PCOS

The impact of oral contraceptives and metformin on anti-Müllerian hormone serum levels in women with polycystic ovary syndrome and biochemical hyperandrogenemia

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Abstract

Objective. To assess the impact of metformin and of two different oral contraceptives (OCs) containing cyproterone acetate and drospirenone, on serum anti-Müllerian hormone (AMH) levels, in a cohort of women with polycystic ovary syndrome (PCOS) with hyperandrogenism.

Design. Prospective randomised study.

Setting. Division of Endocrinology and Human Reproduction, Aristotle University of Thessaloniki.

Patients. Forty-five (45) women with PCOS diagnosed according to the criteria proposed in 1990 by the NIH.

Interventions. Women with PCOS were randomised into three groups, all treated for 6 months: Group A received an OC containing 35 µg ethinylestradiol plus 2 mg cyproterone acetate, Group B received an OC containing 30 µg ethinylestradiol plus 3 mg drospirenone and Group C received metformin 850 mg × 2.

Main outcome measure(s). Anti-Müllerian hormone levels were measured by a specific ELISA.

Results. AMH was significantly decreased under treatment with 35 µg ethinylestradiol plus 2 mg cyproterone acetate (p = 0.002 at 3 months and p < 0.001 at 6 months). Treatment with 30 µg ethinylestradiol plus 3 mg drospirenone, and treatment with metformin 850 mg × 2 did not significantly affect serum AMH levels. AMH was significantly decreased under OCs treatment compared to metformin 850 mg × 2 (p = 0.005).

Conclusion(s). AMH serum levels were significantly decreased under treatment with 35 µg ethinylestradiol plus 2 mg cyproterone acetate, due to decrease in androgens and suppression of gonadotropins.

Keywords: Polycystic ovary syndrome, hyperandrogenism, anti-Müllerian Hormone, oral contraceptives, metformin

Introduction

Polycystic ovary syndrome (PCOS) is the most common endocrinopathy of reproductive age women [1] and is the leading cause of anovulatory infertility in women [2]. PCOS is characterized by hyperandrogenism (hirsutism and/or biochemical hyperandrogenemia) and oligo/anovulation, and is also highly associated with obesity and insulin resistance (IR) [3]. Oligo/anovulation in PCOS is, apparently, due to an excessive early follicular growth and a subsequent follicular arrest as the selection of a follicle from the increased pool of growing/selectable follicles and its further maturation to a dominant follicle does not occur [4].

Anti-Müllerian hormone (AMH) is a member of the transforming growth factor-β (TGF-β) superfamily of glycoproteins that has been found to play an important role in chronic anovulation by inhibiting the initial recruitment of primordial follicles [5] and by promoting follicular arrest [6]. Indeed, we [7] and others [8–10] have found increased AMH levels in both serum [7–10] and follicular fluid [11] of women with PCOS.

Women with PCOS are usually treated with an oral contraceptive (OC), while both lean and obese PCOS with IR might benefit from treatment with metformin. Treatment with OCs is known to normalise menstrual function and to ameliorate hirsutism and acne, while the effects on IR are controversial [12]. On the opposite, treatment with metformin is beneficial for weight and IR reduction, still the effect on menstrual cycle and hyperandrogenism is rather weak. Few data are available on the impact of these treatment modalities on serum AMH levels in women with PCOS. The use of OCs was shown not to affect serum AMH levels both in normal women [13], and in women with PCOS [14], while metformin treatment was shown to decrease AMH serum levels [15].

The aim of the present study was to assess the impact of metformin and of two different regimens of OCs containing different progestins, namely cyproterone acetate and drospirenone, on serum AMH levels in a well characterised cohort PCOS women with hyperandrogenenemia recruited...
on the basis of the stricter criteria proposed in 1990 by the National Institute of Child Health and Human Development Conference on PCOS [16].

Materials and methods

Subjects

The study included forty-five (45) women with PCOS, mean age 21.07 ± 3.21 years and mean BMI 21.52 ± 1.01 kg/m², which were recruited from the outpatient endocrine clinic. Only normal weight (BMI < 25 kg/m²) were included in the study. Diagnosis of PCOS was based on the presence of: chronic anovulation (fewer than six spontaneous bleeding episodes per year), and biochemical hyperandrogenemia in accordance with the criteria proposed in 1990 by the National Institute of Child Health and Human Development Conference on PCOS [16]. All women presented polycystic ovarian morphology on ultrasound examination. Hyperandrogenemia was defined as testosterone levels > 60 ng/ml. This value was derived from the mean value ± 2SD of 100 control women. Other common causes of hyperandrogenism such as prolactinoma, congenital adrenal hyperplasia, Cushing syndrome and virilizing ovarian or adrenal tumours were excluded.

The study was prospective and randomised. Randomisation was non-blind and was based on patients’ chronological presence at the outpatient endocrine infirmary, namely the first one in Group A the second in Group B, the third in Group C, etc. For each patient a prescription of the recommended medication was given in order of appearance for each patient. A written informed consent was obtained from all study Groups. Analysis of data was done by using the statistical package Statistics

Methods

At baseline, blood samples were collected between the third and seventh day after the beginning of a spontaneous bleeding episode, at 09:00, after an overnight fast. Under treatment, blood samples were collected during OC treatment between the fifth and the seventh day of the fourth and seventh cycle (reflecting 3 and 6 months of treatment), while patients were on OCs or metformin.

All assays of hormonal levels and plasma glucose were carried out at the Department of Biochemistry of the Aristotle University of Thessaloniki School of Medicine.

Plasma glucose concentrations were measured using a glucose oxidase technique with an auto analyser (Roche/Hitachi 902; Roche Diagnostics GmbH, Manheim, Germany). Luteinizing Hormone (LH), Follicle Stimulating Hormone (FSH) and prolactin levels were measured with an enzyme-linked immunoassay (ELIA), using commercial kits (Nichols Institute Diagnostics, CA). Testosterone was measured with a Direct RIA kit (Sorin, Biomedica); D4-androstenedione with a Gamma Coat [125I] RIA kit (Inctar Corp.); Dihydroepiandrosterone Sulfate (DHEA-S) with direct RIA solid-phase coated tubes (Zer Science Based Industries Ltd); 17α-OH-progesterone with an ImmuChem Double Antibody [125I] RIA kit (ICN Pharmaceuticals, Inc.); insulin with a Coat-A-count Insulin kit (Diagnostic Products Corp.); and sex-hormone binding globulin (SHBG) with an immunoradiometric assay (IRMA) kit (SHBG: [125I] IRMA Kit, Orion Diagnostica). The intra-assay coefficients of variation (CV) were 1.5% for FSH, 0.7% for LH, 2.7% for prolactin, 3.8% for insulin, 4.1% for 17α-OH-progesterone, 1.3% for testosterone, 5.9% for androstenedione, 9.4% for DHEA-S and 5.8% for SHBG. The average inter-assay CV were 3.2% for FSH, 1.7% for LH, 3.4% for prolactin, 4.4% for insulin, 6.3% for 17α-OH-progesterone, 2.2% for testosterone, 9.2% for androstenedione, 12.1% for DHEA-S, 7.8% for SHBG.

AMH concentrations were measured with an enzymatically amplified two-side immunoassay [DSL-10-14400 Active Mullerian Inhibiting Substance/AMH enzymelinked immunosorbent (ELISA) kit, DSL laboratories, Webster, TX]. The theoretical sensitivity of the method is 0.006 ng/ml, the intra-assay coefficient of variation for high values 3.3% and the inter-assay coefficient of variation for high values 6.7%.

Free androgen index (FAI) was calculated according to the equation: testosterone (nmol/l) × 0.0347 × 100/SHBG (nmol/l). HOMA-IR was derived from the equation: glucose (mmol/l) × insulin (mIU/ml)/22.5. QUICKI was derived from the equation: 1/log (fasting insulin) + log (fasting glucose).

Statistics

All analyses were performed using the statistical package SPSS, v.16.0 (SPSS Inc., Chicago, IL). We performed a sample size calculation for the difference in mean values for serum AMH under different treatment regimens. A total of 15 patients will enter this crossover study. By including a minimum of 15 patients in each study group, the probability is 90% that the study will detect a treatment difference at a two sided 5.0% significance level.

We prefer to use non-parametric methods as fewer assumptions have to be made and especially because of the small number of patients that consists the three different study Groups. Analysis of data was done by using Kruskal–Wallis one way analysis of variance. The Mann–Whitney test was used to evaluate differences between groups. All parameters are shown as the mean ± SD. Correlations were assessed by using Spearman’s rank correlation. Correlations with a critical value of p < 0.05 were considered significant.

Results

The basal hormonal features for all women with PCOS as well as for the three different groups of treatment are summarised in Table I. Concerning all these parameters,
no statistical significant difference was detected between all PCOS groups at baseline. No statistical significant difference in Weight and BMI was noted for all treatment groups after 6 months of treatment (Table I). A tendency for a slight decrease in weight was noted for women under metformin treatment ($60.52 \pm 5.53$ versus $57.77 \pm 4.16$, $p = 0.168$). Menstrual function was normalised in all patients under treatment with an OC (Groups A and B) (mean cycle length $28 \pm 2$). Under metformin treatment in 10 out of 15 patients (66.66%) menstrual function was normalised with a menstrual cycle length between 27 and 32 days. Concerning the rest five patients under metformin treatment three experienced successively ovulatory versus anovulatory cycles, while for the rest two menstrual cycles remained anovulatory during the whole period of treatment.

Six months of treatment with $35 \mu g$ ethinylestradiol plus $2 \mu g$ cyproterone acetate (Group A) led to a significant decrease in serum LH ($p < 0.002$), FSH ($p < 0.001$), testosterone ($p < 0.003$), androstenedione ($p = 0.033$), 17-OH-progesterone ($p = 0.038$), FAI ($p < 0.001$), glucose/insulin ratio ($p = 0.007$) and QUICKI ($p = 0.049$), as well as to a significant increase in SHBG ($p < 0.001$).

Six months of treatment with $30 \mu g$ ethinylestradiol plus $3 \mu g$ drospirenone acetate (Group B) led to a significant decrease in serum LH ($p = 0.001$), FSH ($p = 0.012$), testosterone ($p < 0.001$), SHBG ($p < 0.001$) as well as to serum Glucose ($p = 0.002$) (Table I).

Six months of treatment with metformin $850 \mu g \times 2$ (Group C) led to a statistically significant decrease only in fasting glucose ($p = 0.005$) (Table I).

Under treatment with an OC containing $35 \mu g$ ethinylestradiol plus $2 \mu g$ cyproterone acetate (Group A), AMH serum levels were significantly decreased from 9.10 $\pm$ 2.95 ng/ml at baseline to 7.45 $\pm$ 2.36 ng/ml after 3 months and to 5.28 $\pm$ 0.84 ng/ml after 6 months of treatment ($p < 0.001$ and $p = 0.002$, respectively) (Figure 1).

Under treatment with an OC containing $30 \mu g$ ethinylestradiol plus $3 \mu g$ drospirenone (Group B), AMH serum levels ranged from 8.90 $\pm$ 3.49 ng/ml at baseline to 7.06 $\pm$ 3.12 ng/ml after 3 months and to 8.42 $\pm$ 3.57 ng/ml after 6 months of treatment ($p = ns$) (Figure 1).

Under treatment with metformin $850 \mu g \times 2$ (Group C), AMH serum levels ranged from 9.24 $\pm$ 3.70 ng/ml at baseline to 9.92 $\pm$ 3.80 ng/ml after 3 months and to 7.77 $\pm$ 2.82 ng/ml after 6 months of treatment ($p = ns$) (Figure 1).

Serum AMH levels were significantly decreased under treatment with an OC containing $35 \mu g$ ethinylestradiol plus $2 \mu g$ cyproterone acetate (Group A) compared to treatment with metformin $850 \mu g \times 2$ (Group C) both at 3 and at 6 months ($p = 0.041$ and $p = 0.005$, respectively) (Figure 1). Serum AMH levels were also significantly decreased under treatment with an OC containing $30 \mu g$ ethinylestradiol plus $3 \mu g$ drospirenone (Group B) compared to treatment with metformin $850 \mu g \times 2$ (Group C) both at 3 and at 6 months ($p = 0.032$ and $p = 0.005$, respectively) (Figure 1).

Concerning serum AMH levels at baseline and their change under the influence of different treatment regimens the following correlations were detected: at baseline, in Group A, serum AMH correlated only with serum 17-OH-progesterone (Spearman’s $r = 0.560$, $p = 0.030$) and the observed change of serum AMH levels after 6 months did not correlate with the observed change of serum 17-OH-Progesterone (Spearman’s $r = 0.493$, $p = 0.062$). In Group B, serum AMH levels at baseline did not correlate significantly with any parameter. In Group C, serum
AMH levels correlated at baseline with serum testosterone (Spearman’s $r = 0.533$, $p = 0.041$), fasting glucose (Spearman’s $r = 0.587$, $p = 0.021$), HOMA-IR (Spearman’s $r = 0.568$, $p = 0.027$), and QUICKI (Spearman’s $r = -0.568$, $p = 0.027$). After 6 months of treatment with metformin, the observed change in serum AMH levels correlated only with the observed change in fasting insulin (Spearman’s $r = 0.762$, $p = 0.001$).

Finally, after 6 months of treatment, serum AMH levels were positively correlated to testosterone ($r = 0.021$), 17-OH-progesterone ($r = 0.032$) and FAI ($r = 0.046$) in the group of women with PCOS under 35 $\mu g$ ethinylestradiol plus 2 mg cyproterone acetate (Group A).

Discussion

AMH is produced by the granulosa cells of early developing follicles [17] and reflects the continuous, non-cycling growth of small follicles in the ovary [18]. AMH has been found to be increased in the serum of women with PCOS [6–10]. AMH levels are not influenced by hormonal fluctuations and remain constant throughout the menstrual cycle, making it a promising diagnostic marker for patients with PCOS [19]. Women with PCOS are usually treated with an OC, while obese patients with PCOS, especially those with IR might benefit from treatment with metformin.

Several studies have suggested that the use of OCs aggravates IR and worsens glucose tolerance in women with PCOS. On the contrary, metformin improves insulin sensitivity and, in addition, may decrease circulating androgen levels and may improve menstrual cyclicity, thus addressing the traditional goals of long-term treatment [20].

The aim of the present study was to assess the impact of metformin and of two different regimens of OCs containing different progestins, namely cyproterone acetate and drospirenone, on serum AMH levels in women with PCOS.

The data of the present study clearly demonstrate a significant decrease of serum AMH levels under treatment with an OC containing 35 $\mu g$ ethinylestradiol plus 2 mg cyproterone acetate, while no statistically significant change was noted under treatment with an OC containing 30 $\mu g$ ethinylestradiol plus 3 mg drospirenone or under treatment with metformin 850 $mg \times 2$. Our data are clearly contradictory to the data previously presented by Somunkiran et al. [14], who reported no change in serum AMH levels after 6 months of treatment with 35 $\mu g$ ethinylestradiol plus 2 mg cyproterone acetate. This discrepancy might be attributed to the different selection of patients with PCOS between the two studies. Somunkiran et al. recruited their PCOS patients according to the criteria of the Rotterdam PCOS [21] consensus workshop group, namely the presence of two of the following three criteria: oligomenorrhea or amenorrhea, clinical or biochemical signs of hyperandrogenism, and ultrasonographic polycystic ovarian morphology [14], while patients with PCOS in our study were recruited to fulfil (and) the stricter criteria proposed in 1990 by the National Institute of Child Health and Human Development Conference on PCOS [16]. As all women with PCOS included in this study had also ultrasonographic polycystic ovarian morphology they belonged to the 1A category according to the Rotterdam criteria which is the most severe form of the syndrome. As a consequence, all patients in the present study presented hyperandrogenemia, while in the study of Somunkiran et al. only 56.6% had clinical or biochemical signs of hyperandrogenism. Nevertheless, the decrease in serum testosterone levels achieved was not as significant as in the present study ($p = 0.05$ versus $p = 0.003$), although in both studies the same regimen was used. The decrease in serum AMH levels in this study was mostly attributed to the additional influence of cyproterone acetate, the antiandrogenic progestin component of the OC used. Indeed, with the use of cyproterone acetate serum androgen levels were significantly reduced compared to treatment with both an OC containing drospirenone and metformin. The decrease in serum androgen levels with the use of an OC containing drospirenone, although significant, was not at the magnitude achieved with the use of cyproterone acetate and only concerned testosterone and SHBG and not androstenedione and 17-OH-progesterone. Nevertheless, in the present study, serum AMH levels after 6 months of treatment with 35 $\mu g$ ethinylestradiol plus 2 mg cyproterone acetate were significantly related to serum testosterone and 17-OH-Progesterone levels, as well as to FAI.

In a previous study, we have shown that AMH levels were higher in anovulatory and hyperandrogenemic women with NIH-defined ‘classical’ PCOS, compared to both ovulatory women with PCOS morphology on ultrasound and hyperandrogenemia and to anovulatory women with PCOS morphology on ultrasound but normal androgen levels.

Figure 1. Serum AMH levels at baseline and after 3 and 6 months treatment with 35 $\mu g$ ethinylestradiol + 2 mg cyproterone acetate, with 30 $\mu g$ ethinylestradiol + 3 mg drospirenone and with metformin 850 $mg \times 2$. Values are mean ± SD.
levels [7]. In this study, the strong positive association between LH and AMH levels, the significantly higher LH concentrations in women with ‘severe’ PCOS along with the highest levels of serum AMH, could not possibly be accounted for by any other PCOS-associated hormonal or metabolic defect other than hyperandrogenemia and chronic anovulation [7]. Therefore, the findings of the present study add additional evidence that AMH levels reflect the severity of PCOS, traditionally defined by its two cardinal elements, i.e. oligo-anovulation and hyperandrogenemia [16]. Somunkiran et al. [14], presented data concerning women with PCOS from whom 80% presented menstrual dysfunction, while our cohort of women with PCOS were all anovulatory raising the possibility that the discrepancy in the results recorded could be partly attributed, besides the differences in hyperandrogenemia and to differences in menstrual function.

Ovulatory disorders in women with PCOS are caused by an increased early follicular growth, resulting in a larger reserve of follicles and/or a defective follicular selection, leading to follicular arrest [4]. Since intra-ovarian androgens are also responsible for defective follicular selection and follicular arrest, it has been proposed that the intra-ovarian hyperandrogenism by increasing the AMH intra-ovarian level could exert an inhibiting effect on the selection process [8]. The excess in AMH production by polycystic ovaries might be the result of the increased number of follicles 2–9 mm in diameter caused by the intra-ovarian excess of androgens [4], still it might not be the sole determinant of serum AMH, as we have previously shown that the total number of 2–9 mm-sized follicles, although an independent determinant, contributed only an additional 5.3% to the variance of AMH levels, as opposed to 18% by LH levels alone and an additional 9.5% by serum testosterone [7].

It should be noted that serum testosterone might not accurately reflect intra-ovarian androgen levels, still we might speculate that the observed correlation between serum AMH and androgens levels might be even more pronounced in the intra-ovarian level.

An additional effect of OC administration on serum AMH levels could be attributed to the suppression of pituitary gonadotropins. In particular, the administration of 35 μg ethinylestradiol plus 2 mg cyproterone acetate led to the most significant suppression of both LH and FSH, while the administration of metformin had no effect at all on pituitary gonadotropins. We have previously shown that increased serum LH levels was the most significant independent link between PCOS-associated disorders of ovulation and the observed increase in serum AMH as it has previously been demonstrated that the total number of 2–9 mm-sized follicles, although an independent determinant, contributed only an additional 5.3% to the variance of AMH levels, as opposed to 18% by LH levels alone and an additional 9.5% by serum testosterone [7].

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