Effect of actinomycin D on ovarian reserve in low-risk gestational trophoblastic neoplasia

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ABSTRACT

Objective This study aimed to explore the single-agent chemotherapy actinomycin D on ovarian reserve by measuring the anti-Mullerian hormone (AMH) levels before, during, and after chemotherapy.

Methods This study recruited premenopausal women aged 15 to 45 with a newly diagnosed low-risk gestational trophoblastic neoplasia needing actinomycin D. AMH was measured at baseline, during chemotherapy, and 1, 3, and 6 months after the last chemotherapy. The reproductive outcomes were also documented.

Results Of the 42 women recruited, we analyzed 37 (median: 29 years; range 19–45) with a complete dataset. The follow-up was 36 months (range 34–39). Actinomycin D significantly decreased AMH concentrations during treatment, from 2.38±0.92 ng/mL to 1.02±0.96 ng/mL ($p<0.05$). Partial recovery was seen at 1 month and 3 months after treatment. Full recovery was reached 6 months after treatment among patients younger than 35 years. The only factor correlated with the extent of AMH reduction at 3 months was age ($r=0.447$, $p<0.05$). Notably, the number of courses of actinomycin D was not associated with the extent of AMH reduction. A total of 18 (90%) of 20 patients who had a desire to conceive had live births with no adverse pregnancy outcomes.

Conclusion Actinomycin D has a transient and minor effect on ovarian function. Age is the only factor that impacts the patient's rate of recovery. Patients will achieve favorable reproductive outcomes after actinomycin D treatment.

WHAT IS ALREADY KNOWN ON THIS TOPIC

⇒ Gestational trophoblastic neoplasia mainly affects women of childbearing age who are typically low risk and only require single-agent chemotherapy, with a favorable prognosis. Actinomycin D, while commonly used, has uncertain gonadotoxicity.

WHAT THIS STUDY ADDS

⇒ The evaluation of ovarian function before and following actinomycin D revealed transient reductions in AMH levels, which typically normalize within 6 months of discontinuing treatment. While slight disparities in post-chemotherapy AMH levels were observed between younger and older patients, we determined these variances to be of no clinical significance.

HOW THIS STUDY MIGHT AFFECT RESEARCH, PRACTICE OR POLICY

⇒ Actinomycin D's minimal impact on ovarian reserve provides clinicians with greater confidence in its use for treating low-risk gestational trophoblastic neoplasia.

INTRODUCTION

Gestational trophoblastic neoplasia covers a range of malignant pregnancy-related disorders, including invasive mole, choriocarcinoma, placental site trophoblastic tumor, and epithelioid trophoblastic tumor. They are typically treated with either single or multi-agent chemotherapy with a cure rate above 90%.[1] Patients are stratified to receive these treatments using the International Federation of Gynecology and Obstetrics (FIGO) prognostic scoring system. Women scoring 0–6 account for approximately 95% of patients and are treated using single-agent chemotherapy. The most commonly used first-line agents are methotrexate and actinomycin D. The updated Cochrane Review published in 2016 concluded that actinomycin D was more likely to achieve a primary cure.[2]

The cure rate approaches 100% in low-risk gestational trophoblastic neoplasia. Since women of childbearing age are the most affected, future reproduction becomes a significant concern during treatment decision-making. Chemotherapy is toxic to the gonad, and to what extent it affects the reproductive function varies by the type of the drug, dosage, and duration of treatment.[3] The patient's age and baseline ovarian reserve may also be involved. A meta-analysis evaluating the reproductive and obstetric outcomes after chemotherapy for gestational trophoblastic neoplasia found a fertility rate of 86.7% among women who wished to conceive after chemotherapy. Still, the rate was significantly higher in single-agent chemotherapy than in the multi-agent group.[4] Regarding single-agent therapy, Savage et al found no increased risk of premature menopause after methotrexate.[5] Cioffi et al and Wong et al reported a 97.5–100% rate of menstrual recovery after methotrexate for the low-risk group.[6] Reports on ovarian reserve after single-agent actinomycin D are more challenging to identify. Most studies report on actinomycin D in combination with other drugs or as second-line treatment after methotrexate failure.

Anti-Müllerian hormone (AMH) is a member of the transforming growth factor beta family and is

expressed by the small (< 8 mm) preantral and early antral follicles. The AMH level correlates with the size of the primordial follicle pool and may be the best biomarker of ovarian reserve.[7] In adult women, it remains relatively stable during the menstrual cycle and gradually declines with age,[8] it is undetectable at menopause.[7] No study explored the AMH response to single-agent chemotherapy. Therefore, our study aimed to investigate the ovarian reserve of gestational trophoblastic neoplasia patients before, during, and after actinomycin D chemotherapy by AMH.

METHODS

Participants

Study participants were enrolled from June 2017 to June 2018 in Peking Union Medical College Hospital and were observed longitudinally assessing AMH before, during, and after single-agent actinomycin D chemotherapy. The ethics committee of Peking Union Medical College Hospital approved this study. Each patient's informed consent was obtained before enrollment. The inclusion criteria were females aged ≤45 years with regular menstruation before detecting low-risk gestational trophoblastic neoplasia. Patients were chemotherapy or radiotherapy naïve. Each patient was required to have an intact uterus and no previous ovarian surgery. Before enrollment, demographics, menstrual and reproductive history, and tumor status were collected.

Study Design and Measurements

The chemotherapy regimen was a single-agent actinomycin D protocol. The dosing was calculated as 1.25 mg/m2 body surface area intravenously every 2 weeks (maximum single dose no more than 2 mg). Remission was achieved when β-hCG levels reached and remained normal for at least three consecutive weeks using the FIGO criteria. After attaining an average β-hCG level, one to three courses of consolidation therapy were administered according to metastatic status and FIGO score. All patients were followed until June 2021. After the last course of chemotherapy, levels of β-hCG were checked monthly for at least 1 year. All patients were interviewed by telephone regarding their menstrual periods and obstetric outcomes. Study measurements were performed within 1 week before initiation of chemotherapy, after two courses of chemotherapy, 1 month, 3 months, and 6 months after completion of chemotherapy. Serum samples were obtained at each time point and stored at −80°C until measurement. AMH was measured by Elecsys AMH (Roche).

Statistics

Continuous data are given as means ±SD or medians (minimum, maximum). Mann-Whitney or Fisher's exact test was used for continuous or categorical parameters. The normality of the variable distributions was assessed using the Kolmogorov–Smirnov test. Statistical analysis was performed using Statistical Product and Service Solutions (SPSS) software (IBM SPSS Statistics for Windows Version 28.0; IBM Corp., Armonk, NY, USA). Spearman's correlation was used in univariate analysis. The significance level was set as $p<0.05$.

RESULTS

Patient Characteristics

Forty-two patients with a median age of 29 years (range 19 to 45) were enrolled in this study. Five patients were excluded for drug resistance and change of regimen (1), loss to follow-up (2), and missing data (2), leaving 37 patients with a complete set of data for analysis. A total of 28 (75.7%) patients were younger than 35 years, whereas 9 (24.3%) patients were above age 35. Four of the nine patients were above age 40. The demographics and clinical characteristics of the subjects are displayed in Table 1.

Table 1 Patient demographics and clinical characteristics

	n=37 (Patients with a full set of data)
Median age (years) at diagnosis(range)	29 (19–45)
Median body mass index (kg/m^2) (range)	22.3 (16.1–33.6)
Median gravidity (range)	2 (1-8)
Median parity (range)	0 (0–2)
FIGO staging	
Stage I	18
Stage III	19
Median FIGO scores (range)	1 (0–4)
Median serum β-hCG before chemotherapy (IU/L) (range)	672 (57–37580)
Number of actinomycin D courses (range)	6 (2, 8)

FIGO, International Federation of Gynecology and Obstetrics.

AMH Response to Actinomycin D

Baseline serum AMH levels are inversely correlated with the patient's age ($r=-0.53$; $P < 0.05$), as shown in Figure 1. The patients' AMH response to actinomycin D is shown in Figure 2. Among the 37 patients, the level of AMH decreased significantly to the lowest point during chemotherapy by 57% lower than baseline (1.02±0.96 ng/mL vs 2.38±0.92 ng/mL before treatment, $p<0.05$). One month and 3 months after the end of chemotherapy, the AMH level gradually recovered and reached 1.30±0.98 ng/mL vs 2.05±0.98 ng/mL, respectively. No significance was observed between baseline and 6 month groups (2.38±0.92 ng/mL vs 2.50±1.31 ng/mL, $p=0.116$).

We further divided the patients into two groups based on age 35 years. We found that among patients younger than 35 years, the AMH level 6 months after chemotherapy was significantly higher than at baseline (2.86±1.27 ng/mL vs 2.64±0.94, $p < 0.05$) whereas, among patients older than 35 years, the level 6 months after chemotherapy was significantly lower than at baseline (1.36±0.59 ng/mL vs 1.58±0.51, $p < 0.05$).

Correlation analysis of all potential factors that could influence the extent of this decline revealed that age ($r=0.447$, $p<0.05$) had a positive correlation. Still, there was no correlation between the decrease in AMH level and the number of courses of actinomycin D at 3 months ($r=0.222$, $p=0.19$).

Menstrual Pattern and Reproductive Outcomes

During 3 years of follow-up, one patient under 35 years and three over 35 years reported shortened menstrual lengths. Five patients under 35 years reported decreased menstrual volume. One 45-year-old patient reported menopause within 1 year after the completion of chemotherapy. Of the 20 patients who desired

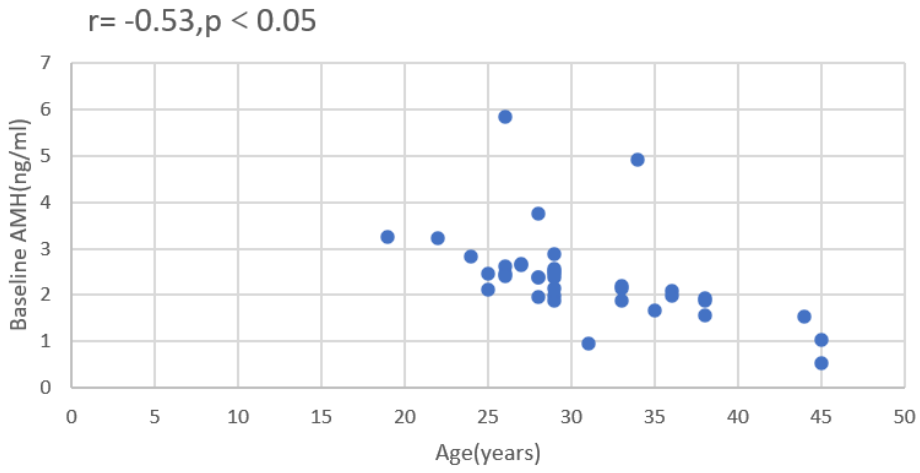

Figure 1 Baseline serum AMH levels are inversely correlated with the women's age.

to conceive, 17 were under 35 years, 18 (90%) patients had live births and no adverse pregnancy outcomes. Two of three patients over 35 years had natural pregnancies and delivered full-term live births. There was one (5%) spontaneous abortion. Of the 18 live births, one patient conceived within 6 months after chemotherapy, and no newborn abnormalities were reported. Of the 17 patients who had no intention of getting pregnant, four were hesistant to get pregnant, two were unmarried, one had acute promyelocytic leukemia (M3) 2 years after completion of chemotherapy, and 10 already had children.

DISCUSSION

Summary of Main Results

According to our research, using actinomycin D resulted in a temporary decrease of 57% in AMH levels, suggesting a potential impact on ovarian function. However, AMH levels returned to baseline within 6 months after chemotherapy. It is important to note that the degree of recovery may differ between younger and older patients. The rate and magnitude of recovery were found to be solely linked to age rather than the number of chemotherapy sessions. It is worth mentioning that patients who received actinomycin D therapy had favorable chances of achieving positive reproductive outcomes.

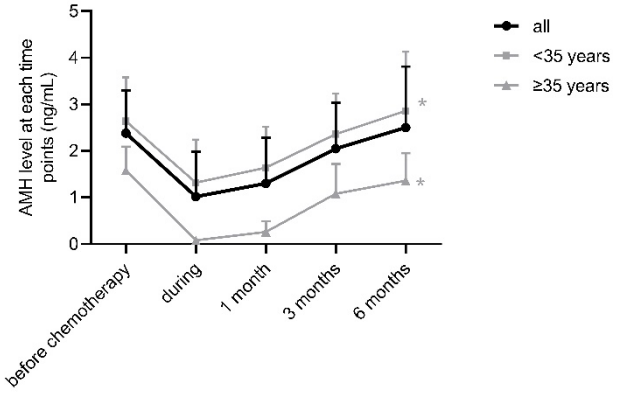

Figure 2 The patients' serum showing a AMH response to actinomycin D over time.

Results in the Context of Published Literature

AMH is frequently assessed in evaluating fertility potential. AMH is a surrogate marker for ovarian reserve and can aid in predicting response to ovarian stimulation. Compared with other commonly considered biomarkers, such as follicle-stimulating hormone (FSH), AMH exhibits more stability throughout the menstrual cycle, thus accounting for its prevalent use as the most reliable predictor of ovarian reserve. Research examining the levels of AMH following cancer treatment has primarily focused on women with breast cancer who received multi-agent therapy. These studies have revealed that AMH levels plummet rapidly to undetectable levels, but the extent of recovery varies among individuals.[9 10] This rapid decline can be explained by the immediate elimination of AMH-producing growing follicles. Moreover, a marked reduction in serum AMH levels were observed in patients with gestational trophoblastic neoplasia receiving etoposide-containing treatment regimens.[11]

Actinomycin D is an anti-tumor antibiotic that can be used as a single agent or combined with other agents in chemotherapy to manage gestational trophoblastic neoplasia. Its primary mechanism of action involves direct binding to DNA to inhibit RNA polymerases, resulting in decreased transcription.[12 13] As to the side effect of fertility, it is classified as limited risk (<20%) of amenorrhea.[14] But no studies have been published regarding ovarian reserve and reproductive outcomes, specifically after actinomycin-D chemotherapy.

As ovarian reserve declines with increasing age [7], and as a representative of growing follicles, AMH strongly correlates with the primordial follicle pool, and we divided the patients into two subgroups based on 35 years as a cut-off. In subgroup analysis, AMH levels were higher 6 months after chemotherapy than baseline in patients <35 years old but were lower 6 months after chemotherapy than at baseline in patients ≥35 years old.

For young patients, an increase in AMH levels may indicate a recovery of ovarian function. However, this does not imply that actinomycin D can improve ovarian function. Nevertheless, the

rise in AMH levels can reassure oncologists when using it among younger patients. It seems unnecessary to routinely follow AMH levels during and shortly after single-agent chemotherapy in younger patients. The modest elevation in AMH levels observed in this study is likely due to the suppressive effect of elevated β-hCG levels before chemotherapy on AMH production. AMH is primarily secreted by the granulosa cells of ovarian follicles, with antral follicles measuring 5–8 mm contributing approximately 60% to the circulating concentration of AMH, 2.1–5 mm follicles contributing 20–25%, and follicles more significant than 8 mm contributing 15–20%.[15] Folliculogenesis largely depends on FSH, and the increase in estrogen and progesterone levels triggered by β-hCG can inhibit FSH secretion.[16] This can decrease overall antral follicle count and shift toward smaller follicle subgroups, a phenomenon also observed in individuals using oral contraceptives.[17]

Similarly, our study was consistent with radioactive iodine treatment on AMH levels, that the effect was more pronounced in patients aged >35 years.[18–20] Although a small yet statistically significant decline in AMH levels was observed among older patients, from 1.58±0.51 ng/mL before chemotherapy to 1.36±0.59 ng/mL, the clinical implications of this finding are uncertain. Faddy's "broken stick" regression theory suggests an abrupt change in the exponential rate of follicle loss at the age of 38 years.[21] In the Chinese population, the reference value for AMH is 2.12 (1.4–3.86) ng/mL for individuals aged 30–34 years but decreases rapidly to 1.22 (0.37–1.59) ng/mL for those aged 35–39 years.[22] Furthermore, since there is more than a year between the start and the end of chemotherapy at 6 months, the decrease in AMH in the last year may be partially due to ovarian aging.

The effect of actinomycin D on older patients may add insult to injury, but it seems that the clinical impact is not significant. The number of participants aged 35 and above is limited, comprising only nine cases, with four patients aged 40 years and above. Consequently, drawing robust conclusions is challenging. The scarcity of cases in the older age groups may be attributed to the higher incidence of gestational trophoblastic neoplasia in younger individuals. On the other hand, low-risk gestational trophoblastic neoplasia frequently arises due to the malignant transformation of the hydatidiform mole to an invasive mole postpregnancy, predominantly affecting younger individuals actively attempting to conceive. Age functions as a factor within the FIGO evaluation framework for gestational trophoblastic tumors, where patients surpassing 40 years of age garner increased ratings, consequently, they are more likely to be classified in the high-risk category.

Our study aims to examine changes in AMH levels during single-agent actinomycin D chemotherapy to treat gestational trophoblastic neoplasia, thereby validating the low reproductive toxicity of actinomycin D and filling the existing knowledge gap. Our findings indicate that routinely monitoring AMH levels may not be necessary for patients undergoing single-agent actinomycin D chemotherapy. The treatment exhibits minimal impact on ovarian reserve and reproductive outcomes. However, women undergoing more dose-intensive or multi-agent therapies may benefit from closely monitoring AMH levels. These treatments could have a more pronounced effect on ovarian reserve and fertility.

Strengths and Weaknesses

This is a prospective preliminary study of single-agent chemotherapy of actinomycin D-treated low-risk gestational trophoblastic neoplasia patients. The gestational trophoblastic neoplasia center where the study was conducted provided standardized chemotherapy management, thereby ensuring the quality of treatment. The detailed diagnosis, treatment, oncologic, and reproductive outcome information were well documented. However, our study had some weaknesses. First, the number of patients included was small due to the rarity of this disease. Second, although all the cases were scored as low-risk, there were differences in β-hCG levels.

Implications for Practice and Future Research

The extent of AMH reduction 3 months after chemotherapy was higher in older patients. There were no significant differences in AMH levels regarding different courses, which indicated that the ovarian reserve was not correlated with cumulative actinomycin D dosage in our study. Therefore, clinically the dosage of actinomycin D to reach a complete remission for low-risk gestational trophoblastic neoplasia patients is relatively safe for ovarian function. Despite the transient decline in ovarian reserve during and shortly after chemotherapy, there was no significant decline in fertility according to the menstruation conditions and obstetric outcomes. AMH measurement is not routinely recommended during and after chemotherapy.

Further research is needed in this area as understanding the implications of various chemotherapeutic regimens on fertility preservation is crucial for guiding clinical practice and patient counseling. Additionally, it is essential to recognize that the ovarian reserve constitutes just one aspect of fertility. It should be noted that low AMH levels are not indicative of short-term fertility.[23 24] Likewise, a weak correlation exists between AMH levels and fertility in cancer survivors who have resumed spontaneous menstruation.[25] Caution is still necessary when interpreting low AMH levels after chemotherapy, and the relationship between AMH and fertility in women with gestational trophoblastic neoplasia requires extensive research.

CONCLUSIONS

In summary, actinomycin D transiently affects ovarian reserve, with full recovery 6 months after chemotherapy among younger low-risk gestational trophoblastic neoplasia patients. Age is the only factor that affects the rate of recovery. Patients receiving single-agent actinomycin D could achieve favorable reproductive outcomes.

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Contributors Conception and design: WX, WC, JY, YX; Acquisition of data: WX, WC, JZ, XW, FF, TR, LQ, JY, YX; Analysis and interpretation of data: WX, WC, JY, YX; Manuscript writing: WX, WC, JY, YX. Guarantors: WX, WC, JY, YX. Manuscript review and approval: all authors.

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Competing interests None declared.

Patient consent for publication Consent obtained directly from patient(s).

Ethics approval This study involves human participants and was approved by the ethics committee of Peking Union Medical College Hospital (ZS-1381). Participants gave informed consent to participate in the study before taking part.

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