DOES ANTI-MULLERIAN HORMONE LEVEL VARY AT DIFFERENT RECOMBINANT FSH DOSES?

ANTI-MÜLLERYEN HORMON SEVİYESİ FARKLI RECOMBİNANT FSH DOZLARI İLE DEĞİŞİR Mİ?

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ABSTRACT

The aim of this study was to compare intracycle variability of Anti-Mullerian Hormone (AMH) throughout controlled ovarian hyper stimulation (COH) with follicle stimulating hormone (FSH) treatment in women with Polycystic Ovary Syndrome (PCOS) and normoresponder infertile patients. Infertile PCOS (n=30) patients were grouped into two. In the first group 15 patients were treated with long luteal gonadotropin releasing hormone analogue (GnRHa) and invitro fertilization (IVF) and 15 PCOS patients were treated with COH-intrauterine insemination (IUI). Non-PCOS (n=30) patients were treated with COH-IUI. AMH was measured three times in all groups (basal, mid-cycle, luteal). AMH levels in PCOS and non-PCOS patients underwent different amount of exogenous FSH treatment were compared. AMH levels in the PCOS patients were 2-2.5 times higher than non-PCOS patients. Total gonodotropin dose used was higher in the PCOS patients (p<0.001). In PCOS patients intracycle AMH variability throughout the cycle wasn't significant but in non-PCOS patients there was declining AMH levels throughout the cycle (p=0.039). Our findings confirm that AMH levels were higher, remain steady and unaffected by high doses of FSH treatment in PCOS patients. On the other hand, in non-PCOS patients there was a small decline in AMH levels with FSH. Clinical pregnancy rates were similar in the groups.

Keywords: *Anti-Mullerian Hormone, Polycystic Ovary Syndrome, Female infertility, In Vitro Fertilization, Pregnancy Rate*

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ÖZ

Bu çalışmanın amacı polikistik over sendromlu (PCOS) ve normoresponder hastalarda follikül stimule edici hormone (FSH) tedavisinin anti-mülleryen hormone (AMH) seviyelerini etkileyip etkilemediğini araştırmaktır. Bu amaçla PCOS'li infertil hastalar (n=30) iki gruba ayrıldı. Birinci gruba long luteal gonodotropin releasing hormon (GnRH) ve invitrofertilizasyon (IVF) (n=15) diğer gruba ise kontrollü overyen hiperstimulasyon ve intrauterin inseminasyon (COH-IUI) tedavisi uygulandı. Normoresponder non-PCOS (n=30) hasta grubu da COH-IUI tedavisi aldı. Tüm gruplarda AMH düzeyleri siklus içinde 3 kez ölçüldü (bazal, midsiklus, luteal). PCOS'li ve non-PCOS'li hastalarda AMH seviyesinin farklı FSH dozlarından etkilenip etkilenmediği araştırıldı. Kullanılan total gonadotropin dozu PCOS'li hastalarda daha yüksekti (p<0.001). PCOS'li hastalarda siklus içi AMH variabilitesi izlenmezken, non-PCOS hasta grubunda AMH seviyesi siklus boyunca giderek azaldı (p=0.039). PCOS'li hastalarda AMH seviyesi daha yüksekti ve yüksek doz ekzojen FSH tedavisi ile değişiklik göstermedi. Diğer taraftan non-PCOS'li hastalarda ekzojen FSH tedavisi ile AMH seviyelerinde hafif bir azalma görüldü. Gruplar arasında klinik gebelik oranları benzerdi.

Anahtar Kelimeler: *Anti-Mülleryen Hormone, Polikistik Over Sendromu, Kadın İnfertilitesi, In Vitro Fertilizasyon, Gebelik Oranı*

INTRODUCTION

Polycystic ovary syndrome (PCOS) is the most common endocrine disorder of reproductive age and it is seen in 6-8% of women (1-2). PCOS disrupts folliculogenesis, prevents dominant follicle progression and causes anovulation. Antimullerian hormone (AMH) produced by granulosa cells from pre-antral and antral follicles and is functionally related with the development of primary follicle and selection of the dominant follicle. It has been shown that serum AMH concentrations might provide information about ovarian reserve in infertile patients. Serum AMH levels has been shown to be two to three-fold higher in women with PCOS than in women with normal ovaries (3) and remain high even after treatment with oral contraceptives (OC) (4). This is mostly due to overproduction of AMH from granulosa cells of the primordial follicles. It is also found that AMH levels are constant through the cycle and not affected by sex steroids as oral contraceptives in non-PCOS women (5).

The aim of this study was to investigate intra-cycle variability of AMH levels in PCOS and non-PCOS patients under different doses of exogenous FSH.

MATERIALS AND METHODS

A total of 60 patients with infertility, 30 PCOS and 30 non-PCOS, were recruited. All patients were informed before participating in the study. This prospective case control study was conducted at Etlik Zübeyde Hanim Maternity and Training Hospital between 2009-2011.

PCOS patients were included to the study according to the Roterdam-2003 criteria (6). Main inclusion criteria for PCOS patients who underwent IVF were: 1. 18-35 years of age, 2. FSH<15mIU/ml, 3. no other endocrine problem, 4. both ovaries present on ultrasound. In addition to these criteria for PCOS patients who underwent controlled ovarian stimulation and intrauterine insemination were: 1. tubal patency, 2. normal male partner sperm parameters (7). Non-PCOS patients were chosen from ovulatory (mid-luteal progesterone>3 ng/dl) patients with bilateral tubal patency who had cyclic menstruation but could not achieved pregnancy after three cycles of clomiphene citrate (CC) although sperm parameters were normal according to WHO data.

Baseline serum AMH, FSH, LH, E2 levels were measured at cycle day 3 prior to the IVF or ovarian stimulation cycles. In PCOS patients who underwent IVF, additional AMH measurement were made at ovarian pick up day and at the day embryo transferred. PCOS patients and non-PCOS patients who were treated with controlled ovarian stimu-

lation and intrauterine insemination (COH-IUI), 2nd and 3rd AMH measurements were made at the day of the IUI and 7 days after IUI.

AMH measurements were made in an accredited laboratory with active MIS/AMH enzyme-linked immunosorbent assay (ELISA) kit according to the manufacturer's manual (Abbexa, Cambridge, UK).

PCOS-IVF group used OC for 21 days at the cycle before IVF (0.03 mg ethynil estradiol- 0.15 mg desogestrel, Desolett, Organon, Istanbul, Turkey). Gonadotropin-releasing hormone agonist (GnRHa), leuprolide acetate 1 mg/d S.C. (Lucrin® , Cedex, France) beginning on the 21st day of the previous cycle and continued until hCG (Ovitrelle® 250mcg, Serono, Swiss) injection. Gonodotropin stimulation started at day 3 with follitropin α (Gonal F®, Serono, Swiss) with a dosage of 150-225 IUdaily. Dose adjustment was made according to the daily transvaginal ultrasonography (Logiq P5, GE Healthcare, USA) evaluation of the follicular growth and E2 levels. hCG (Ovitrelle® 250mcg, Serono, Swiss) was administrated for oocyte maturation when more than two follicles with a diameter >17 mm was observed and ≈35.5 h later, ovum pick-up was performed. If embryo development was succeeded, transfer was performed at 8 days after OPU.
PCOS-IUI and non-PCOS-IUI groups underwent ovarian stimulation with follitropin α (Gonal F®, Serono, Swiss) 75-150 IU at day 3. Dose adjustment was made according to the ovarian response evaluated by serial transvaginal ultrasonography. HCG (Ovitrelle® 250mcg, Serono, Swiss) injection was made when at least one follicle with a diameter of>17 mm was detected. IUI was performed 36 hours after hCG injection (Ovitrelle® 250mcg, Serono, Swiss).

In all patients βhCG measurements were made 12 days after embryo transfer or IUI. Positive results were called for confirmation after 2 days. Presence of fetal heart beat was accepted as clinical pregnancy.

Statistics

Group characteristics were calculated and compared using the arithmetical means and the standard deviations. Independent sample t-test was used for comparison of parametric variables and Chi-square test was used where appropriate. Values with normal distribution and repeating analysis were made with One Way Anova. Statistically significant results underwent Bonferroni correction and Mann-Whitney U test was used. For asymmetrically distributed values Kruskal Wallis was used. A p value <0.05 was considered statistically significant.

RESULTS

There was no significant difference in terms of mean age, body mass index (BMI), and duration of infertility. Table I summarizes some of the clinical and demographic characteristics of the patients.

Table I: Comparison of demographic and clinical parameters

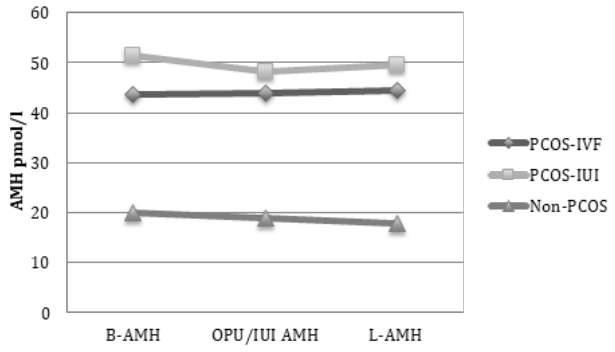
	PCOS-IVF	PCOS-IUI	Non-PCOS	p value
Age (year)	29.27±4.5	27.6±3.4	29.8±3.9	0.239
BMI(kg/m2)	28.9±5.3	27.8±4.9	25.7±3.9	0.76
Infertility (months)	81.6±56.4	75.6±36.0	68.4±8.2	0.689
FSH (mIU/ml)	5.46±1.6	5.5±0.82	6.69±1.6	0.01
LH (mIU/ml)	6.34±2.6	10.9±3.3	5.66±1.7	<0.001
E2 (pg/ml)	32.4±13.9	47.71±17.3	41.21±22.4	0.104
B-AMH (pmol/l)	43.67±10.6	51.54±13.3	20.12±9.01	<0.001
OPU/IUI AMH (pmol/l)	43.88±9.8	48.16±11.08	19.01±8.53	<0.001
L-AMH (pmol/l)	44.51±8.43	49.65±8.99	17.87±7.42	<0.001
Total recFSH dose(IU)	1745.83±613.9	920±369.26	763.75±585.38	<0.001
Clinical Pregnancy (%)	%26.7 (n=4)	%13.3 (n=2)	%13.3 (n=4)	0.487

BMI: body mass index; FSH: follicle stimulating hormone; LH: luteinizing hormone; E2: estradiol; B-AMH: day3 antimullerian hormone; OPU/IUI AMH: mid-cycle antimullerian hormone; L-AMH: luteal antimullerian hormone. p<0.05 is significant. Values are given as mean±SD

As it is seen the table I all three AMH measurements are higher in PCOS patients than normal women (p<0.001). Total gonadotropin dose required for ovarian stimulation was almost 2 times higher in PCOS patients who underwent IVF treatment (p<0.001).

Intra-cycle AMH level change is summarized in figure 1. The change in AMH levels in PCOS patients treated with either IVF or COH-IUI was not statistically significant but in non-PCOS patients there was a small but statistically significant decline (p=0.039).

Figure 1: Intra-cycle AMH change with exogenous follitropin α treatment.



The clinical pregnancy rates were similar in the groups (p=0.487). In PCOS patients there was no significant difference between AMH levels in women who get pregnant or not. However, in non-PCOS patients all AMH measurements were higher in patients who achieved clinical pregnancy (Table II).

Table II: AMH levels of patients with clinical pregnancy or not.

	Clinical Pregnancy		
PCOS	(-)	(+)	P value
B-AMH	46.76±12.36	50.98±13.75	0.47
OPU/IUIAMH	44.70±10.27	51.30±11.08	0.176
L-AMH	46.36±8.92	49.98±9.28	0.384
	Clinical Pregnancy		
Non-PCOS	(-)	(+)	P value
B-AMH	18.08±7.46	33.35±7.29	<0.001
OPU/IUIAMH	17.30±7.17	30.15±9.19	0.003
L-AMH	15.94±5.29	30.48±7.37	<0.001

B-AMH: day3 Antimullerian hormone; OPU/IUI AMH: mid-cycle Antimullerian hormone; L-AMH: luteal Antimullerian hormone. p<0.05 is significant.

DISCUSSION

In this study, we investigated whether AMH levels were altered in patients with FSH at different doses. For this purpose, we included PCOS and non-PCOS patients who received different doses of recFSH in the study. It was observed that AMH levels tended to decline during treatment in non-PCOS patients, despite lower doses of FSH treatment.

AMH is not only a reliable indicator of ovarian reserve and but also a promising tool for diagnosis of PCOS since AMH levels are 2-3 times higher in PCOS patients than normal women (8-10). It is generally accepted that AMH levels do not change from cycle to cycle and are not affected by exogenous interventions. Even so in the literature there are conflicting results for AMH levels measured during menstrual cycle. For example, La Marca et al. reported that serum AMH levels do not change significantly throughout the menstrual cycle (11). In contrary, Wunder et al. reported that in normal ovulatory women AMH levels are higher in the late follicular phase compared with the day of ovulation or the early luteal phase (12). Hadlow et al. also reported that in cycling women, the variability in AMH should be considered by clinicians, especially if a result is close to a clinical cut-off (13).

There are conflicting results for effects of exogenous medications on AMH levels. Doleman et al. reported that oral contraceptives may have a reducing effect on AMH levels (14). In contrary Somunkıran et al reported that hormonal contraception did not alter AMH levels (15). Lie Fong et al. reported that POCS patients had stable AMH levels throughout the ovarian stimulation cycles (16). AMH measurements were made at three times under exogenous follitropin treatment similar to our study. However, our study has a different aspect as it also involves an IVF group including patients who are exposed to higher doses of FSH. In the PCOS patients AMH levels do not vary throughout the cycle and the amount of recFSH used did not alter this finding.

In this report, we observed a small decrease in AMH levels in non-PCOS patients during the ovarian stimulation. It is quite difficult to conclude based on this finding due to the restricted number of the patients. Larger group of patients are really necessary to verify this finding. There is another study conducted on 124 patients undergoing ovarian hyper stimulation supports this finding. Li et al. reported that there was a decrease in the AMH levels throughout the cy-

cle in controlled ovarian hyper stimulation. Main contrast of this study is that the decrease in AMH was shown in both PCOS and non-PCOS patients (17). This small decline in the non-PCOS patients may be due to the unaltered ovarian physiology and HPA axis in these patients.

The value of AMH in prediction of pregnancy has been investigated in various studies by different researchers with conflicting results. Some studies suggest that serum AMH level is associated with pregnancy rates (18-19) whereas others suggest no relationship (20-21). Sahmay et al. in their trial investigated 150 patients with PCOS and concluded that AMH levels has no value in clinical pregnancy prediction (22). In our study, we verified this finding in PCOS patients. Although AMH levels in 2 non-PCOS patients who became clinically pregnant were higher than the rest it is difficult to conclude that AMH could predict pregnancy in these patients because of the small sample size.

CONCLUSION

In conclusion, AMH levels did not change with both low dose and high dose recFSH. In addition, AMH measured at different times of the cycle did not predict clinical pregnancy.

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