=0.04$ ), and c-erbB-2 positivity and ERs positivity ( $p=0.01$ ) were statistically moderate significant. There were also statistically moderate significant relationships

between tumor size and lymph node positivity ( $p=0.023$ ), and age and ERs positivity ( $p=0.027$ ) (Table 3).

### Discussion

Breast cancer mortality has been decreasing as a result of the screening programs leading to early diagnose stages of breast cancer and depending on the progress of targeted therapy [7]. The targeted therapy including "hormonal therapy" is related to the hormone receptor levels, tyrosine kinase inhibitors, and presence of c-erbB-2, and reported as the most effective treatment option for the hormone positive patients [8,9]. Immunohistochemistry (IHC), Fluorescence In Situ Hybridization (FISH), Slot Blots, etc., are currently using techniques for determining ERs, PRs and c-erbB-2 level in breast tumor tissue [10, 11]. IHC has some advantages such as cost affectivity, allows working with conventional microscopes, and stores the stained preparations easily. Although the c-erbB-2 levels detecting by FISH method is considered the gold standard, once the standard applications are made by IHC, the compatible results between FISH and IHC techniques (98%) are be obtained [12]. All of the c-erbB-2 results made by IHC in advance were double checked by FISH, in our study. ERs, PRs and c-erbB-2 receptors remain stable in pH 7.4 medium but if an ischemic situation occurs, pH medium value shifts to acidosis, and it may affect the expression of the receptors. Therefore, the factors such as environment temperature, pH, osmolarity, and quality of the fixing solution, ratio of the fixing solution to the specimen volume, the specimen's residence phase in the fixing solution, the tissue thickness and size seem to be the most important factors for the exact postoperative evaluation of the cancer tissue [13]. Hormone receptor positivity is typically determined about 75 - 80% in breast cancer patients [14]. In our study, the ratio was 55% in group 1, 50% in group 2, respectively in the terms of ERs, and 70% in group 1, 65% in group 2, respectively in the terms of PRs. These values are coherent with the literature. ERs positivity and age have a positive correlation, and hormone receptor positivity have higher rate in the postmenopausal patients than premenopausal patients [15]. Also PRs levels are mostly associated with the menopause statement [16] and many studies showed that prognosis and steroid receptor positivity improve with age [17]. In our study, 9 patients who are older than 60 years old had 75% ERs positivity and 2 patients who are younger than 60 years old had 25% ERs positivity. ERs were positive in 11 cases in group 1, whereas ERs were positive in 10 cases in group 2 and the sensitivity-specificity of these techniques by ROC resulted as

the sensitivity; 1 (100%) and specificity; 0.9 (90%). This statistical data indicated that ERs positive cases of group 2 certainly carry the Estrogen Receptors. However, ERs negative value belonging to group 2 demonstrated that over 10% of these cases could be obtained as false negative.

Fourteen patients had PRs positivity in group 1 and 13 patients had PRs positivity in group 2. The sensitivity-specificity analysis of these methods by ROC resulted as sensitivity 100% and specificity 86%. It indicated that PRs positive cases in group 2 absolutely carry the progesterone receptors. PRs negative cases in group 2 may possibly be the false negative cases with a rate of 14%. As a result, according to the treatment strategy based on ERs and PRs regular follow-up results, 10% of ERs negative cases and 14% of PRs negative cases would not have been received the targeted hormonal therapy in case of our study results. c-erbB-2 over-expressions were 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-erbB-2 was positive in 7 patients (35%) in group 1, and in 4 patients (20%) positive in group 2, and 70% of them had hormone receptor positivity. The ROC analysis showed that positive results in group 2 and the negative results in group 1 were totally the same. As a result; c-erbB-2 positivity in group 2 shows 100% sensitivity. On the other hand, if c-erbB-2 was resulted as negative at the end of the regular-follow up technique, there might have had a 43% rate false negativity according to our study. Due to regular follow up results in our study, the trastuzumab therapy would have not been given to the three cases that had only positive c-erbB-2 results in frozen technique. We concluded that c-erbB-2 activity could be easily affected from ischemia even more than the steroid receptors, and thus in order to get efficient results, it is crucial taking the samples in appropriate conditions and performing the test in the shortest time. ER-PR receptors and c-erbB-2 negative cases defined as "Triple Negative" breast cancer which has a worse prognosis and it approximately contains 10-15% of the breast cancer cases [20]. Comparing with hormone receptor positive breast cancer, this group has less treatment options and has more potential of aggressive spreading. Two patients (10%) had "Triple-negative" breast cancer with positive axillary lymph nodes and grade 3 invasive lobular breast cancers in our study. There were no steroid receptors or c-erbB-2 changing in this group in the current study. We performed correlation analysis between variables such as cell type, grade, tumor size, lymph node, ERs, PRs, c-erbB-2 frozen and regular follow-up results' and FISH results. In our study, there were positive relations between lymph node positivity and tumor size; primer tumor size, and incidence of nodal metastasis (Table 3). This correlation is also showed in a study in the literature [21]. In our study, three patients of grade 3 were 100% ERs negative. Six of grade 1 and 2 patients were 35% ER negative. In addition, our results were relevant with the literature and we determined the negative correlation between ERs and tumor grade in our study ( $p=0.03$ ). The absence of ERs in tumor tissue seemed to be directly related with increased cellular replication, tumor de-differentiation and eventually with the tumor anaplasty which are the main determinants of tumor grade. There were negative relationship between PRs and lymph node in the correlation analysis ( $p=0.04$ ). Ten PRs positive patients had negative lymph node (72%). In addition, 4 patients who had negative PRs, had positive lymph node (67%) which were statistically moderate significant in our study.

Kumar et al. [22] reported that the presence of c-erbB-2 had a positive relationship between the absence of ERs and PRs similar to our study where 4 of the 7 patients who had positive c-erbB-2,

also had negative ERs (57%). A limitation of the present study was the poor number of patient. Nevertheless, with no doubt further prospective randomized clinical studies that have more number of patients can be useful. In our study, it is shown that the steroid receptors and c-erbB-2 levels may interference with tissue ischemia. The evaluation of the mastectomy specimen in the shortest ischemia time and in the most suitable condition is considered to be extremely crucial. The ischemia time seems to have a negative effect on the evaluation of steroid receptors and c-erbB-2 levels to our study. Based on these findings, taking the mastectomy material with most suitable conditions and evaluating the material as soon as possible may let the ischemia time decrease are suggested to be the most useful way to detect accurate steroid receptors and c-erbB-2 levels which could increase number of patients who could benefit from targeted therapy. Numerous randomized controlled studies which have a large number of patients and with miscellaneous parameters are needed.

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