

## Original Article

# STATUS OF ESTROGEN AND PROGESTERONE RECEPTORS IN VARIOUS PHASES OF THE MENSTRUAL CYCLE IN BREAST CANCER

Mohammad Vasei MD\*, Negar Azarpira MD\*\*, Abdolrasoul Talei MD\*\*\*

**Background:** Expression of hormone receptors is routinely evaluated in predicting tumor response to hormone therapy in breast cancer patients. Normal female genital organs show cyclic changes in the expression of estrogen and progesterone receptors. This study was designed to assess variations in estrogen and progesterone receptor expression rates in breast cancer patients, who were operated in different phases of the menstrual cycle.

**Methods:** From 2001 through 2004, 161 premenopausal patients with breast cancer, who were operated on, were enrolled into this study. Immunohistochemistry for the expression of estrogen and progesterone receptors was performed on their tumor paraffin blocks, using antibodies against estrogen and progesterone receptors.

**Results:** Estrogen receptor expression was seen in 24 out of 30 cases (80%) in early luteal phase, which was significantly higher than that of those operated in early follicular (53%), late follicular (51%) and late luteal (49%) phases ( $P < 0.05$ ). Progesterone receptor expression also showed a rising trend in the early luteal phase (87%), as compared with the other phases ( $P = 0.09$ ).

**Conclusion:** Expression of estrogen/progesterone receptor shows cyclic changes in breast cancer patients, being highest in the early luteal phase of the menstrual cycle. This variation implies that this phase of the cycle could be the golden time for evaluation of estrogen/progesterone receptor status.

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**Keywords:** Estrogen receptor • progesterone receptor • menstrual phase

## Introduction

Development of the breast is hormonally regulated in puberty. Histology and the size of the breast are subject to cyclic changes during each menstrual cycle. Cyclic hormonal changes have structural effects on the breast lobules, ducts, and stroma.<sup>1</sup> These changes are clinically manifested by fluctuations in the breast size and consistency. Cyclic expression of estrogen receptor (ER) and progesterone receptor (PR) in the normal breast has been observed during different phases of the menstrual cycle in healthy

women.<sup>2, 3</sup> Cyclic variation has also been described in the expression of some other molecules such as epidermal growth factor, HER-2/neu, and Ki67.<sup>4, 5</sup> Breast cancer cells express ER and PR with higher frequencies than normal breast epithelium. Interestingly, breast cancer in postmenopausal women shows a higher rate of ER and PR expression than in premenopausal women, suggesting a possible hormonal influence on their expression.<sup>6, 7</sup> The ER/PR expression status is an important factor for determining the prognosis and decision making for tamoxifen therapy in breast cancer patients. Whether ER and PR expression in breast cancer is also influenced by the menstrual cycle is not well-understood by immunohistochemistry (IHC). We carried out this study to assess the frequencies of ER and PR expression in different phases of the menstrual cycle in patients with breast cancer.

**Authors' affiliations:** \*Department of Pathology, \*\*Transplant Research Center, \*\*\*Department of Surgery, Cancer Research Center, Shiraz University of Medical Sciences, Shiraz, Iran.

**Corresponding author and reprints:** Mohammad Vasei MD, Department of Pathology, Cancer Research Center, Shiraz University of Medical Sciences, Shiraz, Iran.

Fax: +98-711-230-1784, E-mail: vaseim@sums.ac.ir.

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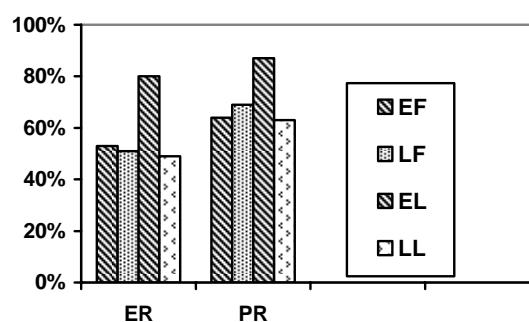
## Patients and Methods

The study group consisted of premenopausal women diagnosed as having breast ductal carcinoma who underwent mastectomy in hospitals affiliated to Shiraz University of Medical Sciences from 2001 through 2004. Based on the days lasting from the first day of the last menstrual period at the operation time, the patients were categorized into four groups: early follicular (days 0 – 7, EF), late follicular (days 8 – 14, LF), early luteal (days 15 – 21, EL), and late luteal (days 22 – 33, LL) phases. The patients who had a recent menstrual cycle in excess of 34 days were excluded from the study.

The appropriate paraffin block was chosen for IHC. After blocking the endogenous peroxidase and nonspecific binding, antigen retrieval was performed by boiling the slides in citrate buffer (pH 6.0) for 40 min. The receptor analysis was performed, using diluted monoclonal antibody (1:100) for ER and prediluted polyclonal antibody for PR (Dako, Denmark), and visualized by the universal Streptavidin/Biotin kit (LSAB Kit, Dako) followed by diaminobenzidine colorization. The slides were then lightly counterstained with hematoxylin. For statistical evaluation  $\chi^2$  test was used to compare the frequencies of expression rates of the receptors. The significance level of  $P$  value was set at  $<0.05$ .

## Results

There were 161 patients with a mean age of 39 (range: 23 – 52) years. PR was detected in 112 (69%) and ER in 91 (56%) patients. In the EL phase, ER positivity was seen in 24 of 30 (80%) patients, which was significantly higher than that



**Figure 1.** Relative frequencies of ER and PR expression in breast cancer in different phases of the menstrual cycle. EF = early follicular; LF = late follicular; EL = early luteal; LL = late luteal.

observed in other phases ( $P < 0.05$ ). ER was expressed in 24 of 45, 20 of 39, and 23 of 47 patients, in EF, LF, and LL, respectively (Figure 1). PR expression also showed a rising trend in the EL phase (26 of 30 cases; 87%), though not statistically significant ( $P = 0.09$ ). PR expressions were relatively equal in other phases, as 29 of 45, 27 of 39, and 30 of 47 patients in EF, LF, and LL, respectively. When we divided the whole cycle to the follicular (days 0 – 14) and secretory (days 15 – 30) phases, the positivity rates of the receptors were higher in the secretory phase for both PR (66% vs. 72%) and ER (52% vs. 61%). The differences, however, were not statistically significant.

## Discussion

We found an increase in the expression rates of ER and PR in patients operated in the EL phase of the menstrual cycle. Cyclic changes of ER and PR have been observed in normal female breast.<sup>8, 9</sup> There are conflicting data addressing the cyclic

**Table 1.** Summary of the available data on ER/PR frequencies in different phases of the menstrual cycle.

Reference	ER expression rate	PR expression rate	Method	No. of cases	Phases
Coradini (1984) <sup>10</sup>	No cyclic change	EL ↑	DCC	290	4 phases
Saez (1978) <sup>11</sup>	Last 10 days ↑	Last 10 days ↓	DCC	77	F/O/L
Weimer (1987) <sup>12</sup>	No cyclic change*	L phase ↑	DCC	78	F/L
Axelrod (1988) <sup>14</sup>	L phase ↑	Not determined	DCC	75	F/L
Markopoulos (1988) <sup>13</sup>	No cyclic change	No cyclic change	DCC	83	F/L
Holli (1995) <sup>19</sup>	EF and LL phases ↑	No cyclic change	DCC	267	4 phases
Mangia (1998) <sup>15</sup>	No cyclic change**	No cyclic change	DCC	248	F/L
Pujol (1998) <sup>7</sup>	F phase ↑	L phase ↑	DCC	575	F/L
Cooper (1999) <sup>16</sup>	No cyclic change	No cyclic change	IHC	112	F/L
Atalay (2002) <sup>17</sup>	F and LL phase ↑	No cyclic change	IHC	88	3 phases

EL = early luteal; L = luteal; EF = early follicular; LL = late luteal; F = follicular; DCC = dextran-coated charcoal; IHC = immunohistochemistry; \* = in this study, the mean concentration of ER and PR showed elevation in the luteal phase; \*\* = in this study the concentration of ER showed a rise in the 4<sup>th</sup> week and PR in the 3<sup>rd</sup> week of the menstrual cycle.

changes of hormone receptors in breast cancer.<sup>7, 10–17</sup> Most of the studies revealed variation in the ER expression in different phases. No consistent menstrual cycle-related pattern for ER could be figured out from these data. Meanwhile, the majority of data support the increase of PR in breast cancers operated in the luteal phase (Table 1).

One of the sources of these controversial results could be originated from the assay methods used in these studies. In most of these studies, the assay method is based on the measurement of cytosolic estrogen and progesterone receptors by the dextran-coated charcoal ligand-binding assay (DCC) method. In this method, the tumor tissue is inevitably diluted by the surrounding stroma and occasionally by necrotic material, giving erroneously low or negative results. Conversely, positive results could be falsely obtained by mixing the normal adjacent tissue with the tumor samples. Nowadays, sex steroid receptor assessment of breast carcinomas is usually performed by IHC. Although the cytosolic assay shows correlation with IHC results, the latter technique gives better results than cytosolic assay because it is more closely related to patient outcome.<sup>18</sup> In the IHC method, tumor cells are exactly visualized and differentiated from the normal surrounding tissue, hence, do not give the problem of false negative or positive results of DCC.

The other source of variability of these results can be explained by the difference in the definition of the various phases of the menstrual cycle (Table 1). In some of these studies in which no significance cyclic changes in positivity rate was observed, the whole menstrual cycle was divided into follicular and secretory phases. In our study, division of the cycle into two phases also showed higher frequencies of both receptors in the luteal phase, though with lower *P* values.

It is claimed that surgery at the luteal phase will increase the chance of disease and recurrence-free survival in patients.<sup>19, 20</sup> There are several hypotheses that might explain the effect of timing of surgery, some of them addressing the influence of endocrine milieu on tumor handling and spread during surgery. We, hereby, introduce another explanation for the impact of timing of surgery on the survival. Breast cancers containing ER and have a better prognosis as compared with ER-negative tumors. Eighty percent of the tumors with expression of both receptors respond to hormonal

manipulation. However, this figure decreases to 40%, when only one receptor is expressed.<sup>21</sup> The results of our study showed a rise in the expression rate of ER and PR in the 3<sup>rd</sup> week of the cycle. By selection of EL phase for operation, the probability of the detection of expression of both ER and PR is higher and the tumor will not be falsely regarded as a receptor-negative tumor. Considering this cyclic change, the patients undergoing operation in the follicular phase may show a decrease in the expression of ER/PR and would not benefit from tamoxifen therapy.

We should appreciate the menstrual cycle changes in the evaluation of the hormone receptor status in patients with breast cancer. To document this cyclic change, evaluation of ER/PR has to be performed in at least two different phases in the same cycle. Usually, needle biopsy or fine-needle aspiration is performed before the main surgery on the patients. If the lag time between the first sampling and the operation puts the sampling time in two different phases of the cycle, these two samples will be the best samples for assessing these cyclic changes.

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