

## Research Article

## The Effect of Cyclophosphamide Chemotherapy on Ovarian Anti-Müllerian Hormone Levels in Breast Cancer Patients

### *Efek Kemoterapi Siklofosfamid terhadap Kadar Hormon Anti-Müllerian Ovarium pada Penderita Kanker Payudara*

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#### Abstract

**Objective:** To evaluate cyclophosphamide effects on Anti-Müllerian hormone (AMH) levels in breast cancer patients treated with cyclophosphamide chemotherapy.

**Methods:** This cohort prospective study was conducted in Dr. Wahidin Sudirohusodo Hospital in the Department of Obstetrics and Gynecology in collaboration with Department of Surgery, Faculty of Medicine, Universitas Hasanuddin between September 2015 and June 2016. Serum levels of AMH from forty breast cancer patients received three series of cyclophosphamide chemotherapy determined by Enzyme Linked Immuno Sorbent Assay (ELISA).

**Results:** Serum AMH levels decreased significant in the first series (from  $2.09 \pm 2.04$   $\mu\text{g/ml}$  to  $0.65 \pm 1.06$   $\mu\text{g/ml}$ ;  $p < 0.05$ ) through the third series (from  $1.53 \pm 1.34$   $\mu\text{g/ml}$  to  $0.5 \pm 0.65$   $\mu\text{g/ml}$ ;  $p < 0.05$ ) of cyclophosphamide chemotherapy.

**Conclusion:** AMH levels decreased significant after cyclophosphamide indicated that cyclophosphamide decrease ovarian reserve.

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**Keywords:** anti-müllerian hormone, breast cancer, cyclophosphamide, ovarian reserve

#### Abstrak

**Tujuan:** Menilai efek kemoterapi siklofosfamid terhadap kadar hormon Anti-Müllerian pada pasien kanker payudara yang dikemoterapi dengan siklofosfamid.

**Metode:** Penelitian kohort prospektif ini dilakukan di RS Dr. Wahidin Sudirohusodo pada Departemen Obstetri dan Ginekologi bekerjasama dengan Departemen Bedah, Fakultas Kedokteran, Universitas Hasanuddin antara September 2015 dan Juni 2016. Pengukuran kadar serum AMH dari 40 pasien kanker yang mendapatkan tiga seri kemoterapi siklofosfamid menggunakan metode Enzyme Linked Immuno Sorbent Assay (ELISA).

**Hasil:** Kadar serum AMH menurun signifikan dari seri pertama (dari  $2,09 \pm 2,04$   $\mu\text{g/ml}$  menjadi  $0,65 \pm 1,06$   $\mu\text{g/ml}$ ;  $p < 0,05$ ) sampai seri ketiga (dari  $1,53 \pm 1,34$   $\mu\text{g/ml}$  menjadi  $0,5 \pm 0,65$   $\mu\text{g/ml}$ ;  $p < 0,05$ ) pada kemoterapi dengan siklofosfamid.

**Kesimpulan:** Kadar AMH menurun signifikan setelah kemoterapi siklofosfamid menunjukkan siklofosfamid menurunkan cadangan ovarium.

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**Kata kunci:** anti-müllerian hormon, cadangan ovarium, hormon, kanker payudara, siklofosfamid

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#### INTRODUCTION

Breast cancer is the most frequency malignancy in women in developed countries. It is estimated that approximately 7% of women with breast cancer are diagnosed before the age of 40 years, and this disease accounts for more than 40% of all cancer in women in this age group.<sup>1</sup> Breast cancer is likely to have a negative impact on reproductive function from the toxic effect of chemotherapy on ovarian follicles.<sup>2</sup> Young women with more primordial oocytes showed a significant reduction of their ovarian reserve following chemotherapy.<sup>3</sup> Chemotherapy causes acute loss of growing follicles

resulting in premature ovarian failure, shortened reproductive life span and hormone deficiency.<sup>4,5</sup>

Cyclophosphamide, also known as cytophosphane, is a nitrogen mustard alkylating agent from the oxazophorine group. Cyclophosphamide is used to treat various types of cancer and some autoimmune disorders by slowing-down or stopping cell growth.<sup>6</sup> Cyclophosphamide, alone or in combination with other chemotherapy agents, is used for the treatment of lymphomas, some forms of leukemia, and some types of solid tumours including breast cancer. Gonadal failure is a major complication of cyclophosphamide administration,

especially in females.<sup>7</sup> Cyclophosphamide affects primordial follicle density rapidly after injection of this agent. Human oocytes are damaged drastically followed by granulosa cells.<sup>8</sup>

The granulosa cells of primary follicles produced Anti-Müllerian Hormone (AMH).<sup>9,15</sup> AMH is a glycoprotein hormone belonging to transforming growth factor b family. It's levels reflect the continuous non-cyclical growth of small follicles.<sup>10</sup> Women with abnormal level of this hormone impaired the ovarian reserved followed by fertility.<sup>11</sup> This study aimed to evaluate the levels of AMH in breast cancer patients treated with cyclophosphamide chemotherapy.

## METHODS

This cohort prospective study was conducted in Dr. Wahidin Sudirohusodo Hospital in the Department of Obstetrics and Gynecology in collaboration with Department of Surgery, Faculty of Medicine, Universitas Hasanuddin between September 2015 and June 2016. The study protocol was approved by the Health Research Ethics Committee of Faculty of Medicine, Universitas Hasanuddin. Eligible patients were women with breast cancer, aged 20 to 48 years, who were to receive three series of cyclophosphamide treatment but who had not received chemotherapy previously. Prior chemotherapy, radiation therapy, oophorectomy, and patients who did not complete the first series of chemotherapy cycles until the third series were ineligible. Serum levels of AMH in each series of chemotherapy were measured at Prodia Clinical Laboratory Jakarta using AMH Enzyme Linked Immuno Sorbent Assay (ELISA) kits. Statistical analysis was performed using SPSS. A two-sided p value <.05 was considered statistically significant.

## RESULTS

The study included 40 women with breast cancer who completed the three series of cyclophos-

phamide chemotherapy. Table 1 summarizes the characteristics of the patients. Seventy percent of the patients were aged 36-47 years, 52.5% had senior high school education, majority of patients had married (87.5%) and multiparity (51.4%).

**Table 1.** Subject characteristics

Variables	n	%
<b>Age (years)</b>		
29 - 35	12	30.0
36 - 47	28	70.0
<b>Education</b>		
SMP	12	30.0
SMA	21	52.5
College	7	17.5
<b>Marital status</b>		
Married	35	87.5
Unmarried	5	12.5
<b>Parity</b>		
0	3	8.6
1 - 2	14	40.0
> 2	18	51.4

Decreased serum AMH levels were seen after each series of cyclophosphamide chemotherapy; first series from 2.09±2.04 µg/ml to 0.65±1.06 µg/ml, second series from 4.03±3.47 µg/ml to 1.30±1.40 and third series from 1.53±1.34 to 0.50±0.65 µg/ml. There was an overall significant decrease in serum AMH levels (p<0.05) for the three series of chemotherapy (Table 2).

## DISCUSSION

In reproductive-aged women treated with chemotherapy and radiotherapy for malignancy, serum AMH levels could be used to predict the ovarian

**Table 2.** AMH Levels in the Series of Cyclophosphamide Chemotherapy of Breast Cancer

Series of chemotherapy	AMH levels (mean±SD µg/ml)		p
	Prechemotherapy	Postchemotherapy	
First	2.09±2.04	0.65±1.06	0.000
Second	4.03±3.47	1.30±1.40	0.003
Third	1.53±1.34	0.50±0.65	0.010

reserve.<sup>12</sup> Chemotherapy has a negative effect on the ovarium that cause the loss of primordial follicles, thus the levels of serum AMH as a marker for early prediction of the ovarian reserve.<sup>13</sup>

We found that there was a significant decrease in serum AMH levels in breast cancer patient treated with cyclophosphamide in the present study. AMH and inhibin B levels immediately decreased after chemotherapy.<sup>14</sup> AMH hormone is produced predominantly in primary, preantral, and small antral follicles.<sup>15</sup> The growing follicles appeared to be the target of chemotherapy agents.<sup>14</sup> Anders et al. conducted a study in AMH, E2, FSH, inhibin B, and AFC for markers of ovarian reserve in patients who received adjuvant treatment. They observed significantly decreased or undetectable serum AMH levels after chemotherapy similar to other previous studies.<sup>16</sup>

This study also found that AMH levels decreased after the first series and along with the next series of chemotherapy. Our results similar to previous study on breast cancer treated with cyclophosphamide that serum AMH levels decreased from first series ( $2.7 \pm 1.0$  ng/ml) to second series ( $0.4 \pm 0.4$  ng/ml).<sup>14</sup> Another study also assayed AMH and other hormonal markers before, during and after chemotherapy administration and reported the decreased serum AMH levels after chemotherapy indicating direct chemotherapy induced damage to the granulosa cells and hence growing follicles and ultimately the follicle pool resulting in decreased ovarian reserved.<sup>17</sup>

AMH levels differ among breast cancer patients that received chemotherapy due to dose administration. Study found that difference dose correlated with AMH levels 0.52 ng/ml, 1.99 ng/ml and 3.09 ng/ml in high dose, low dose and not exposed to chemotherapy, respectively. Increased dose of chemotherapy has greater reduction in AMH levels (Gracia et al., 2012).<sup>18</sup>

AMH levels variations before, during and after chemotherapy provide information about the degree of follicle loss for each patient according to different chemotherapy regimens. Different patterns of AMH levels during the ovarian recovery phase make it possible to discriminate between high and low gonadotoxic chemotherapy protocols. In addition, pretreatment AMH levels are shown to predict the long-term ovarian function after the end of treatment. These provide a better understanding on the ovarian toxicity mechanisms of

chemotherapy and to predict the degree of the ovarian follicle loss. Therefore, it can be useful for fertility preservation strategies, fertility counseling and future family planning.<sup>19</sup>

## CONCLUSION

AMH levels decreased significant after cyclophosphamide chemotherapy. This is indicated that cyclophosphamide decreased ovarian reserve.

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