Research Report

Gonadotoxic Effect of Combined Chemotherapy on Anti Müllerian Hormone (AMH) Level in non-Gynecologic Cancer Patients in Reproductive Age

Efek gonadotoksik kemoterapi kombinasi terhadap kadar hormon Anti Müllerian pada penderita kanker nonginekologi usia reproduktif

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Abstract

Objective: To assess the effect of combined chemotherapy on levels of Anti-Müllerian Hormone (AMH).

Method: This is a prospective cohort study on 12 non-gynecologic cancer women aged 20 - 40 years who received combined chemotherapy treatment. AMH levels and menstrual pattern before and after three months of chemotherapy were examined. The relationships between age, chemotherapy regimens and the cumulative doses on the change of AMH were also analyzed.

Result: The median age of subjects was 37 years (range 20 - 40 years). Pre chemotherapy AMH analysis revealed an inverse correlation between age and AMH levels (r = -0.715; p = 0.009). AMH levels after 3 months of combined chemotherapy drastically declined to 84.6% (p = 0.002). Multivariate analysis indicated that age and total cumulative dose were the main factors contributing to the AMH levels reduction (r = -0.679; p = 0.002 and r = 0.405; p = 0.027). Ten of 12 subjects (83.3%) experienced amenorrhea after 3 months of chemotherapy and had lower level of pre and post-chemotherapy AMH compared to those who still maintained normal periods (p = 0.03 and 0.02).

Conclusion: AMH levels in non-gynecological cancer women who received combined chemotherapy decreased dramatically after 3 months of chemotherapy. Main factors that contribute to this were the cumulative dose and age. Most of these subjects experienced amenorrhea after 3 months of chemotherapy.

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Keywords: ovarian reserve, chemotherapy, Anti Müllerian Hormone (AMH), ovarian function, gonadotoxic

Abstrak

***Tujuan**: Mengetahui pengaruh kemoterapi kombinasi terhadap kadar Anti Müllerian Hormone (AMH).*

***Metode**: Penelitian ini merupakan studi kohort prospektif pada 12 pasien kanker non ginekologi berusia 20 - 40 tahun yang mendapatkan kemoterapi kombinasi. Kadar AMH dan pola haid sebelum dan setelah tiga bulan kemoterapi diperiksa. Hubungan antara faktor usia, rejimen kemoterapi dan dosis kumulatif terhadap perubahan kadar AMH juga dianalisis.*

***Hasil**: Median usia subjek adalah 37 tahun (rentang 20 - 40 tahun). Analisis AMH pra kemoterapi menunjukkan korelasi terbalik antara usia dengan kadar AMH (r = -0,715; p = 0,009). Kadar AMH setelah 3 bulan pemberian kemoterapi kombinasi menurun drastis hingga 84,6% (p = 0,002). Dari analisis multivariat didapatkan bahwa faktor usia dan dosis total kumulatif kemoterapilah yang berperan terhadap penurunan kadar AMH (r = -0,679; p = 0,002 dan r = 0,405; p = 0,027). Sebanyak 10 dari 12 subjek (83,3%) mengalami amenore setelah 3 bulan pemberian kemoterapi dan memiliki kadar AMH prakemoterapi maupun pascakemoterapi yang lebih rendah dibandingkan kelompok yang tetap memiliki haid yang normal (p = 0,03 dan 0,02).*

***Kesimpulan**: Kadar AMH penderita kanker non ginekologi yang mendapatkan kemoterapi kombinasi menurun drastis setelah 3 bulan pemberian kemoterapi dan faktor-faktor yang berperan terhadap penurunan tersebut adalah dosis kumulatif dan usia. Sebagian besar subjek mengalami amenore setelah 3 bulan pemberian kemoterapi.*

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***Kata kunci**: cadangan ovarium, kemoterapi, Anti Müllerian Hormone (AMH), fungsi ovarium, gonadotoksik*

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INTRODUCTION

Currently, non-gynecologic cancer therapy in women who generally accept combination of high-dose chemotherapy has been widely used and this is able to increase the survival rate especially in young cancer survivor. The survival rate in acute lymphocytic leukemia and breast cancer for a notable example has also been increased up to 67% and 75%.[1] However, some chemotherapeutic agents especially the alkylating agents can significantly deplete the ovarian reserve. Thus result in a reduction in fertility and an increased risk of ovarian failure.[2,3] The risk of menopause and infertility has been shown to be a major concern for young premenopausal cancer patients (as if may give) a significant impact on self-esteem, sexual function, and quality of life.

Ovarian dysfunction due to chemotherapy is characterized by irregular menstrual pattern and amenorrhea. The incidence of amenorrhea due to chemotherapy range from 21% - 71% to a adolescent and 49% - 100% in the elderly. Amenorrhea generally will occur within 6 - 16 months post-chemotherapy in women under 40 years old and 2 - 4 months in women over 40 years old.[4] However, menstrual pattern may not be used as an indicator of ovarian reserve, because follicular depletion may occur despite maintenance of regular menstrual cycles.[5] Therefore, more accurate indicators are needed to predict ovarian reserve.

Recently, AMH seems to represent the most promising predictor of ovarian reserve as it is expressed in the ovarian granulosa cell of primary, preantral and small antral follicles that more closely reflects the primordial follicular pool than other hormones. Additionally, AMH does not vary (within menstrual cycle).[6-8]

Study by Anderson *(et al)* indicated that AMH can be used as an early indicator of ovarian reserve decline after 3 months of chemotherapy in breast cancer patients given alkylating agents. This early decline of AMH concentration preceded the changes of other indicators.[9]

Potential risks of fertility dysfunction attributed by chemotherapy agents are important to inform to the patients before starting chemotherapy procedures. Therefore, accurate ovarian reserve marker is needed in order to know properly their reproductive capacity after treatment and it may also help in considering a particular effort of fertility preservation strategies.

Chemotherapy effects on fertility function in childhood cancer survivor has been widely studied, with the result of a significant decline in AMH levels during adulthood compared with normal women of the same age. But there are still few prospective studies and no study in Indonesia aimed to assess the effects of chemotherapy on fertility function of reproductive age. Therefore, this study was conducted to assess prospectively AMH changes due to chemotherapy effects in non gynecologic cancer patients of reproductive age.

METHOD

The Medical Ethics Review Committee of University of Indonesia approved this study and informed consent was obtained from all participants. Fifteen new cancer patients attending haematology and oncology outpatient clinic of Internal department in RSCM and also in Dharmais hospital between August 2009 and March 2010 with: (i) nongynecologic cancer receiving more than 1 chemotherapy agent; and (ii) between 17 and 40 years of age were included in the present study. Patients were excluded if they had a history of gynecologic disease/cancer, history of radiotherapy and chemotherapy treatment, were currently pregnant, and smoked.

The patients were evaluated at two study time points: prechemotherapy (1 week before initiation of chemotherapy) and 3 months after starting chemotherapy (30 days after the first chemotherapy). At each visit, serum AMH level (5 ml) was collected and menstrual status was assessed. Serum samples for AMH obtained and processed within 2 hours after withdrawal. The samples was then freezed in –20°C until assayed. AMH was measured using ELISA (Beckman Coulter, Inc) with sensitivity 0.08 ng/ml. Serum analysis were performed at MAKMAL laboratory University of Indonesia.

Significant differences between values of AMH levels in each visits were calculated by using paired t-test or the non-parametric Wilcoxon test. This main statistic analysis procedure was run on SPSS version 16. A p-value < 0.05 indicates statistical significance.

RESULT

Within the research periods, 15 patients with median age 37 years (range 20 - 40 years) were recruited to the study. However, 3 of them dropped out. Two of them were died from their disease and one patient was lost to follow up. Finally, 12 women were eligible for analysis. The patients characteristics are summarized in Table 1. Analysis of pretreatment hormone showed significant negative correlations between age and serum AMH ($r = -0.715$; $p = 0.009$) (Fig. 1). The patients were then divided into 3 subgroups according to their treatments and the type of cancer: breast cancer (group FAC, n = 6), NHL (group CHOP; n = 4), and CLL (group COP; n = 2). Chemotherapy regimens administered in 3 - 4 cycles with mean of total cumulative doses were 4484.8 (SD 796.4) mg/m^2.

Table 1. Patients characteristics.

Characteristics	Subject (n=12)
Age (years) (median; range)	37 (20 - 40)
Marital states (%)	
married	9 (75)
unmarried	3 (25)
BMI (kg/m^2) (range ± SD)	22.84 ± 4.97
Parity (%)	
Nulliparity	3 (25)
Primiparity	1 (8.3)
Multiparity	8 (66.7)
Hormonal contraception	
Yes	4 (33.3)
No	8 (66.7)
Cancer type and Chemotherapy regimens (%)	
NHL (CHOP)	4 (33.3)
Breast cancer (FAC)	6 (50)
CLL (COP)	2 (16.7)
Total dose of chemotherapy (mg/m^2) (range ± SD)	4484.8 ± 796.4
Menstrual cycle prechemotherapy (%)	
Normal	8 (66.7)
Oligomenorrhea	2 (16.7)
Amenorrhea	2 (16.7)

Abbreviation: CHOP, cyclophosphamide, doxorubicin, vincristine, prednisolone; COP, cyclophosphamide, vincristine, prednisolone; FAC, fluorouracil, adriamycin/doxorubicin, cyclophosphamide; CLL, Chronic lymphositic leucemia; NHL, Non-Hodgkin lymphoma.

By Wilcoxon test, median AMH concentrations fell significantly from 2.06 ng/ml (range 0.30 - 9.50) to 0.20 ng/ml (range 0.12 - 2.22) ($p = 0.002$) within 3 months after initiation of chemotherapy.

As mentioned earlier, there were 3 main treatment groups: FAC, CHOP and COP. AMH level were compared between treatment groups at the study time points. The COP group was only represented by 2 patients, hence was not included in this analysis. There were no significant differences in age in total cumulative doses, pretreatment AMH (AMH_1) and AMH of 3 months after initial chemotherapy (AMH_2) between both FAC and CHOP groups. However, the change of AMH level was significantly different between these two groups (Table 2).

Table 2. Patients characteristics based on chemotherapeutic regimens.

Regimens	FAC (n=6)	CHOP (n=4)	p value
Age (year)	30.8 ± 7.9	36.5 ± 2.6	0.096
BMI (kg/m²)	24.4 ± 4.6	20.7 ± 6,6	0.636
Cumulative dose (mg/m²)	4982 ± 451.0	4189 ± 843.7	0.414
AMH_1 (ng/ml)	4.48 ± 3.31	1.74 ± 0.40	0.073
AMH_2 (ng/ml)	0.43 ± 0.58 (0.20)*	0.17 ± 0.02 (0.16)*	0.077**
ΔAMH (AMH_1 - AMH_2) (ng/ml)	4.05 ± 2.93	1.57 ± 0.42	0.039

Data are mean ± SD for normal distribution and using unpaired t test;
* *= median for abnormal distribution;*
** *= Mann Whitney U test.*

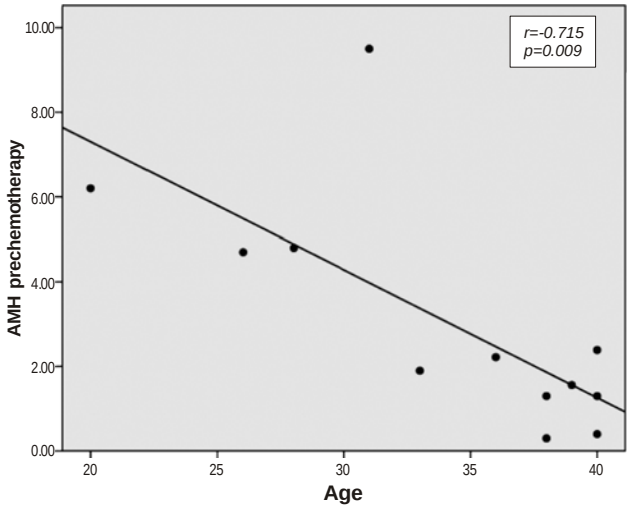

Figure 1. Correlation between age and prechemotherapy AMH level.

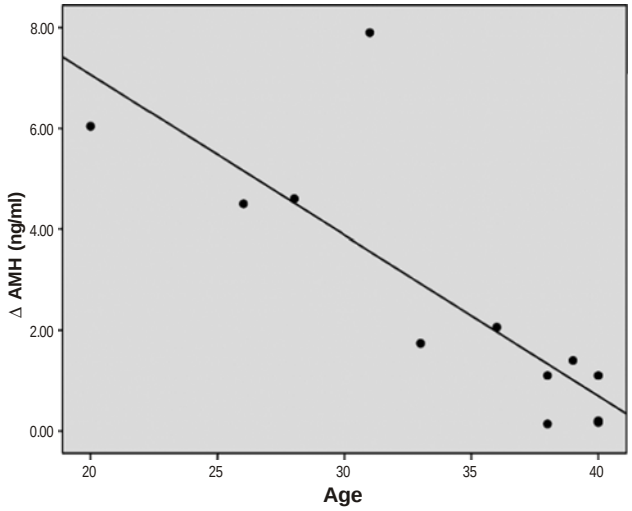

Figure 2b. Correlation between age and ΔAMH.

In Figure 2 it is shown that the correlation between total cumulative doses and age with AMH was decreasing. AMH decline was positively correlated with total doses of chemotherapy (r = 0.610; p = 0.035) and negatively correlated with age (r = -0.817; p = 0.001).

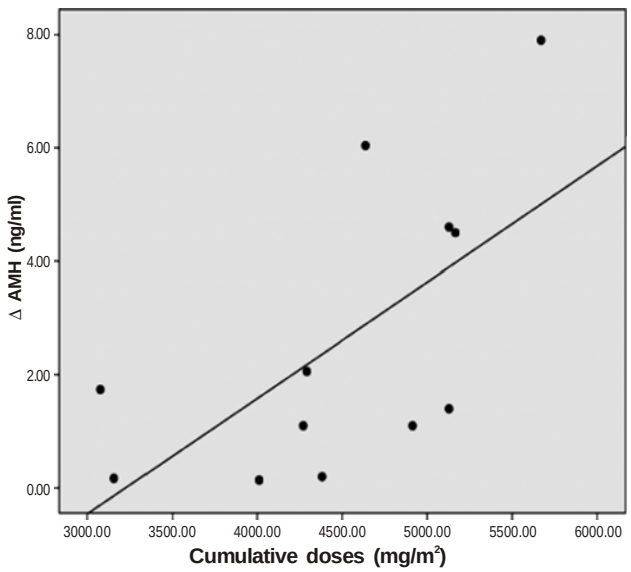

Figure 2a. Correlation between total cumulative doses of chemotherapy and ΔAMH.

Factors affecting the drop in AMH level after 3 months of chemotherapy were analyzed by a multivariate linear regression analysis. On bivariate analysis the chemotherapy regimens had significant relationship with AMH level change. However, age and total cumulative doses of chemotherapy were the only significant factors affecting AMH level decline during 3 months period of chemotherapy on multivariate analysis (Table 3). The AMH level change estimated using the regression equation: Y = 5.776 - 0.265 x age + 0.001 x total cumulative dose.

Prior to chemotherapy, the majority of patients had normal menstrual cycle (10 of 12 patients) with 2 of them were amenorrheic. Three months after the initial chemotherapy, 8 of 10 women from menstruating group became amenorrheic and the two women remained amenorrheic (Table 4).

Pre and post 3 months of chemotherapy, AMH level were found to be significantly lower among amenorrheic women compared to the menstruating groups (2.47 ± 2.03 ng/ml vs 5.95 ± 5.03 ng/ml at baseline, and 0.18 ± 0.02 ng/ml vs 1.91 ± 0.021 at 3 months; p < 0.05 respectively).

Table 3. Multivariate analysis.

	Variable	B	SE	r	p
Level 1	Coefficient	5.661	3.942		
	Chemotherapy regimens	0.041	0.512	0.015	0.938
	Age	- 0.267	0.067	- 0.684	0.004
	Total cumulative dose	0.001	0.001	0.411	0.052
Level 2	Coefficient	5.766	3.505		0.134
	Age	- 0.265	0.060	- 0.679	0.002
	Total cumulative dose	0.001	0.000	0.405	0.027

Table 4. Menstrual cycle characteristics.

Prechemotherapy	Three months after initial chemotherapy	
	Menstruating (n = 2)	Amenorrheic (n = 10)
Menstruating (n = 10)	2	8
Amenorrheic (n = 2)	0	2
Age (years)	35.5 ± 6.3	33.8 ± 6.9

DISCUSSION

This study was conducted in women ages 17 - 40 years. This age selection aimed to assess the effect of combined chemotherapy on AMH level especially to the young cancer survivors (premenopausal age). By knowing the baseline AMH level and its decline after chemotherapy, counseling and treatment related to the aspects of fertility preservation may be considered. Pretreatment fertility state in the current study was shown indirectly from parity for married women, menstrual cycles and no gynecological diseases. The population of this study were nongynecologic cancer patients. In order to minimize the selection bias of fertility disorders due gynecological diseases or malignancies.

The finding in the current study that AMH concentrations decline with age is consistent with the study of de Vat *(et al)*, Van Rooj *(et al)* dan Anderson *(et al)*.[9-11] This decline happened due to the primordial follicles become atresia with advancing age. The approximate time of its decline is when women reach 37 years old. By that time, women may have been menopause around 12 - 14 years later.[11]

Unlike previous studies which investigate chemotherapy effect on ovarian reserve by evaluating some biochemical markers such as FSH, estradiol, inhibin B, AMH and biophysical markers such as basal antral follicles, this study specifically assess the gonadotoxic effects of chemotherapy only by performing the AMH level measurement. AMH is considered a direct marker of ovarian reserve, as it is produced by FSH-sensitive early antral follicles. The decline of AMH level was detected earlier than elevation of FSH level. It has been confirmed by several observational studies on the effects of chemotherapy, including breast cancer treatment protocols using intravenous cyclophospamide.[7,9]

The primary analysis of AMH level changes in this study were confined to the first 3 months, because according to study by Anderson *(et al)* marked fall of AMH level was indicated within 3 months of chemotherapy.[9] In the present study, AMH concentrations fell dramatically by approximately 84.6% within 3 months of treatment. The dramatic falls in AMH concentrations during combined chemotherapy using alkylating agents may reflect severe gonadal toxicity with primordial and preantral follicles as the primary site of toxicity. The other hypothesis developed about mechanism of ovarian reserve depletion by chemotherapy is that it may affect not only on the resting primordial follicles directly but also indirectly by an increased recruitment of primordial follicles. AMH is known to inhibit recruitment of primordial follicles into the pool of growing follicles. Therefore, the absence of AMH with elevated FSH level results in an increased rate of recruitment of primordial follicles which subsequently causes earlier exhaustion of the primordial follicle pool and hence results in a more rapid decline of AMH concentrations.[12]

Multiagents chemotherapy pose a greater risk on ovarian function than the single agents chemotherapy. All subjects in this study were treated with a 3-months (3 - 4 cycles) multiagents chemotherapy that included alkylating agents. As treatment was based on disease staging and pathological grading, patients were not randomized between treatments. The results of this study indicate that ovarian reserve in CHOP group seemed to be less damaged than in FAC group. This is shown as FAC group had significantly larger absolute changes of AMH (ΔAMH) concentrations ($p = 0.039$). Although there was significant difference of the absolute value of ΔAMH concentrations between the two groups, AMH decline was relatively similar between FAC and CHOP groups (90.4% vs 90.2%). It occured as these groups had no significant difference in total cumulative doses and each group consisted of almost similar regimens of chemotherapy.

High total cumulative dose of chemotherapy has been correlated with the premature ovarian failure incidence characterized by amenorrheic condition ≥ 4 months and high FSH level (≥ 40 mIU/ml) occurred at age less than 40 years.[3,13] To date, there were no studies that investigating the correlation between total cumulative doses with ovarian reserve especially AMH concentrations. The majority of previous studies were limited to the investigation of correlation

between cumulative doses of alkylating agents (as a single or multiagents) with premature ovarian failure incidence. The current study demonstrated that there were significant positive correlation between total cumulative doses of chemotherapy regimens with AMH concentrations change ($r = 0.610$; $p = 0.035$).

The risk of ovarian failure in patients who receive chemotherapy is closely related to the age, regimens and higher cumulative doses. The present study found that the main factors correlated to AMH decline during 3 months of chemotherapy based on multivariate analysis were age and total cumulative doses. While chemotherapy regimens did not play a role in AMH decline. This may be explained by quite similar type of regimens and total cumulative doses between treatment groups. This study also demonstrated more pronounced changes in AMH among younger women. This result was consistent with the result of study by Anders *(et al)* (2008) that showed more pronounced AMH concentrations changes found in breast cancer patients aged < 35 years ($p = 0.003$).[14] There is plausible explanation for this finding. In the cylic recruitment, there are more cohort preaantral follicles in younger age than the older women. This results in more affected follicles due to chemotherapy which characterized by more pronounced AMH decline in younger patients.

Normal menstrual cycle after chemotherapy does not always indicate that there is no damage to the ovaries because it is not an "all or nothing" phenomenon. Primordial follicles depletion can occur partially and hence the patients who still can ovulated after chemotherapy administration remain at risk for premature menopause several years thereafter.[15] The degree of ovarian damage will determine whether amenorrhea will be permanent or temporary. If there are minimum amount of oocytes, remains menstrual cycle will have been irregular and the symptoms may develop. On the contrary, if there are no remain oocytes, the menstrual periods will stop.

In the present study, 10 of 12 (83.3%) patients became amenorrheic after 3 months of treatment. This result compares to a 55% rate of amenorrhea among the breast cancer patients receiving combined chemotherapy reported by Anderson *(et al)* (2006).[9]

The AMH baseline in the current study is a useful predictive marker for subsequent menstrual status after chemotherapy. Amenorrheic patients had lower baseline AMH levels compared with menstruating patients (2.47 ± 2.03 ng/ml vs 5.95 ± 5.03 ng/ml, $p = 0.03$). This result contrasts to the study of Bo Yu *(et al)* (2010) which demonstrated no significant different in AMH baseline between amenorrheic patients and menstruating patients.[16] However, our study is supported by 2 previous studies on women with breast cancer. Study by Anderson *(et al)* (2008) illustrated that among 40 premenopausal patients treated with some chemotherapy regimens; prechemotherapy AMH levels were found to be lower among women who became amenorrheic at 6 months after chemotherapy compared to those who resumed menses (0.58 ng/ml vs 1.9 ng/ml; $p = 0.0007$). In the study by Anderson *(et al)*, among women with chemotherapy related amenorrhea, prechemotherapy median AMH levels were lower than those who resumed menses (0.16 ng/ml vs 1.09 ng/ml; $p = 0.02$).[9,14]

Our study has some limitations, including a small sample size and only 3 months of follow up. Due to the short period of follow-up, the menstrual status was only based on the presence or absence of menstruation during this period. We realized that women may resume menses despite low AMH levels after experiencing amenorrhea due to chemotherapy treatment as demonstrated by Rosendahl *(et al)*.[17] Thus, in this study, the longer period menstrual status was not able to establish.

CONCLUSION

These data confirm gonadotoxic effect of combined chemotherapy by significant decreased level of AMH for 3 months since initial treatment. High total cumulative doses and age were the main factors affecting AMH changes. This study also demonstrated the high incidence of amenorrhea during treatment and prechemotherapy AMH level were found to be lower in patients who experienced amenorrhea that menstruating patients.

Further larger and longer period studies were required to evaluate the reversibility of ovarian function by evaluating AMH and menstrual pattern changes after chemotherapy has been completed. It is also necessary to compare the type of chemotherapy regimens (alkylating and non alkylating agents) effects to AMH concentration.

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