Concordant Expression of Estrogen and Progesterone Receptors in Primary and Loco-regional Recurrent Breast Cancer

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Abstract

Background: Malignant neoplastic lesions of the breast are one of the main causes of cancer death among women. Several factors can be influential in the treatment process of breast cancer patients. The expression status of estrogen and progesterone receptors in tumor cells is among the most important determinants affecting the treatment approach and prognosis of these patients. In this paper, we will evaluate changes in the expression of these biomarkers between primary and loco-regional recurrent tumors in breast cancer patients.

Methods: This cross-sectional study included 50 female patients aged 30 to 85 years old who underwent surgical intervention between 1994 and 2011. All experienced loco-regional recurrence of the primary tumor between 6 months and 12 years after the first therapeutic intervention. Detection of estrogen and progesterone receptors was based on the immunohistochemistry staining of tissue samples of malignant neoplastic lesions prepared from tissue biopsies of patients with primary or recurrent breast cancer.

Results: No statistically significant change in the expression of estrogen and progesterone receptors between primary tumors and their matching loco-regional were seen ($P>0.05$).

Conclusion: This research shows no significant changes in estrogen and progesterone receptor expression after loco-regional recurrence of tumors in breast cancer patients. It can be concluded that the assessment of the expression of these biomarkers in primary tumors provides reliable information for the treatment approach of loco-regional tumors.

Keywords: Breast neoplasms, Recurrence, Estrogen receptor, Progesterone receptor

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Introduction

Malignant neoplastic lesions of the breast are the most common form of malignancy diagnosed in women. Breast cancer (after lung cancer) is the second leading cause of cancer death among women. About 14% of cancer deaths among women are caused by breast cancer. Despite geographical patterns of breast cancer incidence and mortality, over the past decades, the incidence of breast cancer has increased worldwide. A small part of this geographical variation is related to genetic differences in people of different races, but it is mainly a consequence of diversity in lifestyle in addition to exposure to various environmental carcinogens.

Risk factors for breast cancer include a younger age at the time of puberty (early menarche), a more advanced age at the time of menopause (late menopause), obesity, a negative history of pregnancy or breast feeding, first delivery at an advanced age (especially more than 35 years old), excessive alcohol consumption, and insufficient physical activity. Noting these risk factors, one realizes that hormonal factors (especially estrogen, endogenous or exogenous) play an important role in breast cancer incidence and the development of its malignant neoplastic lesions. One therapy goal in breast cancer patients, therefore, is to cope with the effects of hormones (especially estrogen) in their bodies.

It should be noted that two thirds of breast cancer patients have tumoral tissues with cells that express estrogen receptor (ER). This feature can be predictive of a patient’s response to hormone therapy against estrogen, and thus affect survival. Unfortunately, resistance to hormone therapy may be seen in these patients (from the beginning or during the treatment process).

Considering the significant role of ER and PR in the treatment approach and prognosis of breast cancer patients, our goal in this study is to assess the expression of ER and PR in primary breast cancer tumors and their matching loco-regional recurrent neoplastic lesions. Then, we evaluate phenotypic changes in the expression of ER and PR between primary and recurrent tumors.

Materials and Methods

This cross-sectional study included 50 female patients aged 30 to 85 years old who underwent surgical intervention (modified radical mastectomy or breast conserving surgery) between 1994 and 2011. All experienced loco-regional recurrence (LRR) of the primary tumor. These patients were selected from the database of the Breast Cancer Clinic at Shahid Motahhari Clinic, Shiraz University of Medical Sciences, Shiraz, Iran. Most, in addition to surgery, based on primary tumor characteristics (size, pathology, nodal involvement, etc.) and hormone receptor expression status had a history of radiotherapy and adjuvant treatments with routine chemotherapy that included such chemotherapeutic regimens as doxorubicin, cyclophosphamide, and taxol, among others, and hormone therapy (tamoxifen).

In this study we defined LRR as a recurrence in cutaneous and/or subcutaneous chest wall tissues in the territory of a previously operated breast, axillary and internal mammary lymph nodes, and lymph nodes located in the supra- or infra-clavicular fossa on the same side as the primary neoplastic lesion.

In this study, detection of ER and PR was based on the immunohistochemistry (IHC) staining of paraffin embedded tissue samples of malignant neoplastic lesions prepared from tissue biopsies of patients with primary or recurrent breast cancer.

Study patients experienced LRR between 6...
months and 12 years after the first therapeutic intervention. We excluded cases that experienced distant metastasis, malignant lesions with new pathology, and recurrence outside the above-mentioned zone for LRR from this study.

For the IHC study, ready-to-use antibodies (antibodies with no need to further process before use) were procured from DAKO Company, Denmark. The IHC staining protocol for tissue samples is explained in Table 1. Statistical analysis was performed by SPSS Statistics software, version 11.5 (Chicago, IL, USA).

### Results

All 50 selected patients experienced LRR. At the time of primary tumor diagnosis, tissue samples from 29 (58%) patients were ER positive and 28 (56%) patients were PR positive. Evaluation of tissue samples from cases with recurrent tumors compared to primary tumors revealed that 26 (52%) patients were ER positive and only 22 (44%) were PR positive.

At the time of recurrence, ER expression in 33 (66% of patients) cases and PR expression in 36 (72%) patients were the same as primary tumors. ER and PR expression, each one, lost in 10 (20%) patients. There were 7 (14%) patients who had new ER expression, and 4 (8%) showed new PR expression.

According to these results, we observed no statistically significant change in the expression of ER and PR between primary tumors and LRR ($P>0.05$). There was no significant difference in the concentration of expressed ER and PR by tumor cells between primary and recurrent tumors. Data are summarized in Tables 2, 3 and 4.

### Discussion

The immunohistochemical properties of tumor cells in breast cancer patients, such as ER and PR expression, play key roles in tumor responsiveness to hormone therapy, recurrence rate, and patient survival. Thus, it is important to identify patients who are more likely to respond to hormone therapy.22,23

Depending on the stage of the disease at the time of diagnosis and the type of treatment received, about 10% to 35% of women with breast cancer will experience LRR.24 Changes in the expression of ER and PR in recurrent tumors impose limitations on the use of hormone therapy for these tumors.25 Today, ER and PR expression is routinely assessed in all newly diagnosed breast cancer patients. Re-evaluation of the expression of these biomarkers at the time of recurrence or distant metastasis to achieve effective and successful treatment remains a controversial issue.26

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**Table 1.** Stepwise IHC protocol for ER and PR staining used in this study.

<table>
<thead>
<tr>
<th>Step</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Cut paraffin sections at 4.0 µm thickness (on sticky slides).</td>
</tr>
<tr>
<td>2.</td>
<td>Put 3 times in xylene for 30 minutes.</td>
</tr>
<tr>
<td>3.</td>
<td>Hydrate sections by placing in decreasing ethanol grades (100%, 96% and 70%).</td>
</tr>
<tr>
<td>4.</td>
<td>Wash slides in PBS* buffer (5-10 minutes).</td>
</tr>
<tr>
<td>5.</td>
<td>Incubate in 3% H2O2 for 3 minutes (inactivation of endogenous peroxidase).</td>
</tr>
<tr>
<td>6.</td>
<td>Wash slides in PBS buffer.</td>
</tr>
<tr>
<td>7.</td>
<td>Use TE** buffer to boil slides.</td>
</tr>
<tr>
<td>9.</td>
<td>Use 10% goat serum to block slides, incubate at room temperature for 20 minutes and use a humidified chamber.</td>
</tr>
<tr>
<td>10.</td>
<td>Incubate slides with primary antibody in a humidified chamber for 1 hour, add 50-100 µl of ready-to-use antibody [Estrogen receptor (ER): 1D5 and progesterone receptor (PR): PgR636, DAKO Company, Denmark].</td>
</tr>
<tr>
<td>11.</td>
<td>Wash in PBS buffer (10 minutes).</td>
</tr>
<tr>
<td>12.</td>
<td>Apply secondary antibody.</td>
</tr>
<tr>
<td>13.</td>
<td>Wash in PBS buffer (10 minutes).</td>
</tr>
<tr>
<td>14.</td>
<td>Add DAB chromogen (5 minutes).</td>
</tr>
<tr>
<td>15.</td>
<td>Counter stain using hematoxylin and eosin.</td>
</tr>
<tr>
<td>16.</td>
<td>Dehydrate slides using serial grades of ethanol, clear in xylene, then mount.</td>
</tr>
</tbody>
</table>

*PBS: phosphate buffered saline; **TE: tris-EDTA
Tumor cells in breast cancer are heterogeneous and composed of different cell clones. ER expression varies in different cell lines.27,28 According to one hypothesis, the cause for change in the phenotype of tumor cells in terms of ER and PR expression during disease progression is linked to the selection of cell lines not expressed in ER, but rather phenomena that occur during the treatment process.29,30

Our study included a precise review of works previously conducted to assess changes in ER and PR expression status of tumor cells following recurrence or metastasis in breast cancer patients. This review showed different results of previous studies. Some favored the concordance in ER and PR expression between primary and recurrent or metastatic tumors. Studies by Gomez-Fernandez et al., D’Andrea et al., Li et al. and Robertson showed no significant changes in ER and/or PR content of tumor cells after recurrence or metastasis. Among the above mentioned studies, the work of Gomez-Fernandez et al. included 278 breast cancer patients and was the most wide-scale research which supported stability of the ER phenotype in breast cancer patients.29, 31-33

By contrast, some researchers have supported the idea that ER and PR expression status can change during tumor progression, recurrence or metastasis. These included works by Rom et al., Pedrini et al., Morimoto et al., Nomura et al. and Idirisinghe et al. Among these, Nomura et al. was the most wide-scale study that assayed ER expression status in 940 breast cancer patients and PR expression status in 773 breast cancer patients who experienced locally recurrent or metastatic tumors.25, 34-37

Differences in evaluation methods of biomarker expression are an important factor that can be related to different results in various studies. Of note, some methodological differences, including biochemical assay versus IHC assay of steroid receptors, method of tissue sampling (surgical biopsy, core needle biopsy, or fine needle aspiration), and inter- or intra-laboratory differences may prevent an accurate assessment of ER and PR during the study process.22, 33, 36 Heterogeneity in receptor expression between different parts of the tumor tissue may also influence results.13 Another confounding factor is the administration of tamoxifen, which binds to the ER on the surface of tumor cells that express this receptor and causes false-negative results.30,33,38 In order to prevent such technical problems in this study, only biopsy specimens of tumor tissue were used, of which these were evaluated by an experienced technician. Biomarkers were assessed by the IHC method, an inexpensive, sensitive method for ER and PR detection widely used as the method of choice in most countries.39

Overall, the results of the present research were similar to those of studies which suggested no significant changes in tumor cell phenotypes after recurrence or metastasis. The concentrations of ER and PR between primary and LRR tumors also showed no significant changes.

**Conclusion**

Considering the results of the present study that

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**Table 2. Estrogen receptor (ER) and progesterone receptor (PR) expression status in cases with primary breast cancer and their loco-regional recurrent tumors.**

<table>
<thead>
<tr>
<th></th>
<th>Primary tumor</th>
<th>Loco-regional recurrent tumor</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>N (%)</td>
<td>N (%)</td>
</tr>
<tr>
<td>ER Positive</td>
<td>29 (58)</td>
<td>26 (52)</td>
</tr>
<tr>
<td>ER Negative</td>
<td>21 (42)</td>
<td>24 (48)</td>
</tr>
<tr>
<td>PR Positive</td>
<td>28 (56)</td>
<td>22 (44)</td>
</tr>
<tr>
<td>PR Negative</td>
<td>22 (44)</td>
<td>28 (56)</td>
</tr>
</tbody>
</table>

**Table 3. Estrogen receptor (ER) and progesterone receptor (PR) expression status in loco-regional recurrent tumors compared with primary tumors.**

<table>
<thead>
<tr>
<th></th>
<th>Number of cases (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Loss of ER exp.*</td>
<td>10 (20)</td>
</tr>
<tr>
<td>Loss of PR exp.</td>
<td>10 (20)</td>
</tr>
<tr>
<td>New exp. of ER</td>
<td>7 (14)</td>
</tr>
<tr>
<td>New exp. of PR</td>
<td>4 (8)</td>
</tr>
<tr>
<td>Stable ER ph. **</td>
<td>33 (66)</td>
</tr>
<tr>
<td>Stable PR ph.</td>
<td>36 (72)</td>
</tr>
</tbody>
</table>

*exp.: Expression; **ph.: Phenotype
suggest no significant changes in ER and PR expression after the LRR of tumors in breast cancer patients, the assessment of the expression of these biomarkers in primary tumors provides reliable information for the treatment approach of loco-regional tumors. But, due to the existence of studies that suggest a significant change in the receptor expression status after recurrence or distant metastasis, it seems reasonable to conduct further studies on a wider scale.

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Conflicts of interest
The authors certify that they have no conflicts of interest.

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