Anti-Mullerian Hormone as a Predictive Factor in Assisted Reproductive Technique of Polycystic Ovary Syndrome Patients

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Abstract- This study aimed to assess the relationship between the serum levels of anti-mullerian hormone (AMH) and other hormonal markers and results of assisted reproductive techniques (ART) in polycystic ovary syndrome (PCOS) patients. This cohort study was conducted on 60 PCOS patients who were candidates for assisted reproductive techniques. In all patients the serum levels of AMH, follicle stimulating hormone (FSH) and luteinizing hormone (LH), estradiol (E2), free testosterone (fT), testosterone (T) and inhibin B were measured in the 3rd day of menstrual cycle. The relationship between serum level of measured hormonal markers with retrieved oocytes, mature oocytes, the number of transferred fetus and pregnancy rate were assessed. The cut-off value for the serum level of AMH and retrieved oocytes were determined. There was a significant direct correlation between the serum mullerian inhibiting substance (MIS) level with number of total picked up oocytes (r=0.412), mature oocytes (r=0.472) and embryo transfer (r=0.291). There was a linear and significant correlation between inhibin B and fertilization (r=0.283) Cut-off point for AMH level according to presence or absence of pregnancy was 4.8 ng/ml and it was not statistically significant (*P*=0.655). Area under curve (AUC) was 0.543. Cut-off point for MIS according to picked up oocytes was 2.7 ng/ml with area under the curve (ROC curve) of 0.724 (CI= 0.591-0.831) (*P*=0.002). Patients with PCOS who had AMH more than 2.7 ng/ml, the number of retrieved oocytes (6 or more) was higher than MIS/AMH <2.7 ng/ml (*P*=0.002). As a marker of ovarian responsiveness to controlled ovarian hyperstimulation (COH) and despite a small sample size of our study, it is revealed that pretreatment MIS/AMH is highly associated with the number of mature oocytes retrieved during COH in PCOS women.

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Keywords: Anti-Mullerian hormone; Polycystic ovary syndrome; Assisted Reproductive technique

Introduction

Anti-Mullerian hormone (AMH) or mullerian inhibiting substance (MIS) is a dimeric glycoprotein member of the transforming growth factor-beta super family. Compared with other known markers, AMH seems to better reflect the continuous decline of the oocyte/follicle pool with age (1,2). AMH appears to offer at least the same level of accuracy and clinical value for the prediction of poor response and non-pregnancy as antral follicle count (AFC) (3). Serum AMH levels may also be predictive of a hyper-response to FSH, and consequently may be useful in the prediction of women at risk of ovarian hyperstimulation syndrome (OHSS) (2). Overall, AMH has the potential to be incorporated in to work-up protocols to predict patient's ovarian response to treatment and to individualize strategies aiming at reducing the cancellation rate and the iatrogenic complications of controlled ovarian hyperstimulation (4).

It is shown that serum AMH levels to be increased in women with polycystic ovary syndrome (PCOS) compared with controls. This is the result of increased synthesis by granulosa cells and secretion of AMH in the polycystic ovaries or disruption in folliculogenesis leading to an excess accumulation of pre-antral and small antral follicles in these patients (2). Although the granulosa cells from polycystic ovaries may continue to produce elevated levels of AMH, possibly because of impaired access of FSH to follicles (5). AMH levels appear to be related to the severity of the syndrome since levels have been observed to be higher in insulin-resistant PCOS women than in patients with normal insulin sensitivity (6). Alternatively, high AMH values could reflect more impaired disruption in folliculogenesis and granulosa cell function in the ovary

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of amenorrheic compared with oligomenorrheic PCOS women (7). According to a case control study, baseline MIS/AMH is a good predictor of the ovarian response to COH in normo-ovulatory women but not in PCOS (8).

However, it is still unclear whether AMH levels may reflect the severity of ovarian function disruption or have a role in predicting the outcome of individual treatment regimens. Due to few available studies in PCOS women, this study aimed to assess the relationship between the serum levels of AMH and other hormonal markers and results of assisted reproductive techniques (ART) in PCOS patients.

Materials and Methods

This cohort study was conducted on 60 polycystic ovary syndrome (PCOS) patients who were candidates for assisted reproductive techniques. The study was conducted in the infertility department of Shariati Hospital, affiliated to Tehran University of Medical Sciences. The project was approved by the ethical committee of the infertility department of the university and was initiated after achieving written consents of the participants. PCOS diagnosis was according to Rotterdam criteria (9). According to the Rotterdam criteria, we accepted the presence of two of the three following characteristics for inclusion in the study: 1) oligomenorrhea/amenorrhea, 2) clinical (hirsutism) or biochemical findings of hyperandrogenism, and 3) polycystic ovaries on transvaginal sonography. All the patients aged less than 35 years with normal prolactin and thyroid hormone levels and normal male spermogram. In all patients the serum levels of AMH, follicle stimulating hormone (FSH) and luteinizing hormone (LH), esradiol (E2), free testosterone (fT), testosterone (T) and inhibin B were measured in the 3rd day of menstrual cycle. Serum levels of FSH and LH were measured by radioimmunoassay (RIA) method (Immunotech, Beckman Counter Co, Czech Republic). Estradiol and testosterone level was measured by Immunoradioimetric assay (IRMA) method (Immunotech, Beckman Counter Co, Czech Republic). Serum AMH was measured by enzyme-linked immunosorbent assay (ELISA) using the MIS/AMH ELISA kit (DRG instruments GmbH, Germany). For each participant a questionnaire was filled by the researchers. Data were collected from questionnaires, clinical, laboratory notes and ultrasound reports.

The patients underwent controlled ovarian hyperstimulation (COH) with Gonadotropin/GnRH-agonist long protocol. All the participants received folic acid 1mg/day (Ruz Daru Co, Iran) before initiating the induction cycle , low dose oral contraceptive pills (Iran Hormone Co, Iran) on day 3 of the previous cycle and doxycycline (Razak Co, Iran) 100 mg twice a day for the first 10 days of the previous cycle. Long term desensitization protocol using the GnRH agonist buserelin 500 micrograms subcutaneously was started at the day 21 of the previous cycle. After complete desensitization, ovarian stimulation using Gonal F (Serono, switzerland) was commenced on day 3 of the next cycle at a daily dose 150 IU. It was replaced by HMG (Ferring, Germany) after the 7 th day of the stimulation. Transvaginal ultrasound (Siemens, Sonoline G20) for follicular development was done every 3-5 days. Final oocyte maturation was triggered when at least 2 follicles with diameter of at least 17 mm was observed, with HCG (Ferring, Germany) 10000 IU administered as a single intramuscular injection. Oocytes were collected 36-38 hours later using transvaginal guided follicle aspiration under general anesthesia. After fertilization through intracytoplasmic sperm injection (ICSI), three to four good quality embryos were transferred transcervically 3 days later. Luteal phase support was started the day after ovum pick up by administration of progesterone suppository Cyclogest (Actavis, UK), 1200 mg daily. Chemical pregnancy was detected by serum beta-hCG analysis 14 days after embryo transfer and transvaginal ultrasound scan was scheduled 2 weeks later to detect the gestational sac of pregnancy.

Age, body mass index (BMI), infertility duration, number of retrieved oocytes, number of mature oocytes, number of gonadotropin injections, estradiol level, chemical pregnancy, clinical pregnancy,ovarian hyperstimulation syndrome (OHSS) and cycle cancellation were considered. The relationship between serum level of measured hormonal markers with retrieved oocytes, mature oocytes, the number of transferred fetus and pregnancy rate were assessed. The cut-off value for the serum level of AMH and retrieved oocytes were determined. SPSS 17 software (SPSS Inc. Chicago IL, USA) was used for data collection and analysis. *t*-test, Mann-Whitney U-test for quantity data, χ^2 and fisher's exact test for quality variables, Pearson test for correlation were used. P value less than 0.05 was considered for statistical significance.

Results

The mean±SD age of these patients was (29.25±5.16) years, the duration of infertility was (6.2±3.12) years. Only 1.64% had secondary infertility.

Table 1. Comparison of hormonal values between pregnant and non pregnant groups (Values are presented as mean ±SD)

	Non pregnant (n=48)	Pregnant (n=12)	*P value*
LH (IU/L)	4.51 ± 2.74	4.3 ± 2.53	0.924
FSH(IU/L)	8.34 ± 2.79	7.64 ± 1.78	0.502
Testosterone (ng/ml)	1.59 ± 2.54	1.28 ± 1.2	0.751
Estradiol (pg/ml)	89.72 ± 287.83	32.27 ± 29.07	0.570
MIS (ng/ml)	5.13 ± 3.28	5.69 ± 3.66	0.651
Inhibin B (pg/ml)	46.13 ± 64.07	32.68 ± 36.9	0.427

The mean ± SD of the following ART parameters (for each participant) were recorded: total picked-up oocytes (11.49±5.75), number of mature oocytes (9.12±4.85), number of 75 unit gonadotropin ampoules (26.23±7), fertilization rate (0.64±0.26 percent) and number of fetus transfer (3.38±1.5), implantation rate (0.67±0.78 percent).Twelve cases had chemical pregnancy (positive beta HCG) and 11 cases had clinical pregnancy (pregnancy sac on ultrasound). Sever Ovarian hyperstimulation syndrome (OHSS) and cycle cancellation were not reported. There was no significant difference between two groups of pregnant and non-pregnant women for LH, FSH, testosterone, free testosterone, E2, MIS and Inhibin B (Table 1).

There was a significant direct correlation between the serum MIS level with number of total picked up oocytes (r=0.412), mature oocytes (r=0.472) and embryo transfer (r=0.291).

There was a linear and significant correlation between inhibin B and fertilization (r=0.283) (Table 2).

The serum level of MIS or AMH in the group with 6 or more retrieved oocytes (5.76±3.46 ng/ml) was significantly higher than in the group with less than 6 retrieved oocytes (3.34±2.36 ng/ml) (*P*=0.015) (Table 3). But there was no significant difference between other hormones in these 2 groups.

Cut-off point for MIS/AMH level according to presence or absence of pregnancy was 4.8 ng/ml and it was not statistically significant (*P*=0.655). Area under curve (AUC) was 0.543 (Figure 1). Although the pregnancy rate was higher in the group with MIS/AMH >4.8 ng/ml, but it was not statistically significant (Table 4).

Cut-off point for MIS according to picked up oocytes was 2.7 ng/ml with area under the curve (ROC curve) of 0.724 (CI=0.591-0.831) (*P*=0.002). Patients with PCOS who had AMH more than 2.7 ng/ml, the frequency of retrieved oocytes (6 or more) was higher than MIS/AMH <2.7 ng/ml (*P*=0.002) (Figure 2).

Table 2. Correlation between hormones and the total number of picked up oocytes , mature oocytes, fertilization rate and the number of transfered fetuses

Variable / Constant	Value	Retrieved oocytes	Mature oocytes	Fertilization rate	Number of transferred embryos
MIS	R	0.412**	0.472**	0.083	0.291*
	P	0.001	0.001	0.538	0.031
LH	R	0.211	0.143	0.011	0.038
	P	0.118	0.294	0.939	0.788
FSH	R	-0.06	-0.135	0.01	-0.054
	P	0.662	0.322	0.941	0.703
Testostrone	R	0.064	0.047	0.11	0.103
	P	0.642	0.729	0.422	0.463
Free-Testostrone	r	-0.015	0.011	0.033	0.081
	P	0.91	0.938	0.812	0.565
E2	r	0.018	0.019	0.049	0.146
	P	0.896	0.892	0.721	0.295
InhibinB	r	-0.038	0.012	.283*	0.101
	P	0.776	0.93	0.033	0.464

* Correlation is significant at the 0.05 level (2-tailed).

** Correlation is significant at the 0.05 level (2-tailed).

Table 3. Hormonal serum levels between two groups of less than six retrieved oocytes and equal or more than six retrieved oocytes

Variable	Retrieved oocytes (<6)	Retrieved oocytes (≥6)	*P value*
LH (IU/L)	4.02 ± 2.1	4.57 ± 2.88	0.534
FSH (IU/L)	8.73 ± 1.86	8.07 ± 2.86	0.286
Testosterone(ng/ml)	1.04 ± 0.89	1.69 ± 2.64	0.502
E2 (pg/ml)	28.02 ± 36.33	95.31 ± 296.84	0.741
MIS (ng/ml)	3.34 ± 2.36	5.76 ± 3.46	0.015
Inhibin B(pg/ml)	23.11 ± 24.52	49.58 ± 66.32	0.17

Table 4. The positive and negative pregnancy percent for patients with less or equal to 4.8 ng/ml AMH serum level and values greater than 4.8 ng/ml

	AMH ≤4.8 ng/ml	AMH >4.8 ng/ml	Sum
Pregnant	5(41.67%)	7 (58.33%)	12 (100%)
Non pregnant	27 (56.25%)	21 (43.75%)	48(100%)
Sum	32 (53.33%)	28 (46.67%)	60 (100%)

P value= 0.520

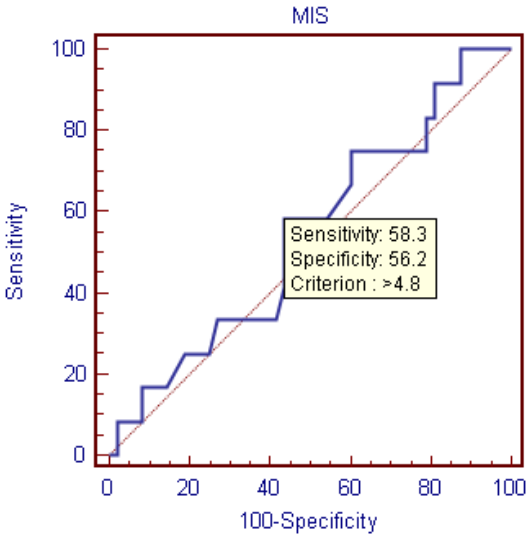

Figure 1. Roc curve of MIS in predicting pregnancy

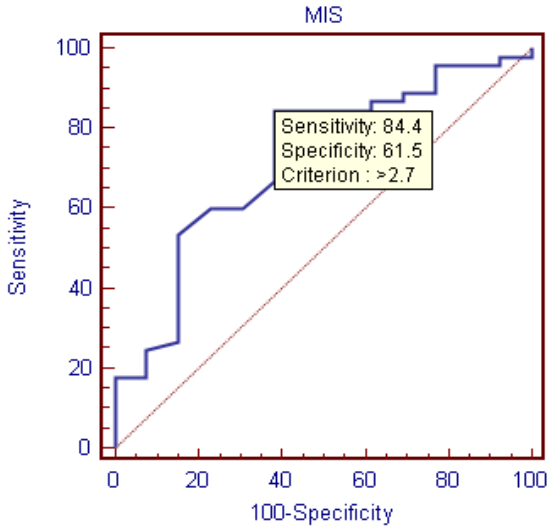

Figure 2. Roc curve of MIS in predicting number of picked up oocytes

Discussion

This study showed that, there was a significant correlation between AMH level and total retrieved oocytes, number of mature oocytes and number of transferred embryos in the PCOS patients. Also it was revealed that fertilization rate had a significant correlation with inhibin B; however; there was no significant correlation between number of retrieved oocytes and other hormonal markers. As a marker of ovarian responsiveness to controlled ovarian hyperstimulation (COH) and despite a small sample size of our study, it is revealed that pretreatment MIS/AMH is highly associated with the number of mature oocytes retrieved during COH in PCOS women. This is in contrast to the study of Wang et. al stating that baseline MIS/AMH is not a good predictor of the ovarian response to COH in PCOS women (8). However there are few published studies confirming the relation between AMH and ovarian response in COH of this group of patients exclusively.

In a recent study to relate follicular fluid AMH and FSH levels in patients with PCOS, it is suggested that the granulosa cells from polycystic ovaries continue to produce elevated levels of AMH, possibly because of impaired access of FSH to follicles. Such an excess in follicular fluid AMH may have harmful consequences

on oocyte quality and final maturation (5). Chu *et al*. concluded that MIS/AMH is more reliable than inhibin B in fertility evaluation (10), our study did not reveal any significant results except for fertilization rate, which could not be explained. Polycystic ovaries have an abnormally rich pool of growing follicles and a disturbance in the selection and subsequent maturation of a dominant follicle. Increased serum AMH is due to increased production per granulosa cell, suggesting an intrinsic GC dysregulation in PCOS. Not only is AMH expression increased, but it also might be protracted in polycystic ovary (PCO) follicles. The potential role of AMH on oocyte quality could be future field of reproduction research.

In the previous studies, it was shown in women undergoing ART with higher serum MIS/AMH level on 3rd day of menstrual cycle have more oocytes retrieved. The mean serum level of MIS/AMH in women with 11 oocytes or more were 2.5 fold in comparison to women with less than 6 oocytes (2.5ng/ml versus1ng/ml). This result was confirmed by Rooji *et al*. in a prospective study. They showed there is a significant relation between MIS/AMH level and oocyte retrieval in 130 patients (11). Muttukrishma *et al.* also concluded that the most useful hormonal marker that correlates with oocyte retrieval was MIS/AMH level (12).

Ficiciglou *et al*. indicated that in 50 patients who underwent ART, the MIS/AMH level was higher in patients with 5 or more retrieved oocytes in comparison to less than 5 oocytes (13). The cut-off point of MIS/AMH in the present study was higher than previous study; this difference may be due to exclusive PCO selection in our study.

Finally, there is not significant relationship between AMH levels and pregnancy rate demonstrated in our study raises the hypothesis of a negative link between AMH and final oocyte maturation. Although we showed MIS/AMH level is a useful predictive marker for ART in PCOS patients but a more comprehensive study is recommended. In the four studies which evaluated the role of MIS/AMH in pregnancy prediction, two studies (14,15) supported it and two studies could not prove (13,16), but all of them indicate that there is a correlation between AMH and ovarian response.

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