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Original Research

Assessment of ovarian reserve with anti-Mullerian hormone in women following allogeneic hematopoietic cell transplantation

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Summary

Introduction: Severe ovarian failure and persistent infertility have could be seen in females following allogeneic hematopoietic cell transplantation (allo-HCT.) In this study, the authors aimed to determine the effectiveness of AMH on assessment of ovarian reserve in long-term survivors after allo-HCT. *Material and Methods:* Female patients, who underwent allo-HCT between August 2009 and February 2016, were retrospectively evaluated for ovarian capacity in long-term follow-up. Twenty-one female patients with a median age of 34 (22-45) years were included in the study. The serum levels of estrogen (E2), follicle stimulated hormone (FSH), luteinizing hormone (LH), and AMH were analysed. *Results:* The median duration of post-transplant follow-up was 37 (12-84) months. Primary ovarian failure (POF) was detected in eight (38,1%) and 19 (90,4%) cases in the pre-transplant and post-transplant period, respectively. It was found that no menstruation cycles were observed in 18 cases with low AMH levels. *Discussion:* Regular menstrual cycles may not guarantee the fertilization in the post-transplant period. Combined analysis of hormonal investigations, antral follicle count by vaginal USG, and evaluation of serum AMH levels may be preferred to demonstrate the presence of POF.

Key words: Anti-Mullerian hormone; Ovarian reserve; Allogeneic stem cell transplantation.

Introduction

Allogeneic hematopoietic cell transplantation (allo-HCT) with its curative potential is an essential treatment modality including but not restricted to haematological malignancies, bone marrow failure syndromes, immuno- deficiencies and metabolic diseases. The past two decades witnessed a global and sustained increase in the use of allo-HCT [1, 2]. In adults, three-year median survival following allo-HCT is between 24-76 % according to the underlying disease [3]. Even patients who survive early complications of the procedure experience psychiatric, endocrine, pulmonary, cardiovascular problems, as well as secondary malignancies, and chronic graft versus host disease (cGvHD) in the long-term follow-up. These long-term complications not only increase the mortality and morbidity of the transplant survivor, but also decrease his or her quality of life [4]. Severe ovarian failure and persistent infertility are encountered over 90% in post-HCT long-term follow-up of females [5, 6]. The most important factors determining the risk of infertility in women after allo-HCT are pre-transplant chemotherapy, radiotherapy (RT), and conditioning regimens [7]. It is known that cyclophosphamide, busulfan, and total body irradiation (TBI), which are commonly used in preparation regimens, have a high risk of ovarian failure [4, 7].

The instrument commonly used to evaluate ovarian reserve is the antral follicle count by ultrasound. In addition, follicle stimulated hormone (FSH), luteinizing hormone (LH), and estrogen (E2) measurements can also be used. However, these methods may be inadequate to fully assess ovarian reserve due to problems such as the necessity of measuring at a certain period of menstrual cycle [8, 9]. In addition, cost and workload are also a major problem for these tests.

Premature ovarian failure (POF) is one type of hypergonatotropic hypogonadism in patients below 40 years of age [10]. The evaluation of ovarian reserve in patients with POF is of utmost importance, because troublesome and expensive assisted reproductive attempts will likely fail if the women do not have functioning ovarian follicles. Recently, anti-Müllerian hormone (AMH) became a sensitive indicator of ovarian reserve directly reflecting the number of follicles. As AMH levels are independent of gonadotropins and have little intra- and inter-cyclic variation, it has been used increasingly to determine the ovarian reserve [11,12]. Achieving the result with a simple blood test independently of the menstrual cycle provides a cost effective and a simple way to show the ovarian reserve for fertility.

There is some evidence that low AMH serum levels can also be used to reflect low ovarian reserve in patients treated for malignant diseases [12, 13]. The use of AMH in allo-HCT setting for post-transplant follow-up is not clear

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[14]. In this study, the authors aimed to determine the effectiveness of AMH on assessment of ovarian reserve in long-term survivors after allo-HCT.

Material and Methods

Twenty-one female patients with a median age of 34 (22-45) years were included in the study. They underwent allo-HCT at the present center between August 2009 and February 2016 and were retrospectively evaluated for ovarian capacity during their post-transplant follow-up period. The exclusion criteria were: patients with a history of post-transplant follow-up period of less than 12 months, recurrent disease, active GvHD and/or receiving immunosuppressive treatment for any reason. All other consecutive female patients who were ≥16 years of age at the time of allo-HCT and ≤ 45 years at the time of evaluation were included in the study. This study was approved by institutional review board of the Ankara Dr. Abdurrahman Yurtaslan Oncology Training and Research Hospital. All patients have provided their written informed consent for participation in this study. The study was conducted in accordance with the Declaration of Helsinki.

The intensity of the preparation regimens was classified according to the consensus criteria [15, 16]. BU-CY (intravenous busulfan 3.2 mg/kg/day for four days, intravenous cyclophosphamide 60 mg/kg/day for two days), and CY-TBI (intravenous CY 60 mg/kg/day for two days, 4-Gy/day TBI in two fractions for three days) were used as myeloablative conditioning regimens (MAC). The reduced intensity conditioning regimens (RIC) were BU-FLU-ATG (intravenous BU 3.2 mg/kg/day for two days, intravenous fludarabine 30 mg/m^2/day for five days, rabbit anti-thymocyte globulin-rATG 2.5 mg/kg/day for four days), and CY-ATG (intravenous CY 50 mg/kg/day for four days, rATG 5 mg/kg/day for four days). All patients received uniform GvHD prophylaxis consisting cyclosporin-A and short term methotrexate. The rATG (10 mg/kg/day for three days) was added to the MAC regimens in unrelated transplants. The rATG preparation during study period was the same for all patients.

The serum levels of E2, FSH, and LH were analysed with an analyser, according to the manufacturer's instruction. Serum samples for AMH were stored at 2-8°C and analyzed within 48 hours at Acıbadem Labmed Clinical Laboratories (Istanbul, Turkey). The results for reproductive hormones included differences in concentration depending on the menstrual cycle and age.

The principle of the LH and FSH measurement was an electrochemiluminescence immunoassay and a sandwich-type assay using monoclonal labelled antibodies directed against two different epitopes of FSH or LH. The measuring range was 0.1–200 U/l. Normal values for FSH (follicular phase) were 3.5–12.5 U/l. The intra-assay and interassay coefficient of variation (CV) were less than 3%. Normal values for LH (follicular phase) were 2.4–12.6 U/l. The intra-assay and interassay CV were less than 4.4%. The ECLIA for E2 was based on the competitive affinity of endogenous hormone and labeled antigen derivative for the binding sites on the specific biotinylated antibody. The measuring range was 18.4-11010 pmol/L. Normal values for E2 (follicular phase) were 196-854 pmol/L. The intra-assay and interassay coefficient of variation (CV) were less than 5%. The serum AMH levels were measured with the AMH enzyme-linked immunosorbent assay kit. The intra-assay and inter-assay variation coefficients were less than 3.5% and 4.2%, respectively, and the lower detection limit was 0.02 ng/mL. The ESHRE criteria was used for defining optimal range of AMH levels being 0.5–6.8 ng/mL [12].

Descriptive statistics, frequency (percentage) and median (min-max), were reported for categorical and quantitative variables, respectively. Comparison for statistical significance was performed by chi-square analysis for categorical variables. The level of significance was set at $p \leq 0.05$. All analyses were performed using the SPSS software version 21.

Results

The median duration of post-transplant follow-up was 37 (range; 12-84) months. The demographic and clinical characteristics of patients are summarized in Table 1. Cases were not under oral contraceptive therapy or receiving any hormonal therapy at the time of analysis. Data on pre-transplant and post-transplant menstrual status, and also hormone levels of 5 (23,8%) single, and 16 (76,2%) married cases are presented in Table 2. POF was detected in eight (38.1%) and 19 (90.4%) cases in the pre-transplant and post-transplant period, respectively. It was found that no menstruation cycles were observed in 18 cases with low AMH levels. In three cases with normal AMH levels, two had oligomenorrhea and one had regular menstruation cycles.

RT was performed in four cases in pre-transplant period and it involved the pelvic region in two of them. Pelvic RT had no impact on post-transplant AMH levels ($p = 0.47$). Following allo-HCT POF was evident in three out of four (75%) and 16 out of 17 (94%) patients with normal and low AMH levels, respectively. The authors observed no correlation between AMH levels and post-transplant development of POF ($p = 0.24$), preparative regimens (MAC and RIC) ($p = 0.59$), TBI-based conditioning ($p = 0.95$) and past history of aGvHD/cGvHD ($p = 0.15$).

All of the married patients desired to be pregnant and to have a child, but, only one had a normal pregnancy and had a live birth, and her AMH level was found to be optimal range. However, pregnancy was not observed in others.

Discussion

Studies have recently been conducted on the efficacy of AMH in assessment of ovarian reserve in patients who have received chemotherapy [12, 13, 17]. In a study involving 25 patients with hematologic malignancies, Lie Fongs *et al.* showed that AMH levels were low despite regular menstruation after chemotherapy [17]. In the same study, 12 patients who underwent allo-HCT had very low levels of AMH and developed POF [17]. In another study, AMH levels were elevated in measurable levels in five of the 11 patients one year after allo-HCT [14]. POF was diagnosed in more than 90% of women who received autologous and allo-HCT for hematological malignancies [18, 19]. In the present study, after a median time of 37 months, POF developed in 90.4% of cases and AMH levels were found to be low in 85.7%. Recent studies emphasize that serum AMH levels tend to decrease following systemic chemotherapy or allo-HCT in patients presenting with hematological cancers [14, 17]. However, the present authors observed optimal range AMH levels in three (14.3%) patients in the late post-transplant period.

Table 1. — *The demographic and clinical characteristics of the study population.*

Primary disease (n, %)	AML: 7 (33.3%) ALL: 5 (23.8%) AA: 3 (14.3%) MDS: 2 (9.5%) HL: 2 (9.5%) CML: 1 (4.8%) MM: 1 (4.8%)
Pre-transplant chemotherapy (median courses, range)	2 (0-5)
Pre-transplant RT(n, %)	No: 4 (19%) Yes: 17 (81%)
Pre-transplant auto-HCT (n, %)	No: 18 (85.7%) Yes: 3 (14.3%)
Donor status (n, %)	Well-matched related: 18 (85.6%) Well-matched unrelated: 2 (9.6%) Haploidentical: 1 (4.8%)
Stem cell source	Peripheral blood: 18 (85.7%) Bone marrow: 3 (14.3%)
Conditioning regimen	Myeloablative: 13 (61.9%) Reduced-intensity conditioning: 8 (38.1%)
TBI as part of the conditioning regimen (n, %)	No: 16 (76.2%) Yes: 5 (23.8%)
Previous history of acute GvHD (n, %)	No: 12 (57.1%) Yes: 9 (42.9%)
Previous history of chronic GvHD (n, %)	No: 14 (66.7%) Yes: 7 (33.3%)

AA: aplastic anemia, ALL: acute lymphoblastic leukemia, AML: acute myeloid leukemia, CML: chronic myeloid leukemia, GvHD: graft versus host disease, HCT: hematopoietic cell transplantation, HL: Hodgkin lymphoma, MM: multiple myeloma, RT: radiotherapy, TBI: total body irradiation.

Table 2. — *Fertility tests and characteristics.*

	Pre-transplant n (%)	Post-transplant n (%)
Menstrual cycle	Regular: 21 (100) Irregular: 0 (0)	Regular: 2 (9,5) Irregular: 19 (90,5)
LH (normal, range) (2.4-12.6 mIU/mL)	Normal: 10 (47,6) < 2.4: 5 (23,8) > 12.6: 6 (28.6)	Normal: 0 (0) < 2.4: 3 (14.3) > 12.6: 18 (85.7)
FSH (normal; range) (3.5-12.5 mIU/mL)	Normal: 10 (47.6) < 3.5: 3 (14.3) > 12.5: 8 (38.1)	Normal: 1 (4.8) < 3.5: 1 (4.8) >12.5: 19 (90.4)
E2 (normal, range) (12.4-233 pg/mL)	Normal: 16 (76.2) < 12.4: 4 (19) > 233: 1 (4.8)	Normal: 9 (42.9) < 12.4: 10 (47.6) > 233: 2 (9.5)
AMH (optimal range) (0.5-6.8 ng/ml)	No data	Optimal range: 3 (14.3) < 0.5: 18 (85.7) > 6.8: 0 (0)

AMH: anti-Mullerian hormone, E2: estrogen, FSH: follicle stimulating hormone, LH: luteinizing hormone.

AMH levels peak at 24 years of age, decrease gradually with aging, and become unmeasurable at menopause [20]. Studies have shown that AMH levels are significantly lower in cases who received pelvic RT or TBI-including conditioning regimens [17, 21]. In this study, the authors found no association between history of pelvic RT ($p = 0.47$) or receiving TBI-including conditioning regimens ($p = 0.94$) and AMH levels. AMH levels were low in all three patients who received autologous-HCT before allo-HCT, and these cases were amenorrheic. This finding may indicate that the POF risk is higher in cases who exposed to more than one conditioning regimens. Some studies reveal that there is less correlation between development of POF and RIC regimens compared to MAC regimens [22, 23], however, others were unable to show an association between POF development and intensity of conditioning regimens [14]. In the present study, there was no significant differences between the conditioning regimens in terms POF development ($p = 0.59$). Although successful pregnancies are reported in cases suffering from cGvHD, it is generally accepted that cGvHD reduce the fertility potential [5, 7]. In the present study, there were no cases with active GvHD. However, there was no significant differences between patients with or without previous history of aGvHD and/or cGvHD in terms of POF ($p = 0.12$). In the post-transplant pe-

riod, fertility is impaired and the probability of becoming pregnant is less than 1% [24]. In the present study only one out of 16 (6.25%) cases with unprotected intercourse had a live birth.

The results of this study are subjected to some limitations. First, it is a single-center study with a relatively small sample size, which might under- or overestimate the results. Second, the study has a retrospective design. More specifically designed prospective studies are needed to externally cross-validate the present findings in a larger cohort of patients. As a result, POF is still a major problem of allo-HCT patients in long-term follow-up. The patients who will proceed to allo-HCT should definitely contact with a physician, who is specialized in fertilization issues and assisted reproduction techniques. In eligible cases, oocyte cryopreservation should be performed. Regular menstrual cycles may not guarantee fertilization in the post-transplant period. In these cases, combined analysis of hormonal investigations, antral follicle count by vaginal USG, as well as measurement of AMH levels may be preferred to demonstrate the presence of POF. In the early post-transplant period, the recovery of fertility in cases presenting with POF is very low, but follow-up can be planned with intermittent AMH measurements.

References

[1] Passweg J.R., Baldomero H., Bader P., Bonini C., Duarte RF., Dufour C., *et al.*: "Use of haploidentical stem cell transplantation continues to increase: the 2015 European Society for Blood and Marrow Transplant activity survey report". *Bone Marrow Transplant.*, 2017, 52, 811.

[2] Tekgündüz E., Şencan İ., Kapuağası A., Ünal D., Öztürk M., Gümüş E., *et al.*: 'Hematopoietic cell transplantation activity of Turkey in 2014: Ongoing increase in HCT rates'. *Transfus. Apher. Sci.*, 2016, 54, 53.

[3] Pasquini M., Zhu X.: "Current uses and outcomes of hematopoietic stem cell transplantation. CIBMTR Summary Slides". 2015:Available at: http://www.cibmtr.org

[4] Majhail N.S., Rizzo J.D., Lee S.J., Aljurf M., Atsuta Y., Bonfim C., *et al.*: "Recommended screening and preventive practices for long-term survivors after hematopoietic cell transplantation". *Rev. Bras. Hematol. Hemoter.*, 2012, *34*, 109.

[5] Salooja N., Szydlo R.M., Socie G., Rio B., Chatterjee R., Ljungman P., *et al.*: "Pregnancy outcomes after peripheral blood or bone marrow transplantation: a retrospective survey". *Lancet*, 2001, *358*, 271.

[6] Carter A., Robison L.L., Francisco L., Smith D., Grant M., Baker K.S., *et al.*: "Prevalence of conception and pregnancy outcomes after hematopoietic cell transplantation: report from the Bone Marrow Transplant Survivor Study". *Bone Marrow Transplant.*, 2006, *37*, 1023.

[7] Loren A.W., Chow E., Jacobsohn D.A., Gilleece M., Halter J., Joshi S., *et al.*: "Pregnancy after hematopoietic cell transplantation: a report from the late effects working committee of the Center for International Blood and Marrow Transplant Research (CIBMTR)". *Biol. Blood Marrow Transplant.*, 2011, *17*, 157.

[8] Singer T., Barad D.H., Weghofer A., Gleicher N.: "Correlation of antimullerian hormone and baseline follicle-stimulating hormone levels". *Fertil. Steril.*, 2009, *91*, 2616.

[9] Scheffer G.J., Broekmans F.J., Dorland M., Habbema J.D., Looman C.W., te Velde E.R.: "Antral follicle counts by transvaginal ultrasonography are related to age in womenwith proven natural fertility". *Fertil. Steril.*, 1999, *72*, 845.

[10] Haller-Kikkatalo K., Uibo R., Kurg A., Salumets A.: "The prevalence and phenotypic characteristics of spontaneous premature ovarian failure: a general population registry based study". *Hum. Reprod.*, 2015, *30*, 1229.

[11] van Disseldorp J., Lambalk C.B., Kwee J., Looman C.W., Eijkemans M.J., Fauser B.C., *et al.*: "Comparison of inter and intra-cycle variability of anti-Mullerian hormone and antral follicle counts". *Hum. Reprod.*, 2010, *25*, 221.

[12] Decanter C., Morschhauser F., Pigny P., Lefebvre C., Gallo C., Dewailly D.: "Anti-Müllerian hormone follow-up in young women treated by chemotherapy for lymphoma: preliminary results". *Reprod. Biomed. Online*, 2010;20:280-285.

[13] Hamy A.S., Porcher R., Cuvier C., Giacchetti S., Schlageter M.H., Coussieu C., *et al.*: "Ovarian reserve in breast cancer: assessment with anti-Mullerian hormone". *Reprod Biomed Online*, 2014, 29, 573.

[14] Nakano H., Ashizawa M., Akahoshi Y., Ugai T., Wada H., Yamasaki R., *et al.*: "Assessment of the ovarian reserve with anti-Müllerian hormone in women who underwent allogeneic hematopoietic stem cell transplantation using reduced-intensity conditioning regimens or myeloablative regimens with ovarian shielding". *Int. J. Hematol.*, 2016, *104*, 110.

[15] Champlin R., Khouri I., Anderlini P., De Lima M., Hosing C., McMannis J., *et al.*: "Nonmyeloablative preparative regimens for allogeneic hematopoietic transplantation. Biology and current indications". *Oncology (Williston Park)*, 2003, 17, 94.

[16] Giralt S., Ballen K., Rizzo D., Bacigalupo A., Horowitz M., Pasquini M., *et al.*: "Reduced-intensity conditioning regimen workshop: defining the dose spectrum. Report of a workshop convened by the center for international blood and marrow transplant research". *Biol. Blood Marrow Transplant.*, 2009, 15, 367.

[17] Lie Fong S., Lugtenburg P.J., Schipper I., Themmen A.P., de Jong F.H., Sonneveld P., *et al.*: "Anti-mullerian hormone as a marker of ovarian function in women after chemotherapy and radiotherapy for haematological malignancies". *Hum Reprod.*, 2008, 23, 674.

[18] Tauchmanovà L., Selleri C., De Rosa G., Esposito M., Orio F. Jr, Palomba S., *et al.*: "Gonadal status in reproductive age women after haematopoietic stem cell transplantation for haematological malignancies". *Hum. Reprod*., 2003, *18*, 1410.

[19] Tauchmanovà L., Selleri C., De Rosa G., Sammartino A., Di Carlo C., Musella T., *et al.*: "Estrogen-progestin therapy in women after stem cell transplant: our experience and literature review". *Menopause*, 2007, *14*, 320.

[20] Seifer D.B., Baker V.L., Leader B.: "Age-specific serum anti-Mullerian hormone values for 17.120 women presenting to fertility centers within the United States". *Fertil. Steril.*, 2011, 95, 747.

[21] Chiodi S., Spinelli S., Bruzzi P., Anserini P., Di Grazia C., Bacigalupo A.: "Menstrual patterns, fertility and main pregnancy outcomes after allogeneic haematopoietic stem cell transplantation". *J. Obstet. Gynaecol.*, 2016, *36*, 783.

[22] Kawano M., Komura H., Kawaguchi H., Shimizu S., Yada-Hashimoto N., Shimizu M., *et al.*: "Ovarian insufficiency following allogeneic hematopoietic stem cell transplantation". *Gynecol. Endocrinol.*, 2017, *33*, 156.

[23] Cheng Y.C., Saliba R.M., Rondón G., Giralt S.A., Lu K.H., Bodurka D.C., *et al.*: "Low prevalence of premature ovarian failure in women given reduced-intensity conditioning regimens for hematopoietic stem-cell transplantation". *Haematologica*, 2005, *90*, 1725.

[24] Frey Tirri B., Häusermann P., Bertz H., Greinix H., Lawitschka A., Schwarze C.P., *et al.*: "Clinical guidelines for gynecologic care after hematopoietic SCT. Report from the international consensus project on clinical practice in chronic GVHD". *Bone Marrow Transplant.*, 2015, *50*, 3.

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