Effects of Oral Contraceptives and Metformin on the Concentrations of Anti-Müllerian Hormone in Women with Polycystic Ovary Syndrome

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Abstract

Background: Whether treatment with oral contraceptives (OC) and metformin can reduce anti-Müllerian hormone (AMH) levels in PCOS remains controversial. This study aims to investigate the effects of OC and metformin on serum AMH concentrations in women with polycystic ovary syndrome (PCOS).

Methods: This prospective study included 120 women with PCOS. Patients were treated with OC (35 µg of ethinyl estradiol plus 2 mg of cyproterone acetate), metformin, or OC plus metformin for 3 months according to their different endocrine/metabolic disturbances. Forty-eight patients with hyperandrogenism (HA) were treated with OC, 32 patients with insulin resistance (IR) were treated with metformin, and 40 patients with HA and IR were treated with a combination of OC and metformin. Serum AMH levels were compared before and after treatment within each group.

Results: AMH levels decreased significantly in both OC groups (from 12.54 ± 5.59 ng/mL before treatment to 9.03 ± 4.49 ng/mL after treatment, $P < 0.01$) as well as in the OC + metformin group (from 10.62 ± 4.57 ng/mL to 7.74 ± 3.19 ng/mL, $P < 0.01$). However, AMH concentrations remained unchanged in the metformin group, although insulin sensitivity was improved.

Conclusion: Treatment with OC alone or OC plus metformin led to a significant reduction of serum AMH in PCOS patients with HA, while metformin treatment alone did not affect AMH levels in patients with IR.

Current Controlled Trials: ChiCTR2000038695

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Plain English Summary

This article discusses the effects of oral contraceptives(OC) and metformin on the concentrations of anti-Müllerian hormone(AMH) in women with polycystic ovary syndrome(PCOS). The level of AMH is 2–3 fold higher in PCOS women compared to healthy women. The increased concentrations of AMH in PCOS is associated with LH, total testosterone and insulin. OC suppress LH secretion and decrease androgen levels, whereas metformin improves insulin sensitivity. In this prospective study, women with PCOS were enrolled to be treated with OC, metformin, or OC plus metformin for 3 months according to their different endocrine /metabolic disturbances. Forty-eight patients with hyperandrogenism (HA) were treated with OC, 32 patients with insulin resistance (IR) were treated with metformin and 40 patients with HA and IR were treated with a combination of OC and metformin. Serum AMH levels were compared before and after treatment within each group. After three months of treatment, AMH decreased significantly in both OC groups, as well as in the OC + metformin group. However, AMH concentrations remained unchanged in the metformin group, although insulin sensitivity was improved. Our conclusion is that treatment with OC alone or OC plus metformin led to a significant reduction of serum AMH in PCOS patients with HA, while metformin treatment did not affect AMH levels in patients with IR.

Background

PCOS is a common endocrinopathy in women and a primary cause of anovulatory infertility. It is typically characterized by HA, elevated luteinizing hormone (LH) concentrations in blood, ovulatory dysfunction, and polycystic ovarian morphology, but may also include insulin resistance (IR) and obesity [1]. Anti-Müllerian hormone (AMH) is specifically synthesized in granulosa cells of the follicle [2]. Serum AMH is widely used as a biomarker; its use is primarily based upon its ability to assess the ovarian follicular pool [3]. The level of AMH is 2–3-fold higher in PCOS women compared with healthy women [4, 5]. According to the triangle theory by Dewailly [6], in patients with PCOS, primordial follicles grow excessively because excessive endogenous androgens make granulosa cells hypersensitive to FSH, resulting in an increase in the number of antral follicles and overexpression of AMH. AMH, in turn, inhibits FSH-induced aromatase activity, thus hindering the development of antral follicles. Although the cause of the overproduction of AMH in PCOS women remains unclear, it has been shown that androgens play a role in the excessive secretion of AMH [7]. In addition, LH significantly stimulates AMH production by granulosa cells in PCOS [4]. Many investigators have reported that the increased concentrations of AMH in PCOS is associated with LH, total testosterone and insulin [8–10]. Therefore, AMH reflects the severity of PCOS [8, 11]. Further, it has recently been suggested that AMH concentration values can be used as a diagnostic and prognostic marker in PCOS patients [12, 13].

The common drugs used to treat PCOS are oral contraceptives (OC) and insulin sensitizers. OC suppress LH secretion and decrease androgen levels [14], whereas metformin, an insulin sensitizer, improves insulin sensitivity and reduces circulating androgen levels in PCOS women [15]. Several studies have evaluated the influence of these therapies on the concentrations of AMH in PCOS, but whether treatment with OC and metformin can reduce AMH levels in PCOS remains controversial. A few studies have reported a significant decrease in AMH levels during therapy with OC and metformin [5, 16]; however, other studies have found no significant change in AMH levels after treatment [17–20].

In this study, we investigated the impact of OC and metformin on the concentration of AMH in PCOS patients.

Methods

Study participants

This study was conducted at Jiaying Maternity and Child Health Care Hospital. In total, 141 women with PCOS (19–35 years of age) with biochemical HA and/or IR were enrolled. We adopted the 2003 Rotterdam criteria for PCOS diagnosis [21]. Total testosterone concentrations higher than the 95th percentile CI of the control women (1.97 nmol/L) were considered as biochemical HA. IR was defined according to the homeostasis model assessment of IR (HOMA-IR). Patients with a HOMA-IR higher than 2.5 were considered to have IR [22]. HOMA-IR was calculated as follows: $\text{HOMA-IR} = (\text{fasting glucose [mmol/L]} \times \text{fasting insulin [mIU/L]}) / 22.5$. Exclusion criteria included hyperprolactinemia and thyroid

dysfunction, as well as other causes of HA, including Cushing syndrome, an androgen secreting tumor, and ovarian drilling or ovulation induction, prior to the study. Patients were also excluded if they wished to conceive. The ethics board of Jiaxing Maternity and Children Health Care Hospital approved this study (No. 2013-17), and all patients provided signed informed consent.

Study design

At baseline, all patients underwent height and weight measurements, laboratory tests, and ultrasonographic examinations on the second–fourth day of the menstrual cycle. After an overnight fast, blood samples were taken from all subjects between 8:00 a.m. and 9:00 a.m. The biochemical assessments included serum AMH, LH, FSH, testosterone, estradiol (E2), insulin, and plasma glucose.

All patients received OC, metformin, or OC plus metformin treatment for 3 months. Then, according to their different endocrine and metabolic disturbances, 141 PCOS patients were assigned to three groups, each receiving different treatments as follows.

The OC group included 56 patients with HA but no insulin resistance. Patients took an OC pill containing 35 µg ethinyl E2 and 2 mg cyproterone acetate (Diane-35®, Bayer Schering, Germany) for 21 days per cycle from the fifth day of menstruation onward.

The metformin group included 38 patients with IR but with normal levels of total testosterone. Patients were treated with 500 mg of metformin twice a day (Glucophage, Sino-American Shanghai Squibb, China) from the first day of menstruation and with 500 mg increments after 1 week. Progesterone was given regularly to induce uterine bleeding.

The OC + metformin group included 47 patients who exhibited not only HA but also IR. Patients were treated with a combination of OC (Diane-35) and metformin at the same doses as above.

After 3 months of therapy, clinical evaluations and hormonal and metabolic measurements were repeated on days 2–4 of the menstrual cycle. AMH levels were compared before and after treatment within each group. The flow chart of the study design is shown in Figure 1.

Assays

Plasma glucose levels were determined by an autoanalyzer (Roche Company, Germany) with the glucose oxidase method. Serum FSH, LH, E2, total testosterone, and insulin were quantified with chemiluminescence immunoassays on an Abbott Architect i2000 system (Abbott Diagnostic, Peachtree City, USA). Serum AMH concentrations were measured by an enzyme-linked immunosorbent assay (ELISA) kit (AMH ELISA; Ansh Labs, UK). The inter-assay and intra-assay coefficients of variation were 15% and 10%, respectively, for AMH.

Statistical analysis

Data were analyzed with SPSS version 18.0 software (SPSS Inc., Chicago, IL, USA). Variables are presented as mean $\pm$ SD. A paired-sample *t*-test or a Wilcoxon signed-ranks test (in case of non-normally distributed data) was used to compare differences between baseline and post-treatment within each group. $P < 0.05$ was considered statistically significant.

Results

Of 141 PCOS patients initially enrolled in the study, 21 patients (8 patients in the OC group, 6 in the metformin group, and 7 in the OC + metformin group) did not complete the study (Fig. 1). Changes in endocrine and metabolic parameters in the three groups following treatment are shown in Table 1. The effects of treatments on serum AMH concentrations in the three groups are summarized in Fig. 2.

A total of 48 patients with HA received OC treatment for 3 months, reducing serum AMH levels significantly from 12.54 ± 5.59 ng/mL to 9.03 ± 4.49 ng/mL ($P < 0.001$). Serum LH and testosterone concentrations also decreased significantly during treatment ($P < 0.001$). BMI and the fasting glucose and insulin levels were not significantly altered.

In the metformin group, the mean level of AMH was 10.33 ± 4.34 ng/mL at baseline and 10.13 ± 4.14 ng/mL after 3 months of treatment ($P = 0.144$). Thus, AMH levels did not change significantly with metformin treatment. However, BMI, plasma glucose, serum insulin, and HOMA-IR decreased significantly ($P < 0.05$). We uncovered no significant changes in any other endocrine parameter.

With respect to the 40 patients in the OC + metformin group, AMH levels were 10.62 ± 4.57 ng/mL at baseline, which decreased significantly to 7.74 ± 3.19 ng/mL after treatment ($P < 0.001$). Furthermore, BMI, HOMA-IR, and concentrations of LH, testosterone, fasting glucose, and fasting insulin were also significantly decreased ($P < 0.001$).

Discussion

PCOS is a complex and heterogeneous syndrome. Women with PCOS who have HA are often treated with OC, while those with obesity, especially with insulin resistance, may gain particular benefits from metformin therapy [11]. Treatment with OC and metformin can reduce androgen levels and improve insulin sensitivity; however, the influence of these treatments on serum AMH concentrations remains debated.

In the present study, we treated patients with OC and metformin according to their different endocrine and metabolic disturbances, following routine therapies used in clinical practice. It is difficult to conduct randomized controlled trials on the effects of different treatments on AMH in patients with PCOS, as PCOS women have heterogeneous manifestations. Thus far, there are few randomized controlled trials with large samples in this field. AMH values were compared before and after treatment within each group

in this study. Importantly, self-controlled trials avoid the influence of individual differences on outcomes. Our results are reliable and have clinical implications for PCOS patients regarding these treatments. Although HA is the major feature and diagnostic component of PCOS, the clinical signs of HA might vary with ethnic differences. The rate of HA is lower in Chinese women with PCOS than in Western women. We herein selected biochemical HA as the criterion for treatment of PCOS, regardless of the symptoms of HA.

Androgens increase the recruitment of follicles but inhibit the selection of the dominant follicle [23], and therefore women with PCOS exhibit excessive numbers of preantral and small antral follicles [24]. This excessive number of small antral follicles induced by androgens and the increase in AMH production per granulosa cell result in elevated serum AMH concentrations in PCOS patients [4, 23]. In addition, high levels of LH stimulate excessive secretion of androgen in polycystic ovaries. Bungum et al. [25] observed a significant positive co-variation between LH and AMH, and this phenomenon suggested that LH was a possible factor involved in the control of AMH secretion. Consequently, AMH was positively correlated with testosterone and LH levels as well as total numbers of follicles [8, 9], and serum AMH levels significantly increased with HA [8, 11, 26].

OC can inhibit LH release and reduce androgen levels. However, it remains debatable whether AMH levels decline during therapy with OC. Fábregues et al. [16] reported that AMH levels significantly decreased during OC treatment, explaining that the reduction in androgens and LH during OC therapy contributed to the significant decrease in serum AMH. Similar results were reported by Panidis et al. [11], who showed a significant diminution in serum AMH in women with PCOS after 6 months of treatment with OC (2 mg of cyproterone acetate plus 35 µg of ethinyl E2). In our study, AMH concentrations significantly decreased after 3 months of OC therapy, with the reduction in AMH being a consequence of the suppression by OC of serum LH and testosterone levels. Our data are therefore in accordance with previous studies [11, 16]. By contrast, Somunkiran et al. [18] did not find significant changes in AMH levels after 6 months of contraceptive treatment in women with PCOS. Our study is not consistent with that of Somunkiran et al. [18], as they did not observe a correlation between AMH levels and LH, FSH, or testosterone.

Birch et al. [27] reported that after adjusting for age, AMH was 19% lower in OC users compared with non-users. However, the inhibitory effect of OC on AMH may be reversed within 3–6 months [28]. Based on our data and the previously aforementioned studies [11, 16], elevated AMH levels decreased significantly during OC treatment, suggesting that AMH is a useful indicator to evaluate OC treatment efficacy in clinical practice. In the current study, BMI, serum insulin levels, and HOMA-IR were not negatively affected by OC use.

Concomitant metabolic disorders constitute another prominent feature of PCOS, including obesity, IR, and metabolic syndrome [21]. IR and hyperinsulinemia are known as important pathogenic factors for PCOS, although they are not included in the definition [1]. AMH levels were found to be positively correlated with insulin and HOMA-IR in several studies [9, 10], suggesting that insulin plays a role in AMH synthesis and secretion [23]. Investigators have reported controversial results regarding the influence of metformin on AMH levels in PCOS patients. Two studies showed that after treatment with metformin, AMH

concentrations were significantly reduced [5, 29]; however, in a study by Nascimento et al. [20], patients with IR received metformin for 8 weeks, and although serum insulin concentrations and androgen levels decreased, AMH levels did not change significantly. Nascimento et al. [20] speculated that the effect of metformin on AMH levels was associated with dosage and duration of treatment. Fleming et al. [30] found that the reduction in AMH levels due to metformin provided a delayed response because elevated serum AMH levels did not change until the second 4-month period. Two other reports showed that metformin treatment significantly decreased AMH levels in hyperinsulinemic patients [31, 32].

In summary, several studies have suggested that the reduction in AMH levels after metformin treatment is primarily observed in highly insulin-resistant patients with PCOS [31, 32], and that the influence on peripheral serum AMH concentrations is most likely related to the dose of metformin and the duration of treatment [20, 30]. In the present study, even though metformin treatment significantly reduced BMI, serum insulin concentrations, and HOMA-IR, AMH levels remained unaffected. Contrary to the research by Romualdi et al. [31]. and Grigoryan et al. [32], we found that metformin treatment failed to decrease circulating AMH levels in PCOS patients with IR. In the present study, although BMI, serum insulin levels, and HOMA-IR were decreased significantly after treatment, the values for these parameters were still abnormal. Serum AMH levels dropped from 10.33 ± 4.34 ng/mL to 10.13 ± 4.14 ng/mL, suggesting a decreasing tendency after metformin treatment. Perhaps a higher dose or a longer duration of metformin treatment is needed to observe clear effects on AMH levels. Thus, the influence of metformin treatment on circulating AMH concentrations in PCOS women requires further study, especially with a larger sample size, in order to reach definitive conclusions.

Combined OC and metformin treatment is superior to OC alone in improving IR and is more effective in decreasing androgen levels than metformin monotherapy [33]. In clinical practice, combined treatment is more effective for those patients with obesity or IR and HA or elevated serum LH levels. However, very few data are available on the impact of the combination of OC and metformin therapy on AMH levels in women with PCOS. A recent study revealed that AMH levels significantly decreased in a cohort of adolescents with PCOS after treatment with metformin plus OC [34]. In our study, combined treatment resulted in a significant decrease in BMI, fasting insulin levels, and HOMA-IR. Furthermore, serum LH and testosterone levels significantly declined. Because AMH levels were positively correlated with both LH and testosterone levels, and LH and testosterone levels were significantly reduced through the use of OC in the combination treatment, serum AMH levels also significantly decreased. In the combination treatment, metformin did not appear to induce an additional change in AMH levels; thus, our findings were similar to those of a previous study [34]. The combined treatment was shown to improve the endocrine disorder status and metabolic aspects and to decrease serum AMH levels in PCOS. We provide additional evidence to support previous reports that AMH concentrations reflect the severity of PCOS [8, 11]. However, the sample size of our study was small, and further studies are needed to confirm the effect of combined treatment on AMH.

In clinical practice, treatment of women who have PCOS with clomiphene (CC) or exogenous gonadotropins for ovulation induction carries a risk of multifollicular development, resulting in ovarian

hyperstimulation and multiple pregnancy [35, 36]. Moreover, cancelation of cycles may be required due to either a poor or an excessive ovarian response. If pretreatment with OC and metformin decreases serum AMH concentrations, and if the value of AMH is useful for estimating treatment efficiency, then AMH may facilitate the optimal determination of subsequent ovulation induction with CC or gonadotropins for women with PCOS [37]. Our findings therefore expand the clinical utility of AMH.

The limitations of our study include the small sample size and the short duration of treatment. Further studies with a larger sample size and longer treatment duration are needed to confirm the impacts of these treatments on serum AMH concentrations.

Conclusion

According to the endocrine / metabolic disorders in patients with PCOS, this study explored the effect of different treatment on serum AMH concentration. Treatment with OC alone or OC plus metformin led to a significant decrease in serum AMH levels in patients with HA, while treatment with metformin alone did not significantly affect serum AMH levels in patients with IR. The study suggested that AMH might be a useful indicator to evaluate OC treatment efficacy in clinical practice.

Abbreviations

PCOS: polycystic ovary syndrome; OC: oral contraceptives; AMH: anti-Müllerian hormone; HA: hyperandrogenism; IR: insulin resistance; LH: elevated luteinizing hormone; HOMA-IR: homeostasis model assessment of insulin resistance; E2: estradiol

Declarations

Acknowledgments

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Authors' contributions

LPW, KH and CXF designed the study. CXF supervised data collection and conducted data analysis. WPF, WWC and HXZ were involved in the data collection. KH and CXF contributed to the development of the full manuscript. All authors read and approved the final manuscript.

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Availability of data and materials

The datasets analysed during the current study are available from the corresponding author on reasonable request.

Ethics approval and consent to participate

The ethics board of Jiaxing Maternity and Child Health Care Hospital approved the study. All the procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki Declaration and its later amendments or comparable ethical standard.

Consent for publication

Informed consent was obtained from all individual participants included in the study.

Competing interests

The authors have no conflicts of interest to disclose.

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Table

Due to technical limitations, table 1 is only available as a download in the Supplemental Files section.

Figures

Figure 1

Flow chart of the study. Data are presented as number of participants. OC, oral contraceptives; HA, hyperandrogenism; AMH, anti-Müllerian hormone.

Figure 2

Comparison of serum AMH concentrations before and after 3 months of treatment within each group. (a) OC group. (b) Metformin group. (c) OC + metformin group. Values are shown as mean $\pm$ SD. OC, oral contraceptives; AMH, anti-Müllerian hormone.

Supplementary Files

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