Anti-Mullerian hormone and antral follicle count as predictors for embryo/oocyte cryopreservation cycle outcomes in breast cancer patients stimulated with letrozole and follicle stimulating hormone

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Abstract

Purpose To predict embryo/oocyte cryopreservation cycle (ECC) outcomes in breast cancer patients stimulated with letrozole and follicle stimulating hormone for fertility preservation based on observed anti-mullerian hormone (AMH) levels and antral follicle counts (AFC).

Methods The correlation between AMH and AFC and ECC outcomes were analyzed retrospectively on forty one women with breast cancer before adjuvant treatment.

Results AMH and AFC had a stronger correlation with the total number of oocytes and the number of mature oocytes than age, FSH, and inhibin B. Subjects were evaluated by the number of mature oocytes retrieved to create cutoff points of AMH level, which identified 1.2 ng/mL as a potential value. Seven of 18 patients with AMH levels ≤ 1.2 ng/mL had low response versus none of 23 with >1.2 ng/mL, ($p=0.001$).

Capsule AMH and AFC are the most reliable serum and biophysical markers of embryo/oocyte cryopreservation cycle outcomes in breast cancer patients stimulated with letrozole and follicle stimulating hormone.

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Conclusions AMH is the most reliable serum marker of ECC outcomes, together with AFC as a biophysical marker, in breast cancer patients. Low response is highly likely when the AMH level is ≤ 1.2 ng/mL.

Keywords Anti-Mullerian hormone (AMH) · Letrozole · Breast cancer · Fertility preservation · Cryopreservation

Introduction

With more than one million cases occurring annually worldwide, breast cancer is the most common invasive cancer seen in women of reproductive age [1, 2]. Before age 40, one out of every 228 women develop breast cancer; and approximately 15% of all breast-cancer cases occur during the reproductive years [3, 4].

In the US, there are about 3 million women who are survivors of breast cancer. This number is likely to grow with earlier diagnosis and adjuvant therapy improvements [5]. In the US, the 5-year survival rates in all stages of breast cancer has reached 89% [6]. As greater emphasis is placed on the quality of life of breast cancer survivors due to higher survival rates, fertility preservation has become a key component of cancer care.

Current options for fertility preservation range from those that are clinically well established and are routinely used, such as embryo cryopreservation, to those that are experimental, such as oocyte or ovarian tissue cryopreservation [7]. Both embryo and oocyte freezing require ovarian stimulation with gonadotropins, which results in excessive levels of estrogen production. To reduce the estrogen exposure during ovarian stimulation in women with breast cancer, we developed novel protocols, COST-

LESS (Controlled Ovarian Stimulation Treatment with Letrozole Supplementation Study) [8, 9] utilizing aromatase inhibitors as an ovarian stimulant.

Essential to the success of fertility preservation by embryo and oocyte freezing is obtaining sufficient quantities of mature, metaphase II (MII) oocytes. If the probability of good ovarian response can be predicted with higher accuracy, patients can make a better informed decision to delay or not to delay their cancer treatment for ovarian stimulation. The delay is generally acceptable as multiple studies showed no effect on survival or recurrence in breast cancer patients if chemotherapy was initiated as late as 12 weeks after breast surgery [10, 11].

Anti-Mullerian hormone (AMH), a member of the transforming growth factor—beta super family, is predominantly produced by the granulosa cells of preantral and early antral ovarian follicles [12]. Recently AMH has been shown to be one of the most sensitive markers of ovarian reserve and a limited number of studies suggested that AMH may be a reliable predictor of ovarian response to stimulation in in vitro fertilization (IVF) [13, 14].

No information is available regarding AMH and its predictive value for embryo/egg cryopreservation cycle (ECC) outcomes in young breast cancer patients. The aim of this study was to predict embryo/oocyte cryopreservation cycle (ECC) outcomes in breast cancer patients stimulated with letrozole and follicle stimulating hormone for fertility preservation based on observed AMH levels and antral follicle count (AFC).

Materials and methods

The inclusion criteria were age <45, breast cancer stage ≤3, no prior chemotherapy, no prior history of ovarian surgery or infertility and availability of serum AMH level. Of those, 126 patients undergoing oocyte or embryo cryopreservation for fertility preservation were treated with COST-LESS cycles and the AMH levels were available in 41 women from March 2007 to December 2009 due to its recent availability as a routine marker. AMH measurement was performed using highly specific mono/mono two-site ELISA method (DSL AMH assay). Under COST-LESS protocol all participants received 5 mg/day of letrozole, starting on the second day of their menstrual cycle until the day of human chorionic gonadotropin (hCG) administration. FSH was added 2 days later at 150–375 IU/day, based on age and body mass index (BMI).

Participants had a baseline blood drawing during the early follicular phase of the menstrual cycle for AMH, FSH and inhibin B measurements before starting the letrozole. Antral follicle counts (AFC) were performed by transvaginal ultrasonography on menstrual cycle day 2. The

number of MII oocytes retrieved, total number of oocytes retrieved, maturation rate (number of mature oocytes/number of total oocytes), fertilization rate (number of fertilized oocytes/number of oocytes undergoing ICSI) and total number of embryos cryopreserved following intracytoplasmic sperm injection (ICSI) were compared retrospectively. Embryo cryopreservation was performed at the pronuclear stage after ICSI.

Statistical analysis was performed with the SPSS 17 for Windows package (SPSS Inc., Chicago, IL) and SAS 9.1 (SAS Institute, Cary, North Carolina, USA). Levene's test of homogeneity of variances ($p < 0.01$) and Kolmogorov-Smirnov test of normality ($p < 0.01$) were performed to choose the appropriate statistical test. Mann-Whitney *U*-test or Student's *t*-test was performed to analyze differences in mean values (presented as mean±SD). Non-parametric correlations were analyzed with Spearman's test and parametric correlations with Pearson's test. When the *P* value was <0.05, the difference was considered statistically significant. Multiple linear regression models were developed using a stepwise procedure introducing variables with a cutoff $p = 0.10$ but requiring $p = 0.05$ in the final model.

Results

The mean age of patients was 34.8 ± 4.7 years (range: 24–44). The mean AMH was 2.5 ± 2.3 ng/mL ranging from <0.1 to 9.7 ng/mL (Table 1). Seven women had oocyte freezing, and 29 had embryo cryopreservation. Four underwent both embryo and oocyte cryopreservation. One cycle was cancelled due to no oocyte yield in a 44 year old patient with an AMH level of 0.2 ng/mL. All correlation values were dictated in Table 2.

AMH had a stronger correlation with the total number of oocytes and the number of MII oocytes than age, FSH, and inhibin B. AMH also had a positive correlation with inhibin B, AFC, and the number of embryos and a negative correlation was seen with FSH. No significant correlation was observed between AMH and BMI, maturation rate, fertilization rate after ICSI, or total dose of gonadotropins. Serum AMH levels of breast cancer patients did not correlate with BRCA, estrogen receptor (ER), progesterone receptor (PR), or HER2-neu status.

FSH levels negatively correlated with total number of oocytes and the number of MII oocytes. Inhibin B had a negative correlation with total dose of gonadotropins and a positive correlation with AFC, total number of oocytes, the number of MII oocytes, total dose of gonadotropins and total number of embryos.

AFC had a positive correlation with inhibin B, total number of oocytes, number of MII oocytes, and the number of embryos. Together with serum AMH level, AFC was

Table 1 Clinical characteristics of patients

	Mean±standard deviation
Age (years)	34.8±4.7
AMH (ng/mL)	2.5±2.3
BMI (kg/m ²)	21.8±2.9
FSH (mIU/mL)	9.7±4.3
Inhibin B (ng/L)	44.5±22.8
AFC	12.2±6.8
Total number of follicles	12.1±9.0
Total dose of gonadotropins (IU)	2207.2±794.6
Total number of oocytes	16.0±10.0
Number of mature oocytes	10.1±6.3
Maturation rate ^a	66.9±23.7
Fertilization rate ^b	79.6±14.7
Number of embryos	7.5±5.1

^a Number of mature oocytes/total number of oocytes^b Number of fertilized oocytes/number of oocytes undergoing ICSI

AMH anti-Mullerian hormone; BMI body mass index; FSH follicular stimulating hormone; AFC antral follicle count

also one of useful markers with strong correlation with parameters of ECC outcomes.

Multiple regression analysis was performed to adjust for AMH, age, FSH, inhibin B, and AFC which had correlations with the number of MII/total oocytes retrieved and embryos cryopreserved. After the adjustment, the correlation between the total number of oocytes and AMH, AFC

and age ($r=0.71$, all $p<0.05$) remained significant. A correlation also remained between the number of MII oocytes and AMH and AFC ($r=0.64$, all $p<0.05$), as well as between the number of embryos and AMH and AFC ($r=0.51$, all $p<0.05$).

Subjects were evaluated by the number of mature oocytes retrieved to create cutoff points of AMH level. When ovarian response to stimulation was classified as low response (<4 mature oocytes retrieved [15]) vs. normal response, which identified 0.8–1.2 ng/mL as a potential cutoff range of serum AMH level. A significantly larger proportion of patients, 7 of 18 (38.9%) with AMH levels ≤ 1.2 ng/mL had low response versus none of 23 (0%) in the patients with >1.2 ng/mL, ($p=0.001$) (Fig. 1). We also found significant differences in the total number of oocytes, FSH, AFC, and number of embryos between the groups with AMH levels ≤ 1.2 ng/mL vs. >1.2 ng/mL (Table 3).

Conclusions

The prediction of stimulation results before controlled ovarian stimulation (COS) is clinically meaningful and helpful when counseling breast cancer patients. If prediction of ECC outcomes is possible by using biological and/or biophysical markers physicians and patients will make better informed decisions regarding the choice and type of fertility preservation and whether or not to delay chemotherapy.

Table 2 Correlation of patient clinical characteristics and ECC outcomes

	Log AMH	Age	FSH	Inhibin B	AFC	Total dose of gonadotropins	Total no. of oocytes retrieved	No. of MII retrieved	No. of embryos cryo-preserved
Log AMH	1.								
Age	NS	1.							
FSH	-.470**	NS	1.						
Inhibin B	.417*	NS	NS	1.					
AFC	.444*	NS	NS	.461*	1.				
Total dose of gonadotropins	NS	NS	NS	-.419*	NS	1.			
Total no. of oocytes retrieved	.454**	-.422**	-.375*	.385*	.658**	NS	1.		
No. of MII retrieved	.583**	-.456**	-.360*	.434*	.605**	NS	.847**	1.	
No. of embryos cryo-preserved	.508**	-.399*	NS	.595**	.588**	NS	.613**	.750**	1.

*Correlation is significant at the 0.005 level (2-tailed)

**Correlation is significant at the 0.001 level (2-tailed)

BMI, Maturation rate, and Fertilization rate had no correlation with all factors

ECC embryo/egg cryopreservation cycle; AMH anti-Mullerian hormone; BMI body mass index; FSH follicular stimulating hormone; AFC antral follicle count; MII oocyte mature, metaphase II oocyte

Fig. 1 Number of MII oocytes in low and good responders based on AMH levels (**a**) and AFC (**b**). *Solid diamonds*: good responders. *Open diamonds*: low responders. Abbreviations: MII oocyte, mature, metaphase II oocyte; AMH, anti-Müllerian hormone; AFC, antral follicle count

Table 3 Comparison of clinical characteristics and ECC outcomes between patients with ≤ 1.2 ng/mL of AMH level and >1.2 ng/mL

	AMH ≤ 1.2 ng/mL ($n=18$)	AMH >1.2 ng/mL ($n=23$)	<i>P</i> value
Age (years)	35.3 $\pm$ 4.3	34.5 $\pm$ 5.1	NS
BMI (kg/m ²)	21.0 $\pm$ 3.0	22.4 $\pm$ 2.7	NS
FSH (mIU/mL)	12.3 $\pm$ 4.0	7.5 $\pm$ 3.2	<0.001
Inhibin B (ng/L)	38.8 $\pm$ 16.0	48.7 $\pm$ 26.5	NS
AFC	9.8 $\pm$ 7.0	13.9 $\pm$ 6.4	0.04
Total dose of gonadotropins (IU)	2270.1 $\pm$ 650.9	2153.2 $\pm$ 912.5	NS
Total No. of oocytes retrieved	11.3 $\pm$ 9.7	19.7 $\pm$ 8.8	<0.01
No. of MII oocytes retrieved	6.8 $\pm$ 5.6	12.7 $\pm$ 5.8	<0.01
Maturation rate ^a	65.9 $\pm$ 29.5	66.2 $\pm$ 19.0	NS
Fertilization rate ^b	81.3 $\pm$ 17.3	78.1 $\pm$ 12.1	NS
No. of embryos cryopreserved	5.6 $\pm$ 4.0	9.4 $\pm$ 5.6	<0.05

Data presented as mean $\pm$ standard deviation

NS no significance

^a Number of mature oocytes/total number of oocytes

^b Number of fertilized oocytes/number of oocytes undergoing ICSI

ECC embryo/egg cryopreservation cycle; AMH anti-Müllerian hormone; BMI body mass index; FSH follicular stimulating hormone; AFC antral follicle count; MII oocyte mature, metaphase II oocyte

Presently AMH is recognized as the best ovarian reserve marker in infertility patients [16–18]. AMH serum levels are relatively stable and consistent, suggesting that it can be used as a menstrual cycle-independent marker of ovarian response to COS. Over age, day-3 FSH, or inhibin B, AMH appears to be a superior marker for predicting ovarian response. Vast majority of studies have also found that AMH and AFC both have similar value in predicting low response to ovarian stimulation [16, 19–21]. A significant positive correlation between AMH levels and the quality [22] and quantity [23–26] of oocytes have been found by several authors, although the value of AMH in predicting oocyte quality is controversial. Guerif et al. reported that serum AMH is a relatively good predictive indicator of ovarian reserve, in terms of quantity but not quality [27].

Consistent with prior studies we found AMH had a positive correlation with numerous other factors such as inhibin B, AFC, and number of embryos and negatively correlated with FSH. After multiple regression analysis, AMH and AFC were significantly correlated with the number of total/MII retrieved and the number of embryos cryopreserved.

We also found that when the AMH level was >1.2 ng/mL before chemotherapy, the possibility of obtaining more than 4 MII oocytes was very high. A significantly larger proportion of patients (7 of 18) with AMH levels ≤ 1.2 ng/mL had low response, however, none of 23 patients if AMH level was >1.2 ng/mL.

No correlation was identified between AMH and BMI, maturation rate, and fertilization rate. Freeman et al. reported that obese women had appreciably lower mean AMH levels compared to non-obese women in their late reproductive years [28]. Most of the patients in this study had a normal BMI, with a mean of 21.8 (range: 16–30). Nevertheless, after correcting our data for BMI our results did not change.

Recently, our research group discovered the association of BRCA1 mutations with diminished ovarian reserve. BRCA1 mutation-positive women produced lower number of eggs and had as many as 38.3 times the relative risk of poor response compared to controls [9]. There were very few patients with BRCA mutations and AMH levels in this group to determine any correlation with BRCA status and egg reserve.

Based on the present results, AMH and AFC appear to be useful to counsel breast cancer patients before ovarian stimulation with COST-LESS for fertility preservation. While our study was the first to report the use of AMH to predict ECC outcomes in cancer patients, in a study by Jayaprakasan et al., AFC and AMH were the most significant predictors of poor response to ovarian stimulation in 135 women with infertility undergoing the first cycle of ART [19]. In a meta-analysis Broer et al. reported that

AMH has at least the same level of accuracy and clinical value in predicting poor response and non-pregnancy as AFC in infertility patients [29]. In addition, data has shown positive correlation between AMH levels and IVF pregnancy rates [26].

Our study has certain limitations. It was a retrospective analysis of a prospective database and the number of patients with AMH measurements was low owing to its recent availability as a routine marker. While our study, for the first time, indicates that AMH is a useful marker in predicting good response to ovarian stimulation in COST-LESS cycles, larger prospective studies will be needed in the future to evaluate the value of AMH as a predictor of fertility preservation success.

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