Evaluation of the effect of surgical timing on systemic response to trauma in premenopausal patients by using cytokine levels

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Summary

Purpose: The aim of the study was to evaluate the severity of systemic responses to trauma; and thus, to determine the most advantageous timing for surgery among different phases of the menstrual cycle. Materials and Methods: 99 women were included in this study. They were patients who were in the postmenopausal period and patients in the different menstrual cycle phases of the premenopausal period. C-reactive protein, interleukin-1β, interleukin-6 and tumor necrosis factor-α levels were measured before and after surgery. Results: Among the menstrual cycle phases, the highest increases in the concentrations of IL-6 and TNF-α during the postoperative period were found in follicular phase patients. Luteal phase patients showed the lowest increases in interleukin-1β and interleukin-6 levels. TNF-α and CRP levels were increased significantly in all phases but not in the luteal phase. Conclusion: Timing of surgery in premenopausal patients seems effective on systemic inflammatory responses to trauma. Performing the operation based on the timing of the menstrual cycle will minimize the risk of operative trauma among premenopausal patients. This may be beneficial for their well-being.

Key words: Breast cancer; Menstrual cycle; Surgery timing; Systemic responses to trauma.

Introduction

In women, breast cancer is the most common malignancy and the second leading cause of death by cancer [1]. The multidisciplinary approach is a very important part of planning treatment for breast cancer patients. Many parameters of the patient and the disease are evaluated by this approach and the most suitable surgical option is provided to the patient. The modified radical mastectomy is currently the most traumatic surgical technique. Many other studies have suggested that trauma is an inflammatory disease and that various mediators in inflammatory response are elevated in severe trauma patients [2]. Hrushesky et al. proposed that menstrual timing of breast cancer surgery had a great impact on the survival of patients [3]. Following these studies, the influence of timing of breast cancer surgery and menstrual cycle on disease-free survival and overall survival were investigated; however, it still remains theoretical [1].

17β-estradiol levels vary in different phases of the menstrual cycle. The roles of estrogen and estrogen-receptor agonists for maintaining functions of organs are more important in the posttraumatic period. Moreover, it has been known that sex steroids modulate posttraumatic cardiovascular responses. Sex steroids regulate inflammatory cytokines and contribute to the protection of multiple organs following a traumatic event. Therefore, altering and modulating the hormonal milieu during an injury are new therapeutic options to get better outcomes for wound healing [4]. While some studies have claimed that this provides an advantage for women, the response of the patient after trauma and sepsis should be evaluated [4, 5]. The reasons for different results found in the literature may be related to the unknown hormonal status of patients during a traumatic event.

In this study, systemic responses to trauma were compared between premenopausal and postmenopausal patients with different estrogen levels who underwent a modified radical mastectomy by evaluating C-reactive protein (CRP), tumor necrosis factor-α (TNF-α), interleukin (IL)-1β and IL-6 levels.

Materials and Methods

The study was conducted between February 2009 February 2010 in Kocaeli University Hospital. After obtaining ethics approval from Kocaeli University Clinical Research Ethics Committee (2008/69), 99 female patients who underwent modified radical mastectomy due to breast cancer were included in the study. All participants in the study provided informed consent. Mean age of premenopausal patients (n = 62) was 42.1 ± 1 years old, and mean age of postmenopausal patients (n = 37) was 59.1 ± 2 years old. Birth dates and last menstrual periods of all patients were recorded and their gonadotropin levels were measured. The patients were classified as pre-
menopausal and postmenopausal. Our main goal was not to compare premenopausal patients with postmenopausal patients, but to determine the differences in response to trauma in the menstrual cycle phases of premenopausal patients. Therefore, we did not consider age distribution. Premenopausal patients were not taking oral contraceptives and postmenopausal patients were not taking hormone replacement therapy. Patients using these were excluded from the study during enrollment. On the other hand, menstrual cycle periods (follicular phase, ovulatory phase, luteal phase and menstruation phase) were determined according to the last menstrual period of premenopausal patients with regular menstruation. Patients with irregular menstruation were not included in the study. No postoperative problems were detected in any patients postoperatively. If developed, they are excluded. One day before and one day after surgical operations, blood samples of all patients were collected. Serum IL-1β, IL-6, TNF-α levels and CRP were measured at the Department of Clinical Biochemistry in Kocaeli University Hospital. Plasma levels of IL-1β, IL-6 and TNF-α were measured by Enzyme Linked Immunosorbent Assay (ELISA) Kit (Invitrogen, ABD) in MicroELISA device (DSXTM Four-Plate Automated ELISA Processing System, Dynex Tecnologies, Virginia, ABD) using ELISA method. CRP was measured by nephelometric method in Immage 800 device (Beckman Coulter, USA) using ready commercial test kits.

Results of each group are shown as mean ± standard error of the mean (SEM). Appropriate parametric or non-parametric test was selected according to the distribution of data and statistical analysis was made by GraphPad Prism 3.0 program. \( p < 0.05 \) indicated statistical significance.

Results

Gonadotropin levels of premenopausal and postmenopausal patients are shown in Figure 1.

Postoperative levels of IL-1β, IL-6 and TNF-α were found to be significantly increased compared to preoperative levels in postmenopausal patients (\( **p < 0.001 \)) (Figure 2). Moreover, statistically significant increases were found in postoperative levels of IL-6, TNF-α (\( **p < 0.001 \)) and IL-1 β (\( *p < 0.05 \)) compared to preoperative levels in premenopausal patients (Figure 2).

When preoperative cytokine levels were compared between both patient groups there were no statistically significant differences for IL-1β and IL-6 levels, but there was statistically significant difference for TNF-α level. When postoperative cytokine levels were compared between each patient group, postmenopausal patients were found to have significantly higher IL-6 (\( *p < 0.05 \)) and TNF-α levels (\( **p < 0.001 \))(Figure 2). IL-1 β, IL-6 and TNF-α levels were evaluated according to the menstrual cycle phases in premenopausal patients.

The cytokine increases in the postoperative period were also assessed compared to the preoperative period. The level of IL-1β was only increased significantly in ovulatory phase patients (\( *p < 0.05 \)). The increases in IL-6 levels in all four phases were statistically significant (\( *p < 0.05, **p < 0.01 \)). The TNF-α level was increased significantly in all phases but not in the luteal phase (\( p > 0.05 \) for luteal phase; \( p < 0.05 \) for other phases) (Figure 3).

Among the menstrual cycle phases, the highest postoperative IL-6 (8.155 ± 2.115 pg/mL) and TNF-α (28.590 ± 1.650 pg/mL) levels were observed in follicular phase patients and the highest increases in the concentrations of IL-6 (5.762 ± 1.265 pg/mL) and TNF-α (16.18 ± 1.849 pg/mL) during postoperative period were found in follicular phase patients (Figure 3).

In the postoperative period compared to preoperative, the smallest increases in IL-1β, IL-6 and TNF-α levels
Figure 3. — Serum IL-1β, IL-6 and TNF-α levels measured pre- and postoperatively in premenopausal patients. (PreOp: preoperative; PostOp: postoperative) *p < 0.05; **p < 0.01; ***p < 0.001; a TNF-α; PostOp-Follicular phase vs. Luteal phase and Menses phase (*p < 0.001); b TNF-α; PostOp-Ovulatory phase vs. Luteal phase (p < 0.001); c TNF-α; PostOp-Menses phase vs. Ovulatory phase and Luteal phase (p < 0.05).

(0.125 ± 0.337, 2.827 ± 0.7296, 0.290 ± 1.022 pg/mL, respectively) were determined to be in the luteal phase compared to the other phases; however, these values were not statistically significant (Figure 3).

TNF-α levels in the postoperative follicular phase were significantly higher than postoperative luteal and menstrual phases (*p < 0.001). TNF-α levels in postoperative ovulatory phase were significantly higher than the postoperative luteal phase (p < 0.001). Moreover, TNF-α levels in the postoperative menstrual phase were found to be significantly higher than the postoperative ovulatory and luteal phases (p < 0.05) (Figure 3).

The increases in CRP levels in the postoperative period were assessed in comparison with the preoperative period. In all patients, CRP levels were observed to be increased significantly in the postoperative period compared to the preoperative period (***p < 0.0001). Postmenopausal patients had significantly higher preoperative CRP values than premenopausal patients (p < 0.0001). When the increase in CRP in the postoperative period was compared, it was determined that it was increased more in postmenopausal patients and this increase was statistically significant (p < 0.0001) (Figure 4).

When preoperative CRP levels were compared between the phases, no statistically significant differences were found. Among the menstrual cycle phases, CRP levels were increased significantly in the postoperative period compared to the preoperative period in all phases except the luteal phase (p > 0.05 for luteal phase; p < 0.05 for other phases). When postoperative CRP levels were compared between the phases, a statistically significant difference was detected in all phases (*p < 0.01) (Figure 5).

Discussion

Following a surgical trauma, such as a modified radical mastectomy, the inflammatory response consists of hormonal, metabolic and immunological compounds. The extent of the inflammatory response correlates with the severity of tissue damage. There is a delicate balance between proand anti-inflammatory mediators following a trauma or uncomplicated surgery [6].

TNF-α, IL-1α, and IL-1β are called “alarm cytokines” which initiate the inflammatory process. Following a trauma, the increase of IL-1β is higher than IL-1α. IL-1 and TNF-α also stimulate the production of both themselves and each other, thereby increasing the rate of inflammatory response. Many cytokines such as IL-1β and TNF-α stimulate IL-6 synthesis and its secretion in serum and different tissue types. IL-1β and IL-6 are responsible for systemic effects such as stimulating acute phase reactions and...
CRP is a commonly used marker of acute-phase inflammatory response. The control of CRP is regulated primarily by the effect of IL-6 on the transcription of CRP gene. The plasma CRP concentration rapidly increases in response to acute inflammation, infection, and tissue damage [9]. CRP levels increase in the first days after surgery [10]. Moreover, circulating levels of CRP are also elevated during infectious diseases, cardiovascular diseases, diabetes, chronic inflammatory diseases, inflammatory bowel diseases, autoimmune disorders, arthritis, and many cancers. Circulating CRP levels in healthy people can vary from 0.1 to 10 mg/l [9]. In our study, we benefited from these cytokines and CRP that indicate inflammation. Clinical studies have shown that premenopausal women have lower pneumonia, sepsis and multi-organ failure incidence than men following a traumatic event [11, 12]. Frink et al. reported that females had a lower incidence of impaired organ function and sepsis associated with lower levels of IL-6 and IL-8 among polytraumatized patients [13].

Other studies reported an increase in proinflammatory serum markers (IL-6, IL-1 and TNF-α) without trauma after menopause and suggested that changes in the immune system were associated with estrogen deprivation with age in the postmenopausal women [14, 15]. Healthy postmenopausal females exhibit substantially greater IL-6 responses to acute stress [16]. Unlike these studies, our patients were diagnosed with breast cancer and were subjected to surgery. In this study, we determined that preoperative TNF-α and CRP values were already higher in postmenopausal patients compared to premenopausal patients. We also observed that postmenopausal patients had more severe systemic inflammatory responses and more aggressive increases in IL-1β, IL-6, TNF-α and CRP levels than premenopausal patients following a modified radical mastectomy procedure. This was suggested to be associated with concomitant chronic diseases and low estrogen and progesterone levels in postmenopausal patients.

Hrushesky et al. claimed that timing of breast cancer surgery at different phases of menstrual cycle was important for the survival of patients [3]. They also stated that premenopausal breast cancer patients, who underwent surgery in the luteal phase of their menstrual cycles, had better disease-free survival and overall survival outcomes than patients who underwent surgery in other phases of the menstrual cycle [3, 17]. Although some studies supported this hypothesis [18, 19], other studies claimed that there was no effect of phase of the menstrual cycle on postsurgical outcomes [1, 20]. Alternatively, some studies demonstrated that patients in the follicular phase had better outcomes [21, 22].

The great differences between the studies in the literature are mostly caused by differences in their methodologies. Most studies in the literature were retrospective in nature [17]. In this prospectively planned study, the phases of the menstrual cycle were confirmed by the date of the last menstrual cycle and by measuring gonadotropin levels. The effects of surgical timing according to the phases of the menstrual cycle were evaluated by determining CRP, IL-1β, IL-6 and TNF-α levels.

Although traumatic events impair functions of organs, it has been claimed that application of a single dose of estradiol (E2) normalizes the organ functions [4]. E2 exerts its effects through α and β subunits of estrogen receptors (ER)-α and -β [5]. It has been shown that tissue-specific subtypes of ER are expressed in various tissues. For instance, ER-α is found in the bowel and particularly in liver and ER-α mediates functions of macrophages and immune cells [4, 23]. In studies focused on T cells, it has been reported that ER-α plays a dominant role in executing physiological effects of E2. ER-α possibly carries out this role via normalizing signal pathways of MAPK, NF-κB, and AP-1. MAPK family induces a serial transcription factor activation and it is involved in the expression of pro-inflammatory and anti-inflammatory cytokines such as IL-6, TNF-α, and IL-10 [24]. All these data explain the increased systemic response in postmenopausal patients after trauma in our study. IL-1β, IL-6, TNF-α and CRP levels were higher in postmenopausal women who had lower estrogen levels. Previous studies have shown that sex steroids can affect expression of chemokine/adhesion molecules; and therefore, they can affect accumulation of neutrophiles within the tissues. Heat shock proteins, heat shock factor 1 and peroxisome proliferator activate the receptor γ coactivator 1 regulated by estrogen receptors; and consequently contributes to the protection of a post-traumatic organ. Sex steroids regulate inflammatory cytokines and affect morbidity and mortality [4]. In one study, it has been claimed that progesterone, which is increased during the luteal phase of the menstrual cycle, acts as an anti-proliferative and provides an advantage for survival [25].

In this study, the timing of surgical treatment among premenopausal patients was found to have an effect on systemic responses to trauma and as it showed alterations in CRP, IL-1β, IL-6 and TNF-α levels. When preoperative cytokines and CRP levels were compared between the phases, no statistically significant differences were observed. This outcome was associated with the comparable ages of the patients in all groups.

Among the menstrual cycle phases, the highest postoperative IL-6, TNF-α and CRP levels were observed in follicular phase patients and the highest increases in the levels of IL-6, TNF-α and CRP during postoperative period were also seen in follicular phase patients. Although TNF-α and CRP levels were increased in all phases, the increase in luteal phase was not statistically significant. When postoperative CRP levels were compared between all phases,
the only statistically significant difference was found in follicular phase compared to luteal phase.

Considering these findings in the context of response to trauma, the most disadvantageous phase was determined to be follicular phase and the most advantageous phase of the menstrual cycle was luteal phase. In luteal phase, both estrogen and progesterone levels are high and together they act synergistically [26]. Although it was statistically significant, the increase in the level of IL-6 was the least during the luteal phase compared to the other phases. The increases in IL-1β and TNF-α levels remained lowest in the luteal phase and they were not statistically significant.

There are some limitations in our study. Since breast cancer mostly occurs at older ages, the majority of the patients in this study were in the postmenopausal period and there was a smaller number of premenopausal patients.

Therefore, the number of patients in both groups was not equal and the age distribution was different. Estrogen and progesterone levels were not measured in premenopausal patients because of their regular menstruation cycles. However, the measurement of estrogen and progesterone levels would make the study results more reliable.

Conclusion

In the present study, systemic response to trauma during different phases of the menstrual cycle in breast cancer patients was evaluated with cytokine levels. Based on our findings, luteal phase was found to be the most advantageous phase to plan operative treatment strategy and to get a better response to trauma due to increased levels of both estrogen and progesterone. Therefore, we recommend to delay the operation to the luteal phase if the case is not urgent. Future prospective studies are also needed to support this hypothesis in premenopausal patients who need surgery. The effect of the timing of surgery on prognosis according to the menstrual cycle needs to evaluate cytokine levels.

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Conflict of interest

The authors declare no conflict of interest.

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