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Research Article

Evaluating the Association of Ovarian Reserve with Age in Women with Polycystic Ovary Syndrome

Abstract

Objectives: Polycystic ovary syndrome (PCOS) is the most common cause of anovulatory infertility. However, they sustained fertility at an advanced age. We aimed to evaluate ovarian reserve of the PCOS patients of advanced age and the control groups.

Material and Methods: A total of 41 patients with PCOS and 28 normo-ovulatory women were included in the study. All subjects were aged 35–42 years. The 2003 Rotterdam criteria were referred, which diagnose PCOS in women possessing two or more of the following criteria: oligomenorrhoea or amenorrhoea; clinical and/or biochemical hyperandrogenism; and polycystic ovaries on ultrasonography. Other possible endocrine disorders were excluded. During the early follicular phase of spontaneous or progestin-induced menses blood samples were collected and antral follicles and ovarian volume were calculated using transvaginal ultrasonographic examinations.

Results: The serum anti-müllerian hormone (AMH) levels, the mean ovarian volume (MOV) and antral follicle count (AFC) were significantly higher in the PCOS group than in the control group (p<0.05). Moreover, the AMH levels revealed a significant positive correlation with AFC values, (r=0.74, p=0.001) and negative correlation with the serum follicle-stimulating hormone FSH levels (r=-0.37, p=0.001).

Conclusion: Ovarian reserve can be preserved better in women with PCOS than in normo-ovulatory women of similar age. Among the tests for ovarian reserve, the AMH levels in the early follicular phase and MOV correlate best with AFC values.

Introduction

Polycystic ovary syndrome (PCOS) is a common endocrine disorder in women of reproductive age, affecting an estimated 6.6 % of women in this age group [1]. PCOS is the most common cause of anovulatory infertility and is characterised by menstrual disruption, increased ovarian androgen production and disordered folliculogenesis. Patients with PCOS may have other metabolic abnormalities including insulin resistance, type 2 diabetes mellitus and cardiovascular diseases [2].

As a result of temporary social trends, an increasing number of women choose to delay their first pregnancy to a later stage in life; consequently, a part of this population may become infertile by the time they opt to conceive. This occurs mainly because of the effects of ageing: decreasing ovarian reserve and low oocyte quality [3]. Patients with PCOS have a larger follicle pool than normo-ovulatory women. Webber et al. (2003) reported that the median density of small follicles at the primordial and primary stages, was six times greater in anovulatory polycystic ovaries than in normal ovaries [4]. The high density of primary follicles in polycystic ovaries supports the hypothesis that these women may have been born with a larger ovarian reserve [4].

Some cross-sectional studies have suggested that menopause which is characterised by exhaustion of the follicle pool and marks the end of the reproductive life span, may occur at a later age in women with PCOS1 [5,6]. Other studies have reported that in patients with PCOS, menstrual cycles often became regular with increasing age [5,7].

The rate of decline in ovarian reserve depends on the number of remaining primordial and small preantral follicles. With advancing age, the follicle pool decreases in both patients with PCOS and normo-ovulatory women. However, as patients with PCOS have a greater initial pool of follicles, their ovarian reserve is unlikely to undergo rapid depletion with age.

The aim of this prospective, observational study was to assess the ovarian reserve in patients with PCOS and normo-

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ovulatory women, above 35 years of age. We used several tests to compare the ovarian reserves such as the levels of FSH, estradiol, anti-müllerian hormone (AMH) and inhibin-B at day 3 of the menstrual cycle; antral follicle count (AFC), the mean ovarian volume (MOV) and clomiphene citrate challenge test (CCCT).

Materials and Methods

This study was approved by the Institutional Ethics Committee and we obtained informed consent from all the participants. The study included 41 patients with PCOS (PCOS group) and 28 age-matched, normo-ovulatory women (controls). Patients who visited the Infertility and Reproductive Medicine Unit of Erciyes University Hospital were enrolled in this study. The participants' age, medical history, surgical history, treatment history, infertility history, height, weight, menstrual patterns, hirsutism scores and gynaecologic examination results were recorded. The 2003 Rotterdam criteria were referred, which diagnose PCOS in women possessing two or more of the following criteria: oligomenorrhoea or amenorrhoea; clinical and/or biochemical hyperandrogenism; and polycystic ovaries on ultrasonography (at least 12 follicles with an individual diameter of 2–9 mm per ovary and/or an ovarian volume >10 cm³). Other possible endocrine disorders, such as thyroid or adrenal disorders, hyperprolactinaemia and androgen-secreting tumours, were excluded. In addition, we eliminated women who reported a history of ovarian surgery, endometriosis, and smoking or received hormonal drugs which interfere with the hypothalamic-pituitary-gonadal axis, in the last three months.

During the early follicular phase of spontaneous or progestin-induced menses, transvaginal ultrasonography was performed using a Shimadzu Portable SDU 350A (TV11R-655U 5 - 8 MHz probe) ultrasound apparatus. The ovarian volume was calculated as $D1 \times D2 \times D3 \times 0.523$. Here the dimensions, D1, D2 and D3 are the length, width and thickness of the ovary, respectively. Antral follicles were calculated using transvaginal ultrasonographic examinations, which were performed by the same operator. At the first visit (cycle day 2,3 or 4), we assayed the circulating levels of FSH, luteinizing hormone (LH), estradiol (E2), thyroid-stimulating hormone (TSH), prolactin, androstenedione (A), total testosterone (t-T), free testosterone (f-T), 17-OH-progesterone (17-OHP), dehydroepiandrosterone sulphate (DHEAS), sexhormone-binding globulin (SHBG), AMH and inhibin-B; we also performed aclinical examination to evaluate the Ferriman–Gallwey score. A CCCT (Navot et al., 1987) was performed by administering 100 mg clomiphene citrate (Klomen 50 mg, Koçak Farma, Turkey) orally from day 5 to day 9 of the same cycle; blood samples were collected on cycle day 10 for measurement of FSH levels.

The circulating levels of fT (RIA DSL-4900, Texas, USA), 17-OHP (RIA DSL-500, Texas, USA), A (RIA DSL-3800,Texas, USA) and DHEAS (Immunotech, Marseille, France) were determined using radioimmunoassay. We determined the circulating levels of FSH, LH, tT, prolactin and estradiol using chemiluminescence immunoassay (ACS: 180,Bayer,Germany). The levels of TSH (KIP1891-KIP1894, TSH IRMA, Biosource,

Belgium) and SHBG (IRMA DSL-7400, Texas, USA) were measured using immunoradiometric assay. The inter-assay coefficients of variation were 9.5%–10.8% for 17-OHP;2.8%–7% for A;4.4%–4.8% for T;6.3%–9.9% for DHEAS;5.2%–5.8% for SHBG;5%–6.2% for LH;2.8%–4.6% for FSH; and9.9%–11.8% for E2. The levels of AMH (DSL-10-14400, Texas, USA) and inhibin-B (DSL-10-84100i, Texas, USA) were determined using enzymeimmunometric assay. The detectionlimit was 0.006 ng/ml; the intra- and inter-assay coefficients of variation for AMH were 5.2% and 9.1%, respectively. The sensitivity of the inhibin-B assay was < 7 pg/ml, and the intra- and inter-assay coefficients of variation were 5.6% and 7.6%, respectively.

We used the following tests to determine the basal ovarian reserve on cycle day 3: levels of FSH, estradiol, AMH, inhibin-B, AFC, MOV and CCCT.

Statistical analysis

The Statistical Package for the Social Sciences (SPSS) 15 was used for all analyses. The Kolmogorov–Smirnov test was used to check for the normal distribution of quantitative data. All values were expressed as mean ± standard deviation (x ± SD). A p value of <0.05 was considered significant. The student t–test was used for comparison between groups. The Pearson correlation coefficient was used to compare the results of the tests for ovarian reserve.

Results

Age and BMI were similar between the PCOS group and the controls; however, the Ferriman–Gallwey scores were higher in PCOS group when compared with the controls (p<0.01). The basal serum levels of LH, prolactin, t-T, f-T, A4 and DHEAS were higher in PCOS group than those in the controls (p<0.01); the FSH and estradiol levels were higher in the controls than those in the PCOS group (p<0.01). No significant differences were observed in the levels of TSH, 17-OHP and SHBG between the two groups (p>0.05). [Table 1].

AMH levels in PCOS group were significantly higher than those in the controls (5.36 ± 3.53 ng/ml vs. 0.93 ± 0.97 ng/ml; p<0.01). In addition, MOV and AFC were significantly higher in the PCOS group (p<0.01). Furthermore, the CCCT results revealed that on cycle day 10, serum FSH levels were higher in the controls than those in the PCOS group (p<0.01). However, inhibin-B levels were similar in both PCOS and control groups (42.65 ±26.48 vs. 39.32 ± 37.93). [Table 2].

The AMH levels were positively correlated with AFC values in all subjects. In addition, the parameters of AMH levels along with MOV were best correlated with AFC values (r=74, p<0.001 vs. r=0.78, p<0.001; n = 69).The basal and day 10 serum FSH levels were negatively correlated to AFC values (r = -0.37, p= 0.001 and r= -0.52,p= 0.001). However, no correlation was observed between AFC values and serum inhibin-B levels (r=0.17, p= 0.16). Although the basal serum estradiol levels were negatively correlated with AFC values, the correlation was not statistically significant (r= -0.09, p= 0.42; [Table 3]).

Discussion

Our results indicate that women with PCOS maintain their quantitative ovarian reserve better than age-matched, normo-ovulatory, eumenorrhoeic women. AFC, MOV and serum AMH levels were significantly higher in the PCOS group, while serum FSH levels on cycle days 3 and 10 and estradiol levels were significantly lower in the PCOS group.

The PCOS group in our study was of the overweight

Table 1: Clinical and endocrine characteristics of the study subjects.

Variables	PCOS (n=41)	Normo-ovulatory controls (n=28)	P -value
BMI (kg/m2)	33.12 ± 5.1	32.78 ± 3.3	0.75
Age	37.14 ± 1.83	36.75 ± 1.64	0.36
FGS	10.21 ± 1.78	5.17 ± 1.38	0.01
FSH (mIU/mL)	5.87±1.49	10.19±1.75	0.01
LH(mIU/mL)	7.97±3.25	4.40±2.25	0.01
E2 (pg/ml)	58.58±21.81	84.79±60.58	0.01
TSH (µIU/ml)	1.16±0.8	2.01±0.9	0.23
Prolactin (ng/mL)	10.33±4.0	6.75±3.5	0.01
t- T (ng/mL)	121.53±53.0	55.35±18.8	0.01
f- T (pg/mL)	3.59±1.05	1.58±0.42	0.01
A4 (ng/mL)	2.87±0.98	1.24±0.31	0.01
17-0H P(ng/mL)	1.04±0.48	0.85±0.45	0.10
DHEASO4(ng/mL)	2922.73±1670.94	1492.89±604.41	0.01
SHBG (nmol/mL)	46.50±16.67	76.14±56.40	0.10

Data given as mean±SD (standard deviation); BMI, Body mass index; FGS, Ferriman–Gallwey score; t-T,total testosterone; f-T, free testosterone; A4, androstenedione; DHEAS, dehydroepiandrosterone sulphate; SHBG, sex hormone binding globulin.

Table 2: Comparison of the results of ovarian reserve tests.

	PCOS (n=41)	Normoovulat y controls (n=28)	P vale
FSH (mIU/mL)	5.87±1.49	10.19±1.75	0.01
E2 (pg/mL)	58.58±21.23	84.79±60.25	0.01
AMH (ng/mL)	5.36 ± 3.53	0.93 ± 0.97	0.01
Inhibin-B (pg/mL)	42.65±26.48	39.32 ± 37.93	0.67
MOV (cm³)	12.16±4,72	4.66±2.37	0.01
AFC	33.04±8,64	8.64±3.91	0.01
Day10FSH(mIU/mL)	3.25±1.44	6.98±2.38	0.01

Data given as mean ± SD; AFC, Antral follicle count; MOV, Mean ovarian volume.

Table 3: Correlation between AFC values and results of other ovarian reserve tests.

	r	p
AMH	0.74	0.001
Inhibin-b	0.17	0.160
FSH	-0.37	0.001
E2	- 0.09	0.420
Day 10 FSH	-0.52	0.001
MOV	0.78	0.001

AMH, Antimullerian hormone; E2, Estradiol; AFC, Antral follicle count; MOV, Mean ovarian volume.

subgroup (BMI, 33.12kg/m²), and their inhibin-B levels were not significantly higher than those of the controls. Cetkovic A et al., reported that inhibin-B levels were not significantly elevated in patients with PCOS [8]. However, Tsiqkaou et al., reported that inhibin-B levels were higher in lean PCOS subgroup than in overweight PCOS subgroup [9].

We observed that the basal and day 10 FSH levels were lower in the PCOS group than in the controls. Moreover, both these levels were negatively correlated with AFC values. In our study, the basal estradiol levels were not correlated with AFC or MOV values. However, Erdem et al., reported a strong negative correlation between basal FSH levels and MOV as well as between basal FSH levels and AFC values [10]. In contrast, Hudecova et al., found that ovarian volume was not correlated with FSH or estradiol levels, and that these last two values were not correlated with AFC values [11]. In addition, Holte et al., reported that basal FSH levels were lower in patients with PCOS than those in the controls at young age [12], moreover Dahlgren et al., found lower FSH levels in 50-year-old women with history of PCOS when compared with age-matched controls [5].

Ovarian reserve indicates a woman's reproductive potential and reflects the number of follicles and quality of oocytes. Despite the initial time is too variable, reduction in the ovarian reserve, to be associated with women age. In normo-ovulatory women, menstrual cycle irregularities due to ageing are dependent on the number of remaining follicles. In normal ovarian ageing, the oocyte quality and quantity rapidly decline in the late thirties [13]. Considering the fixed interval between menopause and the rapid depletion of ovarian reserve[13-15], it was speculated that up to 10% of women in general population undergo early ovarian ageing; thus, screening for early ovarian ageing should be performed in asymptomatic women with associated risk factors such as family history of early menopause, history of ovarian surgery, smoking , severe endometriosis. Several authors suggest that women with PCOS can be excluded from such screening as rapid depletion of ovarian reserve is unlikely in these women [16].

Nevertheless, various human morphometric studies have revealed that there is correlation between the cohort of growing antral follicles and the pool of resting and small pre-antral follicles [17,18]. Webber et al., reported that the median density of small follicles, including those at the primordial and primary stages, was six times greater in polycystic ovaries when compared with normal ovaries [4]. The high density of primary follicles in patients with PCOS indicates that these women may have been born with a larger ovarian reserve.

AFC values have been recognised as a useful, quantitative predictor of ovarian reserve [19]. The AFC values, which are closely related to reproductive age, can indicate the number of remaining primordial follicles [20]. We observed that both AFC and MOV were significantly higher in the PCOS group. Consistent with this observation, Balen et al., ultrasonographically determined that polycystic ovaries are larger and have more follicles. Increased recruitment from the resting follicle pool may occur in polycystic ovaries; hence, polycystic ovaries were

expected to exhibit premature depletion of primordial follicles, leading to an accelerated onset of menopause [21]. However, a recent study suggests that women with PCOS reach menopause two years later than normo-ovulatory women [6]. Moreover, it has been proposed that women with PCOS exhibit delayed ovarian ageing and a sustained reproductive life span [22]. The ovarian reserve of these women is unlikely to deplete rapidly; therefore, while screening for early ovarian ageing, women with PCOS constitute a low-risk group and should be excluded [16]. Authors believed that in women with PCOS, the increased recruitment of follicles from the resting pool may be counterbalanced by the large initial follicle pool and the slow rate of progression through the later stages of preantral follicle development [4,23,24]. On the other hand, in postnatal mouse ovaries, AMH was proven to inhibit initial follicle recruitment and FSH-mediated stimulation of preantral and small antral follicle growth [25]. AMH is expressed in those follicles that are recruited from the primordial follicle pool and have not been selected for dominance. AMH null mice exhibit earlier depletion of the follicle pool and thus, a shorter reproductive life span. AMH levels are an important inhibitor of primordial follicle pool depletion. AMH is a member of the transforming growth factor–beta family and is secreted from granulosa cells in growing follicles (especially preantral and small antral follicles) until they reach the size at which they may be selected for dominance by FSH [26]. Serum AMH levels decrease with increasing age in normo-ovulatory women and are strongly correlated with AFC values. Serum AMH levels have been higher in women the PCOS group than those in the controls and these levels decline over time in both groups; however the reduction is lower in the PCOS group[22].

In addition, AMH has been proposed to be a diagnostic marker of PCOS and has been used to monitor therapeutic response [27]. The high serum AMH levels in the PCOS group are attributable to an increase in the number of small antral follicles as well as to increased AMH production per granulosa cell; subsequently, the levels of AMH are 75 times higher in granulosa cells from polycystic ovaries than in those from normal ovaries. Furthermore, AMH levels appear to be related to the severity of PCOS because higher AMH levels have been observed in insulin–resistant patients with PCOS.

Recent studies have shown that AMH levels may constitute an important and effective measure of ovarian reserve [28–30]. Serum AMH levels of the menstrual cycle in normo-ovulatory premenopausal women show a progressive decrease with age and correlate with AFC, age and FSH levels; however, AMH levels increase and show a slower rate of decline with age in the PCOS group [6,22]. We observed higher AMH levels in the PCOS group than in the controls and a positive correlation between AFC values and AMH levels in all the subjects. In addition, AMH levels together with mean ovarian volume were the parameters best correlated with AFC. Moreover, no correlation was observed between AMH levels and AFC in the PCOS group; however, this observation may be attributable to an insufficient number of patients. Hudecova et al. reported a significant correlation of ovarian volume with AFC and serum AMH levels, but not with serum FSH levels in the PCOS group; they suggested that the

ovarian reserve of the PCOS group may be superior to that of the controls [11]. Another study indicated that women with PCOS maintain their ovarian reserve with advancing age and show better fertility than infertile, eumenorrhoeic women [31]. Weerakiet et al. studied ovarian reserve in women with PCOS and concluded that ovarian reserve was diminished in those who underwent laparoscopic ovarian drilling (LOD) as compared to those who did not undergo this procedure. Moreover, both these PCOS subgroups, regardless of whether they underwent LOD, had a significantly greater ovarian reserve than age-matched, normo-ovulatory controls [32]. Moreover these patients with PCOS experienced spontaneous pregnancies at later stage of the reproductive life span [33].

With increasing age, both oocyte quantity and quality dictate subsequent reproductive events, including decreased fertility, increased abortion rate, cycle irregularities when almost no follicles are left, finally followed by menopause [15].

The present study has a significant disadvantage. The main limitation was low power to show differences. However, significant differences observed in AFC, FSH and AMH levels in PCOS patients, which may have clinical importance. A larger study may resolve this problem.

However, the present study does have some significant strengths. First of all, the major strength is its prospective nature. Another advantage of the present study is the homogenous nature of the study samples and study protocol.

In conclusion, women with PCOS seem to maintain their ovarian reserve better than normo-ovulatory women and may be able to conceive at an advanced age.

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