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Original Article

Cumulative cyclophosphamide dose and serum anti-Mullerian hormone levels in adolescent cancer survivors in Indonesia

Sri Mulatsih[1], Sarrah Ayuandari[2], Naafi Rizqi Rahmawati[3], Rizki Oktasari[3], Agung Dewanto[2]

Abstract

Background As both the prevalence and survival rates of cancer in children and adolescents has risen, longer-term effects of cancer treatment must be investigated. High-risk gonadotoxic chemotherapeutic agents such as cyclophosphamide may affect the ovarian reserve and impact female adolescent fertility. Anti-Mullerian hormone is a reliable marker to assess ovarian reserve.
Objective To assess for a possible correlation between the cumulative dose of cyclophosphamide and serum anti-Mullerian hormone (AMH) levels among adolescent cancer patients.
Methods This cross-sectional study included 12-18-year-old adolescent female cancer patients who had experienced menarche and received cyclophosphamide therapy. We recorded the patients' full history, including menstrual history, computed the cumulative dose of cyclophosphamide received, and measured serum AMH levels. The correlation test was performed to evaluate for a possible correlation between the cumulative dose of cyclophosphamide and ovarian reserve as represented by AMH levels.
Results Out of 12 female adolescent cancer patients, three complained of disturbances in their menstrual cycles. Low levels of AMH (<1.5 ng/mL) were noted in five patients. Median cumulative cyclophosphamide dose was 1,000 mg/m^2 (range 1,000 to 5,250 mg/m^2). Cumulative cyclophosphamide dose was negatively correlated with serum AMH levels, but this correlation was not statistically significant (r=-0.316, P=0.318).
Conclusion This study has not been able to show a correlation between cumulative cyclophosphamide dose and serum AMH level. Regular evaluation of fertility and involvement of fertility team is recommended in adolescents receiving high-risk gonadotoxic chemotherapeutic agents. **[Paediatr Indones. 2023;63:376-82; DOI: https://doi.org/10.14238/pi63.5.2023.376-82**].

Keywords: *cyclophosphamide; AMH; adolescent cancer patient; menstrual cycle*

The total number of childhood cancer cases has grown rapidly through the years. The number of new cancer cases has risen worldwide, from 12.7 million in 2008 to an estimated 22.2 million by 2030.1 Of these, 50% of total pediatric cancer cases were attributed to the Asian population. Although modern multidisciplinary medicine in developed countries has been successful in curing 80% of pediatric cancer cases, a large proportion of the pediatric cancer population resides in low- and middle-income countries, where access is still limited and chances of a cure are still low.[1] As survival rates improve, longer-term effects of cancer treatment are being investigated. The late effects of various treatment modalities which could interfere with development in pediatric cancer survivors include cardiomyopathy, hypothyroidism, obesity, short stature, infertility, and risk of development of other cancers.[2,3]

From the Department of Child Health[1] and Department of Obstetrics and Gynecology[2], Faculty of Medicine, Public Health and Nursing, Universitas Gadjah Mada/Dr. Sardjito General Hospital[3], Yogyakarta, Central Java, Indonesia.

Corresponding author: Sri Mulatsih, Department of Pediatrics, Universitas Gadjah Mada/Dr. Sardjito General Hospital. Jl. Kesehatan No. 1, Sekip, Yogyakarta, Indonesia. Email: smulat@gmail.com.

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Ovarian reserve is defined as the limited number of primordial follicles that form during the second half of intrauterine life, followed by a gradual decrease in number until menopause, around the age of 50 years. Impaired fertility is now recognized as the most prevalent late effect of cancer treatment in young cancer survivors.[4] Each treatment modality has a mechanism of impact on fertility.[5] Chemotherapeutic agents also affect the ovaries in several ways, as oocytes and granulosa cells are most vulnerable. Cyclophosphamide is an example of a chemotherapeutic regimen with a profound effect on the ovarian reserve. Primordial follicle apoptosis peaks within 12 hours after the injection of cyclophosphamide under molecular evaluation, indicating that the initiation of primordial follicle damage occurs almost immediately after exposure to cyclophosphamide.[4]

Antineoplastic agents have been suspected to affect the ovaries, as predicted by the incidence of amenorrhea as well as serum levels of early phase follicle-stimulating hormone (FSH), estradiol, and anti-Mullerian hormone (AMH), and antral follicle count (AFC) determined by transvaginal ultrasound.4 AMH is considered to be the most reliable marker in predicting antral follicular count, given AMH levels are stable throughout the menstrual cycle. AMH, a dimeric glycoprotein belongs to the transforming growth factor-β family, is expressed by small antral follicles in reproductive age women.[6,7]

Our previous study showed that, overall, chemotherapy can affect the ovarian reserve with regards to AMH level, altered menstrual regularity, as well as delayed pubertal development.[8] However, the correlation between cumulative dose of high-risk gonadotoxic agents such as cyclophosphamide and AMH levels has not been studied.

In this study, we aimed to evaluate ovarian reserve in adolescent female cancer survivors who received cyclophosphamide by measuring serum AMH levels and to analyze for a possible correlation between the cumulative dose of cyclophosphamide and serum AMH level.

Methods

This cross-sectional study was held at Dr. Sardjito General Hospital, Yogyakarta from January to August 2019 as part of a research program describing ovarian reserve and reproductive function in pediatric cancer. We recruited subjects from the outpatient clinic, one-day care, and inpatient ward facilities. Inclusion criteria were female pediatric cancer patients aged 12-18 years who had experienced menarche and had received a chemotherapy regimen that included cyclophosphamide at any dose. Critically ill patients were excluded.

Subjects' full clinical histories were recorded, including history of predisposing cancer comorbidities, age at menarche, and detailed menstrual history. Tanner staging was carried out through physical examination to determine the patients' pubertal stage. Serum AMH was measured to estimate ovarian reserve. Blood sampling was performed at a convenient time regardless of which point it was in the patients' menstrual cycle. AMH was measured by an electrochemiluminescence immunoassay (ECLIA) method. The reference value of AMH at our institution was 1.5-4 ng/mL. We did not obtain any ultrasonographic data in this study.

Cumulative cyclophosphamide dose received was computed for each subject and correlated with serum AMH level using Pearson's correlation. Statistical analysis was performed with *SPSS version 25* (*IBM*, Armonk, New York). A P value of <0.05 was accepted as statistically significant.

The study protocol was approved by the Ethics Committee of the Faculty of Medicine, Public Health, and Nursing, Universitas Gadjah Mada. Written informed consent was sought from patients' parents or legal guardians.

Results

A total of 12 adolescent cancer patients who received cyclophosphamide were analyzed in our study. Their median age at the time of recruitment was 14.9 (range 12.1-18.3) years. The time interval between recruitment and the time of diagnosis ranged from 0.3 to 3.2 years. Four patients had high-risk acute lymphoblastic leukemia (HR-ALL), one had relapsed ALL, three had meningeal HR-ALL, two had Hodgkin's lymphoma (HL), one had T-cell type non-Hodgkin's lymphoma (NHL), and one had an ovarian cyst. The median cumulative dose

of cyclophosphamide was 1,000 mg/m^2. All subjects were treated according to standard protocols used in Dr. Sardjito General Hospital for their individual diagnosis; none of them had radiation or surgical therapy. Three patients complained of menstrual cycle disturbance. Based on physical examination, all patients were in a normal pubertal stage for their age (Tanner 3-4). Patients' characteristics, including diagnoses, age, serum AMH levels, and menstrual history are presented in **Table 1**.

Low levels of AMH (<1.5 ng/mL) were noted in 5 (42%) patients. Serum AMH level was lowest in a meningeal HR-ALL patient (0.04 ng/mL) who received a cumulative cyclophosphamide dose of 1,000 mg/m^2. Pearson's correlation showed a negative correlation between cumulative dose of cyclophosphamide and serum AMH, but the correlation was not statistically significant (r=-0.316; P=0.318). The scatterplot between cumulative cyclophosphamide dose and serum AMH is depicted in **Figure 1**.

Discussion

The top three most common pediatric cancers are acute lymphoblastic leukemia (ALL), central nervous system cancers, and neuroblastomas.[5] ALL begins when a lymphocyte transforms into a lymphoblast after a series of mutations. The lymphoblast multiplies uncontrollably, limits other healthy cell development, and spreads into the bloodstream, lymph nodes, spleen, liver, and other organs.[5] In our study, eight out of 12 subjects had ALL in various high-risk forms.

Currently available therapeutic modalities include surgery, chemotherapy, radiation therapy, and bone marrow transplant, depending on the evidence-based treatment of choice for the cancer diagnosis. To mitigate the risk of tumor metastasis and the risk of recurrence, surgical therapy, when indicated, is commonly combined with chemotherapy and/or radiation therapy.[5]

Chemotherapeutic agents affect the ovaries in several ways, with oocytes and granulosa cells most affected, due to their vulnerability. Oocyte cell death through apoptosis is the main laboratory mechanism for germ cell loss and premature ovarian failure in rodent and reproductive-age models of human ovarian xenografts.[4] Each class of chemotherapeutic agents may have different mechanisms of action that induce cancer cell division cycle cessation.[4] In our study, we investigated the effect of a chemotherapeutic agent, cyclophosphamide, on female adolescent fertility. A summary of the common chemotherapeutic agents used in our institution is shown in **Table 2**.

Chemotherapeutic agents damage oocyte DNA. Oocytes try to repair the DNA damage throughout the ataxia-telangiectasia mutated (ATM)-mediated pathway, but some of them cannot be repaired and proceed to the elimination stage, unless the cell is arrested in growth (cell senescence).[4]

Table 1. Subject characteristics

Diagnosis	Age at diagnosis, years	Age at the exam, years	Weeks of chemo	Cumulative cyclophosphamide dose, mg/m^2	AMH, ng/mL	Menstrual cycle
HR-ALL	15.4	17.4	101	2,000	4.23	regular
HR-ALL	16.7	17.9	65	1,000	2.85	regular
HR-ALL	11.4	12.1	33	1,000	4.12	regular
HR- ALL	10.0	13.3	168	1,000	2.43	regular
Relapsed HR-ALL	13.9	16.0	109	1,000	3.76	regular
Meningeal HR-ALL	12.8	13.5	39	1,000	0.04	regular
Meningeal HR-ALL	15.5	16.6	60	2,000	0.47	regular
Meningeal HR-ALL	12.2	13.7	77	1,000	2.41	regular
HL	12.9	13.3	17	4,200	0.57	irregular
HL	16.0	16.3	9	1,800	0.36	irregular
T-cell type NHL	17.0	18.3	68	1,000	2.01	regular
Ovarian cyst	11.8	13.8	102	5,250	1.15	irregular

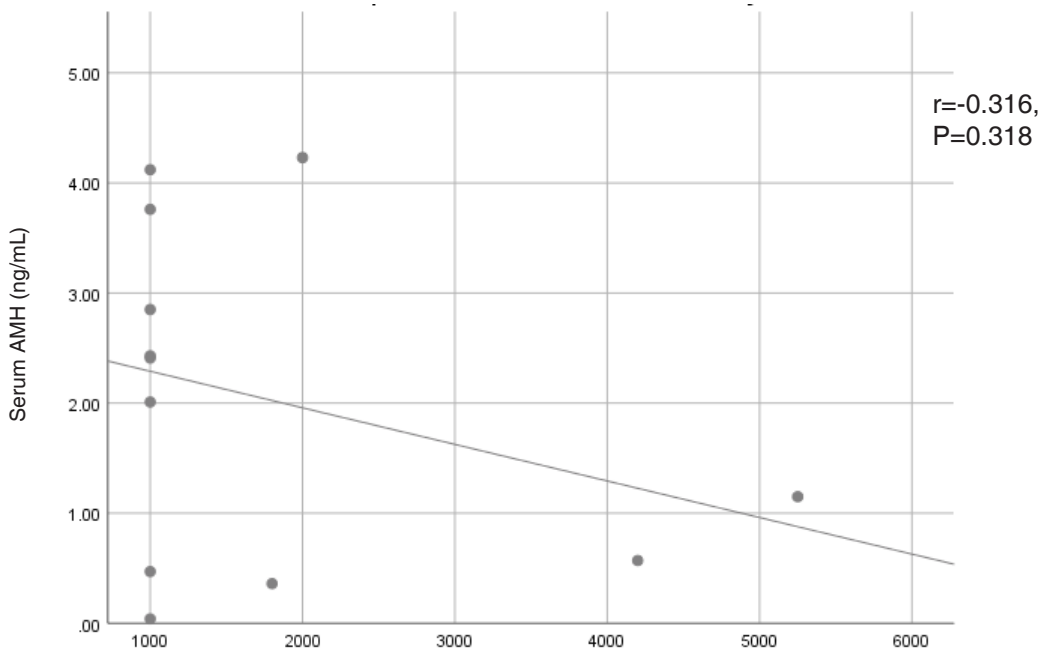

Figure 1. Pearson's correlation scatterplot between of cumulative cyclophosphamide dose and serum AMH

Cyclophosphamide is an example of a chemotherapeutic regimen with profound effects on ovarian reserves. A single dose of cyclophosphamide injected into a human ovarian xenograft causes significant primordial follicle apoptosis, peaking within 12 hours after. Thus, the initiation of primordial follicle damage was almost immediate after exposure to cyclophosphamide.[4] In our study, the total cumulative doses of cyclophosphamide administered ranged from 1,000 to 5,250 mg/m^2. However, the time interval for blood sampling after exposure to cyclophosphamide was not uniformly established among our patients.

The effect of antineoplastic agents on the ovaries can be predicted from the incidence of amenorrhea, serum levels of early phase FSH, estradiol, and AMH, and AFC determined by transvaginal ultrasound.[4] In our study, AMH levels decreased in 5 of 12 (41.7%) patients receiving cyclophosphamide, but we did not perform any ultrasound or other hormone testing. A longitudinal study of three adolescent cancer patients also showed a significant and rapid decrease in serum AMH levels followed by undetectable serum AMH levels independent of the treatment protocol used. All three patients received different type of chemotherapy agents, and some of them also received radiation therapy and/or hematopoietic stem cell transplantation.[6] Besides decreased AMH levels during cancer treatment, FSH levels also increased (>10 mIU/mL), indicating acute gonadal failure.[6] A previous study noted that shortly after exposure to cyclophosphamide, FSHR+AMH+hGL5 cells and their AMH production decreased. However, AMH production recovered 24 hours after administration of an activated form of cyclophosphamide (4-hydroperoxycyclophosphamide, 4HC), while the number of granulosa cells did not recover.[9]

AMH is considered to be the most reliable predictor of AFC, given AMH levels are stable throughout the menstrual cycle.[6,7] AMH levels should be considered more reliable, because of its correlation to AFC levels is more consistent than other ovarian reserve parameters.[7] AMH levels are better at predicting early antral follicle count compared to other conventional hormone parameters and are useful for detecting primary gonadal deficiency, especially in patients without elevated gonadotropin levels.[6,7] The combination of AMH levels and AFC estimates ovarian reserve more precisely in terms of evaluating fertility potential and treatment.[7]

Three of our 12 patients experienced menstrual cycle disorders; all had low AMH levels. Further and more in-depth research on fertility rates is

Table 2. Class of chemotherapy agent and mechanism of action affecting fertility[4]

Class of agent	Examples	Mechanism of action	Infertility risk
Alkylating agents	Cyclophosphamide Mechlorethamine Chlorambucil Busulfan Melphalan	The active metabolites form cross-links with DNA resulting in inhibition of DNA synthesis and function. DNA double-strand breaks and result in P63-mediated apoptotic death in human primordial follicles.	High risk
Platinum-based compounds	Cisplatin Carboplatin	Covalently binds to DNA to form intra- and interstrand DNA cross-links, leading to DNA breakage during replication. This inhibits DNA transcription, synthesis and function. Specific toxicity has not been shown in human primordial follicles.	Intermediate risk
Antimetabolites	Methotrexate 5-fluorouracil Cytarabine	Inhibition of DNA, RNA, thymidylate and purine synthesis. Does not cause DNA damage in human follicles, hence not gonadotoxic.	Low risk
Vinca alkaloids	Vincristine Vinblastine	Inhibition of tubulin polymerization and disruption of microtubule assembly during mitosis. This arrests mitosis during metaphase and leads to cell death. Does not cause DNA damage in human follicles, hence not gonadotoxic.	Low risk
Anthracyclin antibiotics	Daunorubicin Bleomycin Adriamycin (doxorubicin)	Inhibition of DNA synthesis and function. It interferes with DNA transcription. It inhibits topoisomerase II, which leads to DNA breaks. It also forms toxic oxygen-free radicals, which induce DNA strand breaks, thereby inhibiting DNA synthesis and function. Doxorubicin induces DNA double-strand breaks and P63-mediated apoptotic death in human primordial follicles.	Low risk (except Adriamycin: intermediate risk)

needed in populations experiencing similar events. A study demonstrated that after cyclophosphamide exposure in women with breast cancer, ovarian reserve markers such as mean age, AMH levels, and AFC were significantly associated in patients with menstrual disorders than in those who had regular cycles. Patients aged ≥ 32 years with serum AMH levels <3.32 ng/mL and AFC <13 had a significantly higher risk of developing menstrual disturbance such as amenorrhea or oligomenorrhea after cyclophosphamide exposure.[10]

The negative correlation we found between the cumulative dose of cyclophosphamide and serum AMH levels (r=-0.316, P=0.318) was in agreement with the results of another study comparing oral administration of cyclophosphamide in women with granulomatosis with polyangiitis. Decreased AMH level was inversely correlated with the cumulative dose of oral cyclophosphamide (P=0.01); there was an AMH decrease of 0.74 ng/mL for every 10 grams of oral cyclophosphamide dose administered.[11]

As survival rates have improved, the late effects of cancer treatment are also being investigated, as they could interfere in the development of pediatric cancer survivors, leading to conditions such as cardiomyopathy, hypothyroidism, obesity, short stature, infertility, and risk of other cancer diagnoses.[2,3] Impaired fertility is currently recognized as the most prevalent late effect of cancer treatment in young survivors. Emotional stress and concern about the possibility of partial or total infertility during treatment can also impair the quality of life.[4]

Pediatric oncologists generally provide complete and comprehensive information regarding the diagnosis, choice of treatment modality, and prognosis after conducting thorough medical evaluations.[5] Such information can trigger various emotional responses in patients and families, increasing stress and anxiety in the first month after the diagnosis.[13,14]

The *Children's Oncology Group* (COG), an

international working group, has released a risk-based, exposure-related clinical practice guideline, the *Children's Oncology Group Long-Term Follow-Up Guidelines for Survivors of Childhood, Adolescent, and Young Adult Cancers*. It provides personalized, systematic screening, surveillance, and prevention recommendations based on a patient's illness and treatment history to overcome late effects.[15]

The long-term effects of chemotherapy on fertility are starting to gain more attention. Several methods of fertility preservation can be employed to overcome the risk of infertility due to cancer treatment complications including conservative cancer management, and cryopreservation of oocyte, embryo, or ovarian tissue. Embryo and oocyte cryopreservation have been applied at a large scale worldwide and are considered to be established fertility preservation techniques.[4] Although there has been progress in recent years, ovarian tissue cryopreservation is still being reviewed as an experimental technique.[16]

Among our 12 female adolescent cancer patients treated with cyclophosphamide, 42% had low serum AMH levels and 25% had menstrual cycle disturbances. The cumulative dose of cyclophosphamide was negatively correlated with serum AMH levels ($r=-0.316$; $P=0.318$), indicating that the greater the cumulative cyclophosphamide dose, the lower the serum AMH level. Although our results did not reach statistical significance, the AMH level has the potential to serve as a marker of fertility in pediatric cancer patients.

This study has not been able to conclusively show a strong negative correlation between the cumulative dose of cyclophosphamide and AMH level. It is necessary to conduct a study with a larger population or a multicenter study. We need to seriously consider the long-term effects of chemotherapy on fertility in adolescent cancer patients, especially with regard to the decrease in ovarian reserve. Regular evaluation of fertility and involvement of a fertility team may increase fertility rates and reduce morbidity from long-term cancer treatment.

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