Anti-Mullerian hormone and puberty development in girls and adolescents who underwent cancer treatment

Sarrah Ayuandari1 · Agung Dewanto1 · Rizki Oktasari3 · Naafi Rizqi Rahmawati3 · Nurulita Ainun Alma3 · Kuky Cahya Hamurajib3 · Sri Mulatsih2

Received: 27 June 2021 / Accepted: 6 December 2021 / Published online: 19 January 2022
© The Author(s), under exclusive licence to Springer-Verlag GmbH Germany, part of Springer Nature 2021

Abstract

Background High survival rates of children diagnosed with cancer have led to a growing population of women with premature ovarian failure (POF) due to chemotherapy and radiotherapy. The POF process occurs due to the disruption of the hypothalamic–pituitary and gonadal axis followed by the delay of puberty development. Evaluation of reproductive function in children with cancer is essential to determine the fertility preservation plan. This study aimed to describe reproductive functions in children and adolescents with cancer who received chemotherapy based on Tanner stage evaluation, menstrual cycle, and anti-Mullerian hormone (AMH) examination using electro-chemiluminescence immunoassay kit.

Results Twenty-three girls aged 12–18 years old and had menarche who underwent cancer therapy in January–August 2019 in Dr. Sardjito General Hospital were included in the study. Among them, 61% had low AMH levels and were defined as diminished ovarian reserve (DOR). Two subjects with DOR experienced delayed puberty. Regular menstrual cycle was reported in 65.2% of subjects and irregular menstrual cycle in 34.8%, while 21.7% with irregular menstrual cycle encountered secondary amenorrhea.

Conclusion Chemotherapy exposure affected DOR occurrence in 60.9% of patients with childhood and adolescence cancer. Moreover, it also altered menstrual regularity in 34.8% and delayed puberty development in 8.7% subjects.

Keywords Anti-Mullerian hormone · Tanner stage · Puberty · Childhood cancer · Fertility

Abbreviations

POF Premature ovarian failure
HRC High-risk chemotherapy
LRC Low-risk chemotherapy
AMH Anti-Mullerian hormone
ECLIA Electro-chemiluminescence immunoassay
DOR Diminished ovarian reserve
OR Odds ratio
CI Confidence interval
SPSS Statistical package for the social sciences

ALL Acute lymphoblastic leukemia
HL Hodgkin lymphoma
NHL Non-Hodgkin lymphoma
CML Chronic myelogenous leukemia
DNA Deoxyribonucleic acid

Background

Children diagnosed with cancer have a 5-year survival rate of more than 80% [1]. The increased survival rate in children with cancer has led to a growing population of women with premature ovarian failure (POF) due to radiotherapy and chemotherapy exposure in cancer treatment [2]. POF is defined as menopause occurrence before 41 years old and it is marked by increased gonadotropin level and decreased steroid sex hormone [3]. Moreover, chemotherapy and radiotherapy exposure of patients in childhood or adolescence can potentially disrupt pubertal development [4]. Therefore, education and counseling related to fertility preservation...
should be done in childhood cancer patients before receiving chemotherapy and radiotherapy.

Premature ovarian failure in female children and adolescents can be evaluated clinically based on puberty development and menstrual history [5]. During puberty, there is growth spurt, sexual maturity, and attainment of the reproductive capacity process [6]. Puberty is initiated by the release of pulsatile GnRH causing reactivation of the hypothalamic–pituitary and gonadal axis [7]. Therefore, the disruption of this axis that occurs due to tumors, surgery, radiation, and chemotherapy, which are gonadotoxic, can cause endocrine disturbances that lead to disruption of the puberty process [8]. This disruption of puberty can be measured with the Tanner staging which is used to classify the development of secondary sex characteristics of children [9].

Reproduction function measurements in an adult woman can be done by measuring gonadotropin, estradiol, and progesterone levels. However, the hypothalamus–pituitary–gonadal axis has not been activated in prepubertal girls; therefore, those markers are impossible to be measured [5]. Anti-Mullerian hormone (AMH) levels in women can be detected from birth and continue to increase until reaching their peak around the age of 25 years old [10]. AMH is a member of tumor growth factor-beta (TGF-β) produced by granulosa from pre-antral and small antral follicles [11]. AMH hormones can be used as a marker indirectly in ovary follicle reserves with the assumption that the numbers of pre-antral and small antral follicles are related to the number of primordial follicles [12]. Therefore, the AMH level can be used as an alternative parameter of ovarian follicle reserve in children and adolescents [13].

Evaluation of reproductive function in children and adolescents with cancer is essential to determine the fertility preservation plan [14]. Previous studies have stated that there is a negative impact of chemotherapy and radiotherapy agents on fertility function [15]. However, studies of ovarian follicle reserve and puberty development in children and adolescents are limited in practice. Therefore, this research aimed to describe the reproductive function in female children and adolescents with cancer, based on the Tanner evaluation, menstrual cycle, and AMH examination during or after chemotherapy.

Methods

Study design

This study used cross-sectional methods which conducted the data collection of AMH level and pubertal status during and after chemotherapy at a single point of time in patients with malignancy. The data collection was performed during January until August 2019 in the Pediatrics Department of Dr. Sardjito General Hospital, Yogyakarta, Indonesia.

Subjects

The study recruited girls diagnosed with hematological malignancy or solid cancer in Dr. Sardjito General Hospital who underwent chemotherapy during the study period regardless of the chemotherapy phase during study period. Patients were eligible to participate in this study when they met the following criteria: (a) female children and adolescents aged 12–19 years old, (b) who reached menarche, and (c) parents have already given written informed consent to participate. However, children and adolescents who encountered critical states were excluded from the study.

Data collection

Data collection targeted outpatients and inpatients in the Pediatrics Department. The demographic data were collected using a form to document subjects’ data including: age at data collection, age at diagnosis, age at initial administration of chemotherapy, diagnosis, therapy onset, chemotherapy agent used, and menstrual cycle.

Regularity, cycle interval, and duration of menstruation were documented. Prolonged interval of menstrual cycle for more than 3 months was defined as secondary amenorrhea [16].

The AMH level was measured using blood samples and examined using AMH Electrochemiluminescence Immunoassay (ECLIA) Kit (Roche™). The sample analysis was conducted in accordance with the kit’s protocol. The AMH levels were considered decreased and defined as diminished ovarian reserve (DOR) if AMH level was < 1.52 mg/l [17].

Meanwhile, Tanner staging was measured to examine reproductive function. Delayed puberty was defined as the absence of breast growth in a patient more than 13 years old.

Data analysis

Data which had been documented were analyzed using Statistical Package for the Social Sciences (SPSS) 25.0 software (IBM Corp., Chicago). The demographic data, AMH level, menstrual pattern, and puberty development data were analyzed descriptively and summarized by mean or median and proportion value.
Results

Demographic characteristics

This research included 23 girls who underwent cancer therapy in January–August 2019 in Dr. Sardjito General Hospital. Three subjects died before finishing their scheduled therapy. The median of subjects’ age, when checked for AMH level, was 14 years old. Table 1 shows the subjects’ characteristics.

Among subjects, most of them had a hematological malignancy, such as acute lymphoblastic leukemia (ALL), Hodgkin lymphoma (HL), non-Hodgkin lymphoma (NHL), and chronic myelogenous leukemia (CML). Only two patients had a solid tumor in the form of osteosarcoma and ovarian cystoma. In this research, stratification for therapy risk was classified as low risk when received methotrexate, vincristine, actinomycin D, vinblastine, mercaptopurine, or hydroxyurea and high risk when the patient received cyclophosphamide as their treatment regimen. No subject received radiotherapy.

AMH level

The levels of AMH were measured for all subjects. From all subject, mean of AMH level was $1.43 \pm 1.36 \text{ ng/dl}$ (Table 1). The highest AMH level was 4.23 ng/dl, found in an 18-year-old girl who had been receiving cyclophosphamide and undergone maintenance phase during the study period. Meanwhile, the lowest AMH level was 0.01 ng/dl, found in a 13-year-old girl who had been receiving methotrexate, vincristine, prednisone, leucovorin, daunorubicin, and L-asparaginase (Indonesian ALL 2013 Protocol—Standard Risk).

Based on AMH level, 60.9% of the subjects experienced DOR and among them, five subjects received HRC and nine subjects received LRC. Among patients who experienced DOR, 57.1% patients had irregular menstrual cycles, 35.7% encountered secondary amenorrhea, and 42.9% had regular menstrual cycles (Fig. 1).

Menstrual cycle

Among all subjects, average of menstrual cycle interval was 28 days (Table 2). Girls with cancer had average of menstrual duration around 6 days. Fourteen subjects ($n = 14$) in this research had regular menstrual cycle. Almost all subjects who had irregular menstrual cycle were classified as DOR. The longest interval of menstrual cycle which reached 35 days was experienced by a 14-year-old girl in her maintenance phase with history of receiving methotrexate intrathecal, oral prednisone, daunorubicin, cytarabine, cyclophosphamide, and vincristine (based on ALL 2016 protocol).

### Table 1 Subjects’ characteristics

| Characteristics                                      | $(n = 23)$ |
|-----------------------------------------------------|------------|
| Age (median)                                         | 14 years old (13–19) |
| Age at diagnosis (median)                            | 12.9 years old (7–17) |
| Onset chemotherapy at AMH level measurement (median) | 64 weeks (3–289) |
| Diagnosis                                           |            |
| ALL standard risk (ALL SR)                           | 1 (4.3%)  |
| ALL high risk (ALL HR)                               | 8 (34.8%) |
| ALL HR meningeal                                     | 3 (13%)   |
| NHL T-cell                                          | 1 (4.3%)  |
| HL                                                  | 2 (8.7%)  |
| Osteosarcoma                                        | 1 (4.3%)  |
| Ovarian cystoma                                     | 1 (4.3%)  |
| CML                                                 | 6 (26.1%) |
| The gonadotoxicity risk of chemotherapy agent        |            |
| Low gonadotoxicity                                  | 11 (47.8%)|
| High gonadotoxicity                                 | 12 (52.2%)|
| The chemotherapy phase during AMH measurement        |            |
| Induction                                           | 1 (4.3%)  |
| Consolidation                                       | 3 (13.0%) |
| Maintenance                                         | 10 (43.5%)|
| Monitoring                                          | 6 (26.1%) |
| Completed chemotherapy                              | 3 (13.0%) |

*ALL* acute lymphoblastic leukemia, *HL* Hodgkin lymphoma, *NHL* non-Hodgkin lymphoma, *CML* chronic myelogenous leukemia
Protocol Meningeal High Risk. Five girls (21.7%) reported to have experienced secondary amenorrhea and three girls (13.1%) reported infrequent cycles. During menstruation, most of the symptoms that occurred were stomachaches and abdominal cramps in the initial cycles which were normal.

Puberty development

Girls with cancer on average reached menarche at 12 years old. Among all subjects, most of them had normal puberty development according to their age and only two subjects had delayed puberty development. Subjects who had delayed puberty were also found as DOR and received LRC during treatment. One of the subjects was diagnosed with osteosarcoma 2 years before and underwent the 6th cycle of chemotherapy protocol by European Osteosarcoma Inter-group (EOI). Another subject was diagnosed with ALL at 7 years old and during data collection had already completed chemotherapy for 3 years.

Discussion

The increase of childhood and adolescence survival after cancer treatment in recent decades is associated with infertility in adulthood. This study was the first research which evaluated AMH level, puberty disruption, and Tanner stage of girls and adolescents with cancer who underwent cancer treatment in Indonesia. The results were also used as background information for the initiation of fertility preservation services for childhood cancer patients who would receive gonadotoxic therapy in Indonesia.

Chemotherapy agents and radiotherapy could accelerate follicle depletion which would impact on fertility and premature menopause occurrence [18, 19]. This is in accordance with the results of this study which found 61% of subjects had DOR. In this study, there was a higher proportion of DOR experienced by subjects who received LRC. Meanwhile, a different result was found in a previous study by Lunsford et al. in 2013 which showed DOR occurrence was higher in the HRC group [19]. The different result might occur due to the difference in age of the subjects when receiving treatment, the younger age at diagnosis, and subjects had not undergone menarche. In females who reach menarche, there is activation of the hypothalamic–pituitary–gonadal axis. This condition causes a greater number of growing follicles, which are more susceptible to chemotherapy exposure, to undergo atresia. Damage of growing follicles during chemotherapy might occur due to disruption of granulosa cells division through inhibition of replicated DNA [20]. Depletion of growing follicles reduces the production of AMH, which reduces inhibition of primordial follicle development. The increasing growth of follicles will increase follicle atresia and reduce ovarian reserve [21]. Therefore, younger patients who have not experienced menarche will have more follicular reserves and less frequently experience DOR [22].

The decreased growth of follicles during chemotherapy could also lead to menstrual disorders, such as transient or permanent amenorrhea [23]. Among patients with DOR, this recent study identified one patient experienced infrequent menstruation and five patients experienced secondary amenorrhea. Menstrual cycle pattern might be influenced by the timing of chemotherapy within the patients’ menstrual cycle. Patients who received chemotherapy during the follicular phase will experience increased risk of menstrual changes compared with those who received treatment during the menstrual phase. In addition, the duration of chemotherapy more than 6 cycles can also increase the risk of amenorrhea [24]. The amenorrhea relates to the chemotherapy regimen. In a previous study conducted by Casimo et al. in 2004, the use of cyclophosphamide regimen was associated with an increased risk of amenorrhea. However, it is also influenced

Table 2 Evaluation of reproduction function on children and adolescent cancer with chemotherapy treatment

| Parameters                        | n  = 23 |
|-----------------------------------|---------|
| AMH level (mean)                  | 1.43 ± 1.36 |
| Menstrual cycle                   |         |
| Menstrual duration (mean)         | 6.2 ± 0.4 days |
| Interval of menstrual cycle (mean)| 28.4 ± 0.2 days |
| Menstrual regularity              |         |
| Regular menstrual cycle (%)       | 65.2    |
| Irregular menstrual cycle (%)     | 34.8    |
| Delayed puberty development (%)   | 8.7     |
by the accumulated dose and number of chemotherapy cycles which have been received [24].

Cancer treatment, such as chemotherapy, cranium radiation or gonadal radiation, can also increase the risk of puberty disruption due to the hypothalamus–pituitary axis disorder or ovarian failure [25]. The results of this study also found two cases of delayed puberty in patients with DOR. Research conducted by Muller in 2002 showed similar results in which childhood cancer patients in chemotherapy treatment alone did not affect the development of puberty [25].

This study was the first study conducted in our hospital for future identification of fertility and pubertal disturbance among children and adolescents with chemotherapy treatment. Our study has some limitations, such as the small sample size and no follow-up measurement of AMH level and other pubertal parameters after certain period of chemotherapy. A more comprehensive study with larger sample size and a specific AMH evaluation period should be done to confirm our findings. In future study, evaluation of AMH level and menstrual cycle should be monitored before, during, and after chemotherapy to obtain a better description of the menstrual cycle changes.

Conclusion

In conclusion, chemotherapy exposure impacted on DOR occurrence in 60.9% of patients with childhood and adolescence cancer. Moreover, it altered menstrual regularity in 34.8% and delayed puberty development in 8.7% subjects.

Acknowledgements The authors would like to express their gratitude to the staff of Klinik Bahasa for language assistance.

Author contributions SA was involved in the study design, data interpretation, manuscript writing and review; AD was involved in the study design and manuscript review; KH contributed to data analysis, data interpretation, manuscript writing, and editing; NA contributed to data analysis, data interpretation, manuscript writing, and editing; RO was involved in the data collection; NR was involved in the data collection; SM was involved in the study design and manuscript review. All authors have read and approved the final version of the manuscript.

Funding This study received funding from a research grant from Dr. Sardjito Hospital, Yogyakarta to cover AMH level examination cost, and did not receive any other funding from public or commercial sectors.

Availability of data and material Data findings are available from corresponding author upon reasonable request.

Declarations

Conflict of interest Authors declare no conflict of interest.

References

1. Gatta G, Zigon G, Capocaccia R, Coebergh JW, Desandes E, Kaatsch P et al (2009) Survival of European children and young adults with cancer diagnosed 1995–2002. Eur J Cancer 45:992–1005
2. Larsen EC, Muller J, Rechnitzer C, Schmiegelow K, Andersen AN (2003) Diminished ovarian reserve in female childhood cancer survivors with regular menstrual cycles and basal FSH <10 IU/L. Hum Reprod 18(2):417–422
3. Lee SI, Schover LR, Partridge AH, Patrizio P, Wallace WH, Hagerty K et al (2006) American Society of Clinical Oncology recommendations on fertility preservation in cancer patients. J Clin Oncol 24(18):2917–2931
4. Issaoui ME, Giorgione V, Mamsen LS, Rechnitzer C, Birkebak N, Clausen N, Kelsey TW, Andersen CY (2016) Effect of first line cancer treatment on the ovarian reserve and follicular density in girls under the age of 18 years. Fertil Steril 106(7):1757–62.e1
5. Hagen CP, Aksela L, Sörensen K, Main KM, Cleemann L et al (2010) Serum levels of anti-Mullerian hormone as a marker of ovarian function in 926 healthy females from birth to adulthood and in 172 Turner syndrome patients. J Clin Endocrinol Metab 95(1):5003–5010
6. Patton GC, Hemphill SA, Beyers JM, Bond L, Toumbourou JW, McMorris BJ et al (2007) Pubertal stage and deliberate self-harm in adolescents. J Am Acad Child Adolesc Psychiatry 46(4):508–514
7. Herbison AE (2016) Control of puberty onset and fertility by gonadotropin-releasing hormone neurons. Nat Rev Endocrinol 12(8):452–466
8. Bozzola M, Albanese A, Butler GE, Cherubini V, Cicognani A, Caruso-Nicoletti M et al (2001) Unresolved problems in optimal therapy of pubertal disorders in oncological and bone marrow transplanted patients. J Pediatr Endocrinol Metab 14:997–1002
9. Emmanuel M, Bokor BR (2021) Tanner stages [Updated 2020 Dec 18]. In: StatPearls [Internet]. StatPearls Publishing, Treasure Island (FL). Available from: https://www.ncbi.nlm.nih.gov/books/NBK470280/
10. Lie FS, Visser JA, Welt CK, de Rijke YB, Eijkemans MJ, Broekmans FJ et al (2012) Serum anti-Mullerian hormone levels in healthy females: a nomogram ranging from infancy to adulthood. J Clin Endocrinol Metab 97(12):4650–4655
11. Dunlop CE, Anderson RA (2015) Uses of anti-Mullerian hormone (AMH) measurement before and after cancer treatment in women. Maturitas 80(3):245–250
12. Hansen KR, Hodnett GM, Knowlton N, Craig LB (2011) Correlation of ovarian reserve tests with histologically determined primordial follicle number. Fertil Steril 95:170–175
13. Weenen C, Laven JS, Von Bergh AR, Cranfeld M, Groome NP, Visser JA et al (2004) Anti-Mullerian hormone expression pattern

Ethics approval All procedures in this research had been approved by The Medical and Health Research Ethics Committee (MHREC) Faculty of Medicine, Public Health and Nursing Universitas Gadjah Mada—Dr. Sardjito General Hospital, Yogyakarta, Indonesia with reference number: KE/FK/0726/EC/2019.

Consent to participate Written informed consents were obtained from every parent of the patients who participated in the study.

Consent for publications Not applicable.
in the human ovary: potential implications for initial and cyclic follicle recruitment. Mol Hum Reprod 10:77–83
14. Sonigo C, Beau I, Grynberg M, Binart N (2019) Anti-Müllerian hormone in fertility preservation: clinical and therapeutic applications. Clin Med Insights Reprod Health 13:1–7
15. Angarita AM, Johnson CA, Fader AN, Christianson MS (2016) Fertility preservation: a key survivorship issue for young women with cancer. Front Oncol 6:1–10
16. Fraser IA, Critchley HOD, Broder M, Munro MG (2011) The FIGO Recommendations on terminologies and definitions for normal and abnormal uterine bleeding. Semin Reprod Med 29(5):383–390
17. Cui L, Qin Y, Gao X, Lu J, Geng L, Ding L et al (2016) Anti-Müllerian hormone: correlation with age and androgenic and metabolic factors in women from birth to postmenopause. Fertil Steril 105(2):481-485.e1
18. Brougham MFH, Crofton PM, Johnson EJ, Evans N, Anderson RA, Wallace WHB (2012) Anti-Müllerian hormone is a marker of gonadotoxicity in pre- and postpubertal girls treated for cancer: a prospective study. J Clin Endocrinol Metab 97(6):2059–2067
19. Lunsford AJ, Whelan K, McCormick K, McLaren JF (2013) Mullerian hormone as a measure of reproductive function in female childhood cancer survivors. Fertil Steril 101(1):227–231
20. Mitchison TJ (2012) The proliferation rate paradox in antimitotic chemotherapy. MBoC 23(1):1–6
21. Meirow D, Biederman H, Anderson RA, Wallace WHB (2010) Toxicity of chemotherapy and radiation on female reproduction. Clin Obset Gynecol 53(4):727–739
22. Feigin E, Freud E, Fisch B, Orvieto R, Kravarusic D, Avrahami G (2008) Fertility preservation in female adolescents with malignancies. In: Cancer in female adolescents. USA Science Publishers, Hauppauge, pp 38–101
23. Decanter C, Cloquet M, Dassonneville A, Orazio ED, Mailliez A, Pigny P (2018) Different patterns of ovarian recovery after cancer treatment suggest various individual ovarian susceptibilities to chemotherapy. Reprod Biomed Online 36(6):711–718
24. Cosimo SD, Alimonti A, Ferretti G, Sperduti I, Carlini P, Papaldo P et al (2004) Incidence of chemotherapy-induced amenorrhea depending on the timing of treatment by menstrual cycle phase in women with early breast cancer. Breast Cancer 15(7):1065–1071
25. Muller J (2002) Disturbance of pubertal development after cancer treatment. Best Pract Res Clin Endocrinol Metab 16(1):91–103

Publisher’s Note Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.