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Research article

Antimullerian Hormone Levels through the Cycles of Assisted Reproductive Techniques: A Correlational Study

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Abstract

Purpose: The aim of this study was to assess the association of serum antimullerianhormone(AMH) levels in assisted reproductive techniques(ART) cycles with some reproductive measures.
Methods: In a cohort study, 60 patients aged 20-40 years who attended as candidates for ART at an infertility clinic were enrolled. Serum levels of follicle stimulating hormone(FSH), E2 and AMH was assessed using blood samples taken on the third menstrual day.
Results: Serum FSH was negatively correlated with E2 levels(r=-0.28, P<0.05). AMH was moderately correlated with implantation rate(r=0.6, P<0.001). AMH and FSH were not correlated with BMI, antral follicles or oocyte count.
Conclusion: Weak to moderate correlations may exist between AMH and some reproductive factors, but a high prediction power cannot be expected in predicting the pregnancy occurrence for single measurements of AMH.

Introduction

Antimullerian hormone (AMH) is a dimeric glycoprotein belonging to the transforming growth factor beta (TGFb) family and closely related to inhibin and activin[1]. AMH, also known as Mullerian inhibiting substance, is a testicular hormone, secreted by immature sertoli cells and responsible for the regression of Mullerian ducts in male fetuses[2,3]. It is secreted by the Sertoli cells in males and the granulosa cells in postpubertalfemales[4,5]. AMH levels normally are low in primary follicles, increase to maximal levels in large preantral and small antral stages, and then decline during final follicular maturation, becoming restricted to cumulus cells surrounding the oocyte[5]. AMH can be a nice predictor of the number of oocytes retrieved during an ART cycle as well the pregnancy outcome[6-8]. It is also It is the earliest marker of diminishing ovarian reserve[9,10]. In women undergoing treatment for infertility, correct identification of poor responders by assessment of their ovarian reserve before entering an in vitro fertilization (IVF) program is very important[11]. Since AMH is exclusively produced by the growing ovarian follicles, serum levels may be used as a marker for ovarian reserve, representing the quantity and quality of the ovarian follicle pool. Recent reports indeed indicate that AMH levels decline with increasing female age, and that the initial AMH level is associated with ovarian response in IVF patients with normal FSH levels. Analysis for prediction of poor response showed that serum AMH levels had a better predictive value than serum levels of FSH, inhibin B and estradiol[11,12]. The aim of this study was to assess the association of serum AMH levels in ART cycles and some reproductive factors.

Methods

Study was conducted between March 2008 and June 2009 at the infertility clinic of Al- Zahra University hospital in Tabriz, Iran. A total of 60 patients aged 20-40 years attending as candidate for assisted reproductive techniques treatment at infertility clinic were enrolled into this cohort study. The inclusion criteria were as: (1) presence of both ovaries and lack of morphologic abnormalities; 2) no previous history of ovarian surgery 3) no history of hormone therapy during the last six months; 4) no history of ovarian radiotherapy or cytotoxic drug use; 5) adequate visualization of ovaries at transvaginal ultrasound assessment.

Women underwent serum FSH and E2 measurements at, 8 a.m. on cycle day three during the menstrual cycles. Serum E2 and FSH levels were determined by an automated multianalysis system using a chemiluminescence technique. For E2, the lower detection limit was 15 pg/ml, and intra- and inter-assay coefficients of variation were 8 and 9%, respectively. For FSH, the lower detection limit was 0.1 mIU/ml and intra- and inter-assay coefficients of variation were 3% and 5%, respectively[13]. Simultaneously with FSH and E2 measurements, ovarian ultrasound scans were performed using 3.6-8.OMHz multi-frequency transvaginal probe to evaluate pelvis anatomy and the number of ovarian antral follicles 2-5 millimeters. For AMH assay, we used ultra sensitive ELISA (Enzyme-Linked Immunosorbent Active) according to the manufacturer's instructions. This highly specific mono/mono two-site ELISA uses detection and capture antibodies with results available within three hours. The standards cover a range from 0.05 to 15 ng/ml[14].

The patients were all treated with a long protocol for ovarian stimulation. In the long protocol, by administering GnRHagonist(500 micro gram SC) starting from the day 21 of menstrual cycle, then decreasing the dose to 25µg /day when the menstrual bleeding occurred. Thereafter, stimulation was initiated using Menogon and GONAL-f from the second day of their menstrual cycle. Monitoring was carried out by transvaginal ultrasound (HS-4000 Japan) on days

7 and 10 of HMG stimulation. After more than 3-4 follicles larger than 14-16 millimeter (mm) or two follicles larger than 17 mm in diameter were observed, 10,000 IU of human chorionic gonadotropin (HCG) (Pregny15000, Organon) was administered intramuscularly. Thirty-six hours later, follicles were retrieved under general anesthesia by transvaginal ultrasound-guided aspiration. Mature oocytes were retrieved from follicular fluid and after fertilization, embryo was transferred.

All embryo transfers were performed two days after oocyte retrieval using Labotect catheters. Before the transfer, the embryos were evaluated microscopically and the best-quality embryos were selected for the transfer. Fetus viability was definite by ultrasound, meanwhile for support of lethal phase, 100 mg progesterone (oil) was administered daily.

Statistical analysis:

Data were analyzed by SPSS, version 16. The appropriate statistical tests including Student's t test, Chi-square and Fisher exact test, were used to compare the results. A $P<0.05$ was considered statistically significant.

The study was approved by the committee of ethics in Tabriz University of medical sciences. Written informed consent was obtained from all the participants in the study.

Results

Embryos were available for transfer in 58 out of 60 patients who underwent IVF and ICSI. Mean serum AMH level was 4.3(SD 3.6) ng/ml(range 0.4-14.5). fourty-two patients underwent ICSI and 18 received IVF treatments. Overall, 13 out of 60 couples had a positive β-HCG test. Distribution of age, primary infertility period and BMI were similarly distributed between the groups. The difference observed between groups regarding day three serum FSH, number of antral follicles, number of follicles larger than 18 mm, and number of retrieved oocytes was not statistically different. However, serum HCG, day three E2, and clinical pregnancy rate were found to be statistically different between groups(details in table1). AMH levels inversely correlated with day three FSH ($r=-0.28$, $P<0.05$) and with day three E2($r= -0.27$, $p<0.05$). On the other hand AMH also correlated moderately with implantation rate ($r= 0.6$, $p<0.001$). Neither AMH nor FSH were found to be correlated with any of BMI, antral follicles, and oocytes count.

Table 1: Some hormonal and clinical characteristics of participants compared between those with normal versus abnormal AMH

Measurement	Normal AMH	Abnormal AMH	P value
Age(years)	30.8(4.4)	30(5.8)	0.6
BMI	25.5(2.5)	26.7(4.9)	0.3
Primary infertility	33(85%)	17(83%)	0.8
Day-3 FSH(mIU/ml)	11.7(7.9)	14.4(8.9)	0.3
Day-3 E2(pg/ml)	62.6(37.8)	99.7(87.7)	0.03
Number of antral follicles	5.7(2.4)	5.5(3.4)	0.9
Number of antral follicles>8mm	2.7(2)	2.8(4)	0.8
Number of retrieved oocytes	9.1(5.9)	9.1(5.2)	0.9
βHCG	1.55(0.5)	1.95(0.2)	0.003
Clinical pregnancy	5(25%)	0(0)	0.003

Discussion

Study found that AMH levels negatively correlate with E2 and FSH concentrations. Our analysis confirmed that AMH and baseline FSH demonstrate a negative linear relationship that was in line with previous research[5,15,16]. Dumesic and colleagues found that the levels of baseline FSH but not E2 had negative correlation with AMH[5]. Another study showed that the levels of baseline FSH were significantly lower in cycles resulting in a normal ovarian response as well as cycles resulting in clinical pregnancy[15]. Van Rooij et al. demonstrated that the baseline levels of FSH, but not E2, were higher in poor responders and that AMH levels were lower in the poor responders compared to normal responders[17]. As Production of E2 is under direct regulation of FSH the negative relation between AMH and E2 is conceivable in PCOS women where an inverse relationship between E2 and AMH serum levels has been previously established[3,17]. The results of studies conducted on rats treated with GnRH antagonist and FSH have indicated that FSH inhibits AMH and its type II receptor expression in pre-antral and early antral follicles[18]. Some other studies have shown that the levels of FSH and E2, were significantly lower in cycles resulting in a normal ovarian response as well as cycles resulting in clinical pregnancy[19]. Since lower day three FSH levels, represent satisfactory ovarian reserve and higher levels represent declining OR[18]. Our study does not confirm previous observations of association among AMH, antral follicles and retrieved oocyte count[13]. Nevertheless, the inverse correlation between AMH levels and age of women was not significant, possibly due to age variability as the oldest sample in this study was 38 year-old. We observed a positive correlation between serum hCG and AMH levels, suggesting that administering hCG may stimulate early luteal AMH production[13]. We found positive correlation between AMH levels and implantation rate. On the other hand there was a significant difference between the two groups in clinical pregnancy. In the present study, we could not demonstrate any significant difference in antral follicle count (AFC), number of retrieved oocyts and day three FSH between the two groups. All pregnancies occurred in normal AMH level group and there was a significant difference between the two groups in this regard. Similarly, Aflatoonian et al investigated AMH level in patients who had undergone IVF[20]. This study revealed that AMH measurement in ART cycles could provide useful information about prediction of outcome in different procedures.

The ability to predict ovarian reserve and response to ovarian stimulation continues to be an important component of infertility treatment. Nowadays many tests such as FSH, estradiol, inhibins, antral follicle count, testosterone and free testosterone are suggested for predicting ovarian reserve. Thus, despite the different validity of all these tests, there still remain patients who respond poorly to stimulation despite having normal tests of ovarian reserve[21]. Our results supports the assumption of existing an association between AMH and pregnancy rate in ART that can be motivating for future research for detailed assessment of such an association. However, the highest correlation coefficient was 0.6 which is a moderate correlation but is indicative of low prediction power.

Conclusion: Weak to moderate correlations exist between AMH and some reproductive factors, but a high prediction power cannot be expected in predicting the pregnancy occurrence at least for single measurements of AMH.

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Authors' contributions

LF, AG,ZB, and FF were involved in all areas of research design,

conduction interpretation and drafting. MN contributed as above but in fields related to biochemical assessments.

Competing interests: The author(s) declare that they have no competing interests.

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