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Chemotherapy Effect on Estradiol Levels in Patients with Triple-negative Breast Cancer: A Clinical Prospective Study from Indonesia

Rizka Vinkan Septiani[1]*, Widyanti Soewoto[2], Ida Bagus Budhi[3]

[1]Department of Surgery, Faculty of Medicine, Sebelas Maret University, Surakarta, Indonesia; [2]Department of Surgery, Oncology Division, Faculty of Medicine, Sebelas Maret University, Surakarta, Indonesia; [3]Department of Surgery, Digestive Division, Faculty of Medicine, Sebelas Maret University, Surakarta, Indonesia

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***Correspondence:** Rizka Vinkan Septiani, Department of Surgery, Faculty of Medicine, Sebelas Maret University, Surakarta, Indonesia. E-mail: rizkavs@gmail.com
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Abstract

AIM: This study aimed to scrutinize the chemotherapy's effect on estradiol levels in patients with triple-negative breast cancer (TNBC) at low-resource country.

METHODS: This cohort prospective study involved patients with TNBC who had undergone surgery and had never received chemotherapy or hormonal therapy before. Patients were checked for estradiol levels before and after chemotherapy. This study was conducted at the Surgical Oncology Department of Regional Public Hospital Dr. Moewardi, Surakarta, Indonesia, from April 2020 to March 2021. Descriptive data were presented in a frequency table based on age, menopausal status, parity status, breastfeeding status, hormonal contraception, hormonal contraception duration, family history, stage, and histological grade. Differences in estradiol changes before and after chemotherapy (mean ± SD) were then reported.

RESULTS: From a total of 23 patients, 21 patients (91.3%) experienced a decrease in estradiol levels, while 2 patients (8.7%) underwent an increase in serum estradiol levels after chemotherapy. The mean decrease was 11.57 pg/ml. The two samples that experienced an increase in estradiol levels had a mean increase of 16.5 pg/ml. There was a significant difference between estradiol levels before and after undergoing chemotherapy, with $p = 0.001$. Based on the disease stage, changes in estradiol levels showed significant differences, with $p = 0.003$.

CONCLUSION: In this study, chemotherapy reduced estradiol levels in TNBC patients. Statistically significant reductions in estradiol levels were based on the disease stage.

Introduction

Breast cancer is a major burden worldwide, with a high number of new cases, morbidity, and mortality globally [1]. In 2015, Indonesia had an incidence rate of 42.1 per 100,000 population, with an average death rate of 17 per 100,000 population. Specifically, at Dr. Moewardi Hospital, breast cancer outpatient visits in 2016–2020 were 13.935, 15.356, 16.456, 15.224, and 10.604 patients, respectively.

Breast cancer is caused by constant exposure to estrogen circulating in the blood through the binding of estrogen receptors (ERs) and the active uptake of estrogen. The duration of this exposure due to the accumulation of estrogen metabolites in the form of catechol estrogen that is mutagenic is instrumental in the process of carcinogenesis of breast cancer. Therefore, systemic adjuvant therapy is given to improve mortality and increase relapse free and patient survival rates [2], [3]. Zeleniuch-Jacquotte *et al*. and Baglietto *et al*. stated a relationship between estradiol and the risk of breast cancer with negative ER types [4]. In addition, the measurement of hormone levels in serum, one of which is estrogen, is carried out to assess the chemotherapy's indirect effect through the mediation of hormonal action in patients with breast cancer.

Chemotherapy is believed to decrease the estradiol levels. The significance of estradiol with breast cancer prognosis, as said by Soewoto *et al*., is that high estradiol level will decreased the possibility of remission, while normal estradiol level will increased survival in breast cancer patients [5].

TNBC accounts for 10–15% of all breast cancers, tending to be young at diagnosis, with characteristics of ER-, PR-, and HER2-. TNBC is generally an advanced stage, where most of the mutations in TP53 have occurred and are aggressive with a poorer prognosis. TNBC mainly occurs in younger and obese women and has a strong correlation with BRCA1/BRCA2 mutation status, of which 20% are carrier mutations [6], [7]. However, the optimal chemotherapy regimen for the TNBC treatment has not been found; thus, after all, chemotherapy provides advantages in systemic therapy, both in the form of neoadjuvant or adjuvant and in metastatic conditions [8].

The aim of this study is to knowing the effect of chemotherapy on estradiol levels in TNBC patients. Furthermore, we would like to know the changes in estradiol levels in TNBC patients before chemotherapy and after six cycles of chemotherapy, and also assess the effectiveness of chemotherapy treatment using estradiol level parameters.

Materials and Methods

This study was a cohort prospective involving patients with TNBC (immunochemistry characteristics: ER-, progesterone receptor-, and human epidermal growth factor receptor [HER2-]) who had undergone surgery and had never received chemotherapy or hormonal therapy before. TNBC patients regardless of the type of anatomical pathology or stage of the disease will get adjuvant chemotherapy according to the stage of the disease. Patients with accompanying diseases or conditions that can increase estradiol levels and loss of follow-up patients were excluded from the study. The research was carried out at the Surgical Oncology Department, RSDM Surakarta, from April 2020 to March 2021. This study has been approved by the Ethics Committee of Dr. Moewardi Hospital, Surakarta, Indonesia, number 1.394/III/HREC/2020.

The patients were checked for serum estradiol levels twice, before starting adjuvant chemotherapy and after completing six chemotherapy cycles. Both estradiol scores were recorded by including the study subjects' characteristics: Age, menopausal status, parity status, breastfeeding status, hormonal contraception, hormonal contraception duration, stage, histological grade, and family history.

The data were then analyzed statistically to determine the chemotherapy's effect on serum estradiol levels. Descriptive data were presented in the frequency table. Furthermore, differences in serum estradiol changes before and after chemotherapy were reported in terms of mean and standard deviation (mean ± sd). These data analyses utilized SPSS version 25.0 [9].

Results

Patient characteristics

From April 2020 to March 2021, there were 30 new TNBC patients who had never received therapy, chemotherapy, or hormonal therapy before. However, only 23 patients partook in the study, with seven patients losing follow-up due to chemotherapy not according to schedule (improvement of general condition) and stopping the chemotherapy.

Furthermore, the research subjects' characteristics were presented in the frequency distribution value (%). They can be observed in Table 1. By age, most patients were >40 years old (87.0%). The histological grades mainly were Grade II (78.3%). Most of the stages were in the locally advanced breast cancer (LABC) category (43.5%). Regarding menopausal status, most patients were in the postmenopausal category (56.5%). For parity status in this study, most patients had more than 2 children (60.9%). For breastfeeding duration, most patients were fewer than 2 years (52.2%). For the use of contraception, majority of patients used hormonal contraception (82.6%). Furthermore, the hormonal contraception duration in most patients was <5 years (78.3%). Meanwhile, five patients had a family history of cancer in this study (21.7%).

Table 1: The research subjects' characteristics and changes in estradiol levels before and after chemotherapy (n = 23)

Characteristics	n (%)	Decreased estradiol (%)	Increased estradiol (%)	Difference in estradiol change (mean ± sd) in pg/ml	p-value
Age					0.745
<40 years old	3 (13.0%)	2 (66.7%)	1 (33.3%)	−12.94 ± 42.20	
>40 years old	20 (87.0%)	19 (95%)	1 (5%)	−17.57 ± 19.50	
Histological grade					0.293
Grade I	1 (4.3%)	1 (100%)	0	−55.46 ± −0	
Grade II	18 (78.3%)	16 (88.9%)	2 (11.1%)	−15.22 ± 22.65	
Grade III	4 (17.4%)	4 (100%)	0	−15.19 ± 13.78	
Stage					0.003*
EBC	4 (17.4%)	4 (100%)	0	−47.27 ± 14.48	
LABC	10 (43.5%)	10 (100%)	0	−15.05 ± 11.28	
ABC	9 (39.1%)	7 (77.8%)	2 (22.2%)	−5.63 ± 22.92	
Menopausal status					0.917
Pre-menopause	10 (43.5%)	9 (90%)	1 (10%)	−17.53 ± 23.02	
Post-menopause	13 (56.5%)	12 (92.3%)	1 (7.7%)	−16.53 ± 22.49	
Parity status					0.933
<2 children	9 (39.1%)	8 (88.9%)	1 (11.1%)	−17.47 ± 23.56	
>2 children	14 (60.9%)	13 (92.9%)	1 (7.1%)	−16.64 ± 22.18	
Breastfeeding status					0.746
<2 years	12 (52.2%)	12 (100%)	0	−18.45 ± 15.36	
>2 years	11 (47.8%)	9 (81.8%)	2 (18.2%)	−15.34 ± 28.62	
Hormonal contraception					0.123
Yes	19 (82.6%)	17 (89.5%)	2 (10.5%)	−13.67 ± 21.70	
No	4 (17.4%)	4 (100%)	0	−32.63 ± 19.78	
Hormonal contraception					0.339
<5 years	18 (78.3%)	18 (100%)	0	−21.09 ± 14.20	
≥5 years	5 (21.7%)	3 (60%)	2 (40%)	−2.12 ± 38.74	
Family history					0.104
Yes	5 (21.7%)	4 (80%)	1 (20%)	−2.65 ± 24.25	
No	18 (78.3%)	17 (94.4%)	1 (5.6%)	−20.94 ± 20.55	

Note: *Changes in estradiol levels showed significant differences (p < 0.05).

Statistic analysis

From the analysis results, it was revealed that based on age (p = 0.745), histological grade (p = 0.293), menopausal status (p = 0.917), parity status (p = 0.933), breastfeeding status (p = 0.746), hormonal contraception (p = 0.123), hormonal contraception duration (p = 0.339), and family history (p = 0.104), there was no significant difference in changes in post-chemotherapy estradiol levels (p > 0.05).

However, based on stage (p = 0.003), changes in estradiol levels showed significant differences (p < 0.05), with a mean decrease in estradiol levels of −47.27 ± 14.48 in the early breast cancer (EBC) stage,

a mean decrease in estradiol levels of −15.05 ± 11.28 at the LABC stage, and a mean decrease in estradiol levels of −5.63 ± 22.92 at the advanced breast cancer (ABC) stage. Hence, the higher the stage, the less responsive the effect of chemotherapy to reducing estradiol levels.

Before chemotherapy, it was known that the mean estradiol levels were 85.77 ± 69.38. Then, after chemotherapy, the mean estradiol levels were 68.81 ± 64.32. It indicates that there was a difference in the decrease in estradiol levels of −16.96 ± 22.20. In other words, after chemotherapy, the mean estradiol levels were −19.8%. This study also identified that the number of negative ranks was 21, or 21 patients experienced a decrease in estradiol levels. Meanwhile, the number of positive ranks was two people or two people with increased estradiol levels.

The statistical test results obtained p = 0.001 (p < 0.05), meaning that there was a significant difference in estradiol levels before and after chemotherapy. Thus, chemotherapy effectively lowered estradiol levels in TNBC patients. This is summarized in Table 2.

Table 2: Differences in estradiol levels before and after chemotherapy (mean ± SD) in pg/ml

Variable	Pre	Post	p-value	Difference
Estradiol	85.77 ± 69.38	68.81 ± 64.32	0.001*	−16.96 ± 22.20
Negative rank	21			
Positive rank	2			
Ties	0			

Discussion

In this study, the patients' age ranged from 30 to 83 years (mean 52.7 ± 13.4), with details of 3 patients (13%) <40 years and 20 patients (87%) ≥40 years. Three patients aged <40 years consisted of two patients aged 30 years and one patient aged 38 years. It aligns with Lebert *et al*. who asserted that the type of TNBC is aggressive, occurring primarily in younger and obese women, with a mean age of onset of 53 years [6].

Fourteen patients (60.9%) had children >2, and nine patients (39.1%) had children ≤2. Among women with invasive breast cancer, higher parity and short or non-breastfeeding duration were independently associated with TNBC. Shinde *et al*. reported that compared to non-TNBC breast cancer, TNBC was correlated with higher parity. Besides, a total of 12 patients (52.2%) breastfed for a duration of <2 years, and 11 patients (47.8%) breastfed for a duration of ≥2 years. Shinde *et al*. also mentioned that TNBC was linked to shorter breastfeeding duration per child [10].

Regarding hormonal contraception, 19 patients used it (82.6%), while four patients did not use it (17.4%). Five patients (21.7%) used hormonal contraception for more than 5 years (21.7%). Mørch *et al*. affirmed that the risk of breast cancer increases in women who have used or are currently using hormonal contraception, and this risk rises with the duration of hormonal contraception use [11].

There were 4 patients (17.4%) with EBC stage, 10 patients (43.5%) with LABC stage, and 9 patients (39.1%) with ABC stage. These results are consistent with studies, which state that triple-negative breast cancer (TNBC) patients present with advanced stages, and most have mutations in TP53 [6], [7].

For histological grade, 1 patient (4.3%) had Grade I, 18 patients (78.3%) had Grade II, and 4 patients (17.4%) had Grade III. In this regard, TNBC is aggressive and has the worst outcome with a complex genomic sequence. Grade III tumor presentation was higher in the TNBC group (p < 0.001) [12]. Concerning the presence of low-grade TNBC cases, TNBC is highly heterogeneous, with multiple entities, namely, genetic, transcriptional, histological, and clinical differences, with neoplasms in this group ranging in degree from low to high [13].

In this study, there were 2 patients (8.7%) with a history of breast cancer in a nuclear family, 3 patients (13%) with a history of cancer other than breast cancer in a nuclear family, and 18 patients (78.3%) without a relative with a history of cancer. Having a history of breast cancer in a nuclear family was associated with TNBC [14]. TNBC showed a strong correlation with BRCA 1 or BRCA 2 mutation status, and 20% of patients with TNBC are the mutation's carriers [4].

Of the 23 patients, 10 (43.5%) were pre-menopausal, while 13 (56.5%) had already experienced menopause when starting chemotherapy. Out of the 10 patients who were still menstruating, all experienced amenorrhea at different times between the first chemotherapy and the fourth chemotherapy, with the highest incidence occurring at the third chemotherapy. In this case, chemotherapy regimens used in breast cancer can cause permanent or temporary amenorrhea, which is the effect of chemotherapy itself. Chemotherapy-induced amenorrhea can also occur with varying incidence, depending on the chemotherapeutic agent and the dose used [15].

This study found that 21 patients (91.3%) experienced a decrease in estradiol levels, while 2 patients (8.7%) had an increase in serum estradiol levels after chemotherapy. The mean decrease was 11.57 pg/ml. The two samples with increased estradiol levels had a mean increase of 16.5 pg/ml. Thus, there was a significant difference between estradiol levels before and after chemotherapy, with p = 0.001.

In patients with increased serum estradiol levels, it might be due to progressive cancer or an unresponsive to chemotherapy. The possibility of metastatic conditions could also be a cause. In this study, two patients who experienced an increase in serum estradiol levels after chemotherapy were patients with advanced-stage breast cancer.

Serum estrogen and androgen levels in postmenopausal women affect breast cancer risk. At estradiol or testosterone levels above 20–25% of the mean, the breast cancer incidence is 2–4 times higher [16].

In their study, Pribylova *et al*. revealed 72 patients with breast cancer who underwent chemotherapy and were measured for the hormone levels before, during, and after chemotherapy: Estradiol, progesterone, luteinizing hormone, follicle-stimulating hormone, insulin-like growth factor-1 (IGF), and IGF-binding protein-3 levels. That study found no statistically significant results of tumor stage, hormone receptor expression or HER2, and the treatment regimen used with the hormonal changes studied. In addition, hormone levels after chemotherapy compared with status during chemotherapy were only significantly different in estradiol ($p \leq 0.05$). Meanwhile, there were no significant results in the menopausal gap, age, weight, or chemotherapy type [17].

Moreover, the study of Braverman *et al*. in patients with breast cancer who received chemotherapy found that all study subjects experienced amenorrhea at 1–34 months after chemotherapy, and the chemotherapy regimen administered was associated with estradiol levels after amenorrhea [18]. One study also evaluated the effect of anthracycline chemotherapy in patients with ABC and its effect on estradiol and tumor size. The total effective value of the anthracycline group was 64.7%. Meanwhile, the ratio of serum estradiol before treatment was 82.65 ± 5.61 and after treatment was 53.91 ± 4.72 ($p \leq 0.05$). After therapy, tumor volume decreased gradually with increasing treatment time [19].

Further, chemotherapeutic agents are known to influence ovarian follicle cell death. Oktem and Oklay conducted a study on ovarian tissue samples that received chemotherapy and did not receive chemotherapy. The estradiol level on each culture day decreased significantly in the chemotherapy group. Besides, overall, the ovarian cortical regions of patients receiving chemotherapy produced less estradiol [20]. Yu *et al*. assessed the chemotherapy's effect on ovarian function in young women with breast cancer, with one of the markers being estradiol. Estradiol levels decreased during chemotherapy, and at 52 weeks, the levels returned to pre-chemotherapeutic values [21].

Significant differences in estradiol changes before and after chemotherapy by stage

In this study, based on the breast cancer stage, there were significant changes in estradiol levels before and after chemotherapy ($p = 0.003$). It is where the mean estradiol decrease was −47.27 ± 14.48 in the EBC stage, the mean estradiol decrease was −15.05 ± 11.28 in the LABC stage, and the mean estradiol decrease was −5.63 ± 22.92 in ABC stage. Thus, the higher the stage, the less responsive the effect of chemotherapy to reducing estradiol levels.

Aboulkheyr *et al*. mentioned differences in the chemotherapy response of patients with primary and metastatic breast cancer. In the metastatic tumor samples, the response to paclitaxel and doxorubicin was still good, but it was resistant to cisplatin in 2/3 of the samples. Meanwhile, most primary tumors were responsive to chemotherapy [22].

Considerations for administering hormonal therapy after chemotherapy in patients with TNBC

In this study, 20 of 23 patients (87%) had high serum estradiol levels before chemotherapy, and 3 patients (13%) had normal serum estradiol levels before chemotherapy. After undergoing chemotherapy, three patients with normal serum estradiol values became low. Meanwhile, of the 20 patients with high serum estradiol before chemotherapy, two experienced an increase in serum values (stayed high), and 18 patients underwent a decrease in serum estradiol values, with details of three patients being low, one patient being normal, and 14 patients declining, but still in the high-value range.

Serum estradiol value that decreased after chemotherapy, but was still in the high range, can be considered in administering anti-estrogen drugs, such as tamoxifen after chemotherapy, including in the case of TNBC.

About 5–10% of ER-negative breast cancers showed sensitivity to tamoxifen therapy, depending on variations in the expression of estrogen-related receptor alpha, ER-beta subtype (ERβ), tumor microenvironment, and epigenetics [23]. ER-negative breast cancer is not absolute without ER expression. ER-negative status is assigned based on the low expression of the ERα isoform. One study suggested that normal breast glands express more ERβ than ERα, and breast epithelial cells in mice with ERβ inactivated hyperproliferative properties indicate a tumor-suppressive role of ERβ. Another study also uncovered that ER-negative patients with moderate ER expression revealed increased recurrence-free breast cancer survival after adjuvant tamoxifen therapy [23]. This can be a consideration in the administration of estradiol suppressant therapy for TNBC patient.

Conclusion

Chemotherapy affected reducing estradiol levels in TNBC patients before chemotherapy and after six chemotherapy sessions. This statistically significant decrease in estradiol levels was obtained based on stage, namely, in cases of TNBC with EBC stage.

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