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Original Article

Longitudinal observation of serum anti-Müllerian hormone in three girls after cancer treatment

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Abstract. Gonadal dysfunction and infertility are major endocrinological late effects among childhood cancer survivors. Chemotherapy and radiation have gonadotoxic effects and diminish the ovarian reserve. The serum concentration of anti-Müllerian hormone (AMH) is a useful marker of ovarian reserve in survivors. We conducted a longitudinal study to investigate the variations of AMH in evaluating the acute and chronic effects of cancer therapy on the ovary. Three young female patients with different hematological diseases were registered, and their medical records were reviewed. Patient 1 with myelodysplastic syndrome received reduced-intensity hematopoietic stem cell transplantation (HSCT) at 10 yr of age. Breast development and menarche occurred spontaneously after HSCT; however, AMH level became undetectable and gonadotropin did not increase. Patient 2 with acute lymphoblastic leukemia had been receiving chemotherapy since 11 yr of age. AMH level became undetectable but increased after chemotherapy and was associated with regular menstruation. Patient 3 with acute myeloid leukemia received chemotherapy at 13 yr of age and myeloablative HSCT at 14 yr of age. AMH level became undetectable after HSCT, and the patient developed amenorrhea. These different patterns in the recovery phase demonstrated that the AMH level immediately after the end of cancer therapy is inappropriate for the evaluation of the ovarian reserve.

Key words: anti-Müllerian hormone, childhood cancer survivor, gonadal function, fertility, ovarian reserve

Introduction

With the improvement of cure rates in cancer patients, the number of childhood cancer survivors (CCSs) is surmised to be growing rapidly (1). However, this improved survival has been accompanied by the occurrence of treatment-related late complications (late effects) (2–4). Infertility and impaired reproductive capacity after cancer therapy have become important issues pertaining to the quality of life of patients (5–7). Our group previously reported the endocrinological abnormalities and gonadal dysfunction among CCSs at a single hospital (8). In collaboration with members of the CCS Committee of the Japanese Society for Pediatric Endocrinology (JSPE), we conducted

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a questionnaire survey concerning reproductive function in pediatric cancer patients (9). Chemotherapy and radiation therapy are gonadotoxic and can lead to premature ovarian failure and infertility in female patients (10, 11). The long-term follow-up guidelines of the Children's Oncology Group have recommended periodic screening of gonadotropins and sex steroids among cancer survivors (12). The JSPE has also issued a follow-up guide for CCSs (13). However, markers such as regular menstrual cycle, basal FSH values, and pregnancy have limitations with respect to utility. It is difficult to predict gonadal function and reproductive capacity during early childhood, as the serum concentrations of gonadotropins and sex steroids are not informative. Although the recovery of ovarian function (i.e., regular menstruation) may occur after cancer therapy, the ovarian primordial follicle pool may not recover. In this regard, the serum concentration of anti-Müllerian hormone (AMH) might be helpful for testing and interpreting measures of ovarian reserve (14). AMH is secreted by ovarian granulosa cells and does not change significantly during the menstrual cycle (15). AMH levels are reduced after chemotherapy and radiotherapy; therefore, AMH level is currently considered a useful indicator of the long-term impact of cancer therapy on the reproductive capacity of female CCSs (16–18). We have also reported that AMH concentration is a useful marker of ovarian reserve in CCSs for detecting primary gonadal deficiency, particularly among patients without increased gonadotropin levels (19). While the utility of serum AMH is gradually being recognized in Japan, the most appropriate time to measure this marker in pediatric CCSs has not been established.

The aim of this study was to investigate the variations of serum AMH levels longitudinally in determining the acute and chronic effects of cancer therapy on the ovarian reserve, and to establish the appropriate time to measure this marker in Japanese pediatric CCSs.

Subjects and Methods

The ethical committee of Osaka University Hospital approved this study (approval No. 10224). This study complied with the Declaration of Helsinki. Written informed consent for evaluation was obtained from the parents of the patients. The medical records of three girls who had received cancer treatment for hematological disease were reviewed prospectively. They had been treated and were followed regularly in the Pediatric Department of Osaka University Hospital. The patients were questioned about age at breast development and details of menstruation. We did not perform transvaginal ultrasonography for measurement of ovarian antral follicle count because of the young age of the patients or refusal of the parents to consent.

We measured the basal plasma concentrations of FSH. FSH was measured by using a chemiluminescent enzyme immunoassay (EIA) (Access FSH; Beckman Coulter, Tokyo, Japan) with a sensitivity of 0.2 mIU/mL. The reference values of FSH for female patients at our institute are as follows: 4.5–11.0 mIU/mL during the follicular phase, 3.6–20.6 mIU/mL during the ovulatory phase, 1.5–10.8 mIU/mL during the luteal phase, 2.0–21.9 mIU/mL during perimenopause, and 21.5–159.0 mIU/mL during menopause. In this study, high FSH was defined as > 10 mIU/mL before menarche and > 20 mIU/mL for female patients receiving estrogen replacement therapy. Aliquots of serum were frozen at –20°C and subsequently used to measure concomitant AMH concentrations. AMH levels were measured by using highly sensitive ELISA (AMH Gen II, Beckman Coulter Company) from SRL Inc. (Tokyo, Japan). The concentrations were compared to the normal values in healthy pediatric female patients as reported by Hagen *et al.*, with a cutoff value < 2.5th percentile being defined as low AMH (20). The conversion formula offered by SRL Inc. was as follows: AMH Gen II (ng/mL) = 0.189 × EIA AMH (pmol/L) – 0.334.

Table 1 Underlying diseases, treatment regimens, and development of puberty in the three patients

	Patient 1	Patient 2	Patient 3
Diagnosis	Myelodysplastic syndrome	Acute lymphoblastic leukemia	Acute myeloid leukemia
Age at start of cancer therapy	10 yr 0 mo	11 yr 8 mo	13 yr 11 mo
Pubertal stage (breast)	Tanner 1	Tanner 4	Tanner 4
Age at completion of therapy	10 yr 0 mo	12 yr 2 mo	14 yr 6 mo
Duration of follow-up posttherapy	36 mo	30 mo	31 mo
Thelarche	11 yr 1 mo (12 mo after HSCT)	8 yr	NA
Menarche	12 yr 5 mo (28 mo after HSCT)	9 yr	12 yr 0 mo
Age at start of ERT	None	None	16 yr 5 mo (23 mo after HSCT)
Outcome	Alive	Alive	Alive
Chemotherapy (cumulative dose)	Fludarabine 150 mg/m^{2*} Melphalan 180 mg/m^{2*}	Cyclophosphamide 3350 mg/m^2 Pirarubicin 120 mg/m^2 Etoposide 1200 mg/m^2 Vincristine 7 mg/m^2 Methotrexate 20 g/m^2 Cytarabine 32 g/m^2	Melphalan 180 mg/m^{2*} Etoposide 1250 mg/m^2 Mitoxantrone 70 mg/m^2 Idarubicin 10 mg/m^2 Cytarabine 69 g/m^2
Radiation therapy	None	None	Total body irradiation (12 Gy)*
HSCT	Reduced intensity HSCT at 10 yr 0 mo	None	Myeloablative HSCT at 14 yr 6 mo

*Conditioning for HSCT. HSCT, hematopoietic stem cell transplantation; NA, not available; ERT, estrogen replacement therapy.

Results

The underlying diseases and treatment regimens for chemotherapy, radiation therapy, and hematopoietic stem cell transplantation (HSCT) were obtained from medical records and are summarized in Table 1. The treatment regimens were chemotherapy in three, radiotherapy in one, and HSCT in two girls. We surveyed the development of puberty and menstrual cycles. The onset of puberty i.e., breast development and menarche had occurred spontaneously before cancer therapy in two patients and after therapy in one patient. We examined the relationship between AMH and FSH levels in these patients.

Patient 1 was a girl with myelodysplastic syndrome. She received reduced-intensity HSCT at 10 yr of age. Fludarabine (150 mg/m^2) and melphalan (180 mg/m^2) were used for conditioning for HSCT (Table 1). Breast development and menarche occurred spontaneously, and gonadotropin did not increase after HSCT; however, serum AMH levels became undetectable: 1.48 ng/mL pre-HSCT *versus* < 0.10, 0.9, 0.34, and < 0.1 ng/mL at 1–9, 12, 15, and 18–36 mo post-HSCT, respectively (Fig. 1).

Patient 2 was a girl with acute lymphoblastic leukemia. She had been receiving chemotherapy from 11 yr of age (Table 1). The AMH level became undetectable but increased after chemotherapy: 1.85 ng/mL pretreatment *versus* < 0.10, 1.46, 0.6, 1.24, and 1.55 ng/mL at 0, 3, 6–18, 24, and 30 mo posttreatment, respectively (Fig. 2). The FSH level increased during cancer therapy but normalized after treatment. Menstruation continued regularly.

Patient 3 was a girl with acute myeloid leukemia. She began receiving chemotherapy at 13 yr of age and myeloablative HSCT at 14

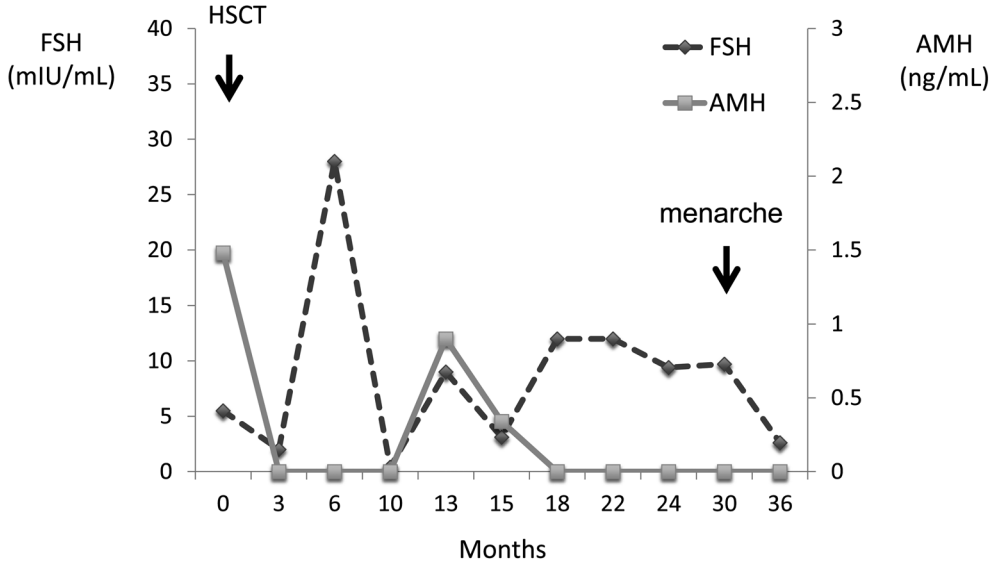

Fig. 1. Serum anti-Müllerian hormone (AMH) levels became undetectable but recovered transiently after hematopoietic stem cell transplantation (HSCT) in patient 1.

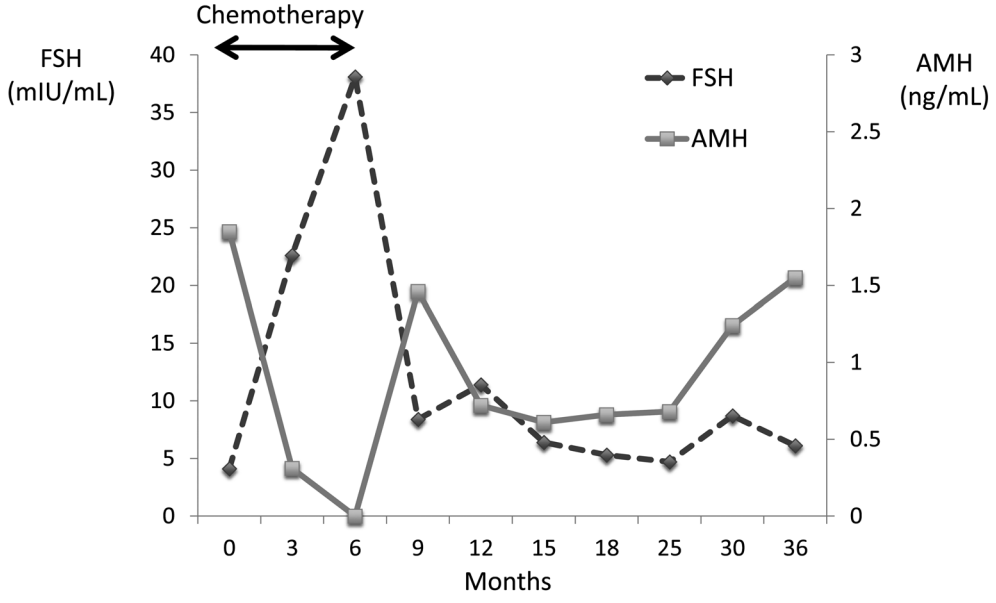

Fig. 2. Serum anti-Müllerian hormone (AMH) levels became undetectable but recovered after chemotherapy in patient 2.

yr of age (Table 1). She received a multiagent chemotherapy regimen, and was given melphalan (180 mg/m^2) and 12 Gy total body irradiation (including both ovaries) for conditioning for HSCT. The FSH levels increased abnormally. AMH level became undetectable and showed no recovery after HSCT: 1.41 ng/mL pre-HSCT *versus* 0.88 ng/mL during therapy and < 0.10 ng/mL at 0–31 mo post-HSCT (Fig. 3). Regular menstrual cycles stopped after the initiation

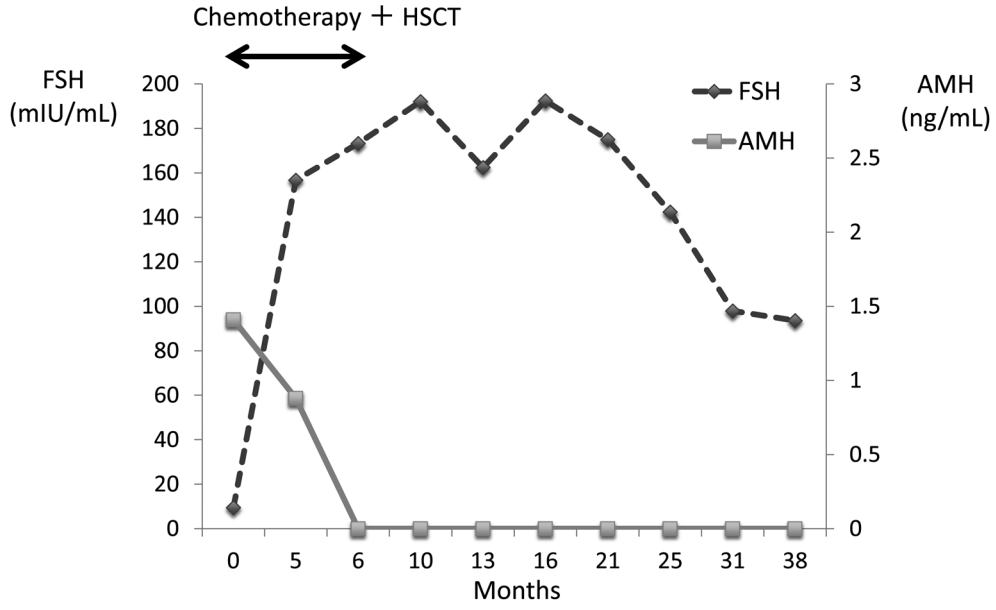

Fig. 3. Serum anti-Müllerian hormone (AMH) levels became undetectable and showed no recovery in patient 3.

of cancer therapy. She developed secondary amenorrhea, and estrogen replacement therapy was started from 16 yr 5 mo of age (23 mo after HSCT).

Discussion

Few studies have been designed to prospectively evaluate the longitudinal variations of AMH levels in pediatric patients treated with cancer therapy (21, 22). A marked and prompt decrease in serum AMH levels was noted in our study, with AMH levels becoming undetectable independently of the treatment protocol used. All three girls had clearly elevated concentrations of FSH (> 10 mIU/mL) and decreased AMH levels during treatment, indicating acute gonadal failure.

The pattern during the recovery phase differed depending on the protocol used. The FSH levels normalized after completion of treatment in patients 1 and 2. Notably, breast development and menarche occurred spontaneously after HSCT in patient 1; however, the AMH level became undetectable (< 0.1 ng/mL). This patient was presumed to have a high risk of premature ovarian failure, and FSH and menstruation were not helpful in this case. As we have reported previously (19), measurement of AMH concentration is useful for detecting primary gonadal deficiency, particularly among patients without increased gonadotropin levels. A biochemical evidence of the recovery of ovarian function was detected on the basis of recovery in AMH levels in patient 2.

The most appropriate timing to measure AMH should be considered for proper medical information and also take into consideration the financial cost. More than 2 yr were needed for recovery and stabilization of the AMH level in patient 2. We usually consider cancer patients as survivors at a minimum of 2 yr after cancer therapy. Therefore, we recommend AMH as suitable for patients who survive for > 2 yr, but not as an acute-phase marker.

The main physiological role of AMH seems to be the inhibition of the initial follicular recruitment from the primordial to the antral pool (15). The recovery in AMH after cancer therapy indicates restoration of the pool of small

growing follicles (23). The absence of a recovery indicates a profound loss of the primordial follicle pool, which is unable to generate sufficient small, growing follicles to secrete a detectable amount of AMH. This primordial follicle loss can arise from a direct elimination effect. In addition, a recent study showed "burn-out" of the ovarian follicle reserve—that is, excessive recruitment of the primordial follicles into the growing pool due to a decrease in AMH after damage of growing follicles (24).

There are various mechanisms of ovarian damage, including apoptotic processes, cortical fibrosis, and blood vessel injury (10, 11). The degree of toxicity is dependent on the treatment variety and dosage, as well as on patient age (25, 26). The toxicity of alkylating agents has been well studied (26, 27). By using the cyclophosphamide equivalent dose (CED), 180 mg/m^2 melphalan is equal to 7200 mg/m^2 CED (40 × cumulative melphalan dose) in patients 1 and 3 (28).

Recently, reduced-intensity stem cell transplantation (RIST) has been used more widely to decrease the risk of complications. Shimizu *et al.* reported that reduced-intensity conditioning consisting of fludarabine/melphalan showed an advantage in the recovery of ovarian function in adolescent and young adult patients (29); however, they did not measure AMH levels. We have shown that the girl treated with RIST (patient 1) had a high risk of premature ovarian failure. Further study is needed in more patients with respect to this finding.

This study may help better understand ovarian toxicities associated with cancer therapy and may help predict the needs for hormone replacement therapy and fertility counseling in the future. However, there are some limitations in this survey. Large numbers of pediatric cancer patients need to be surveyed longitudinally with a more sensitive AMH assay.

In conclusion, a marked and prompt decrease in AMH levels was observed after cancer therapy. Different patterns of AMH level during the recovery phase support the significance of a longitudinal study. The optimal timing to measure serum AMH levels for evaluating ovarian reserve should not be immediately after the end of cancer therapy in CCSs, as levels may recover over time.

Acknowledgments

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