

Metformin Therapy Decreases Hyperandrogenism and Ovarian Volume in Women with Polycystic Ovary Syndrome

Marzieh Farimani Sanoee¹,
Nosrat Neghab¹, Soghra Rabiee¹,
Iraj Amir²

¹Department of Obstetrics and
Gynecology,
Fatemeh Hospital,

²The Center for Molecular Medicine
Research,
Hamedan University of Medical Sciences,
Hamedan, Iran.

Correspondence:

Marzieh Farimani Sanoee MD,
Department of Obstetrics and
Gynecology,
Hamedan University of Medical Sciences,
Hamedan, Iran.

Tel: +98 918 1111240

Fax: +98 811 8283939

Email: dr_farimani@yahoo.com

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Abstract

Background: It is well known that there is a close relationship between elevated androgen plasma levels and the ultrasound findings of stromal hypertrophy in polycystic ovary syndrome (PCOS). The objective of this study was to investigate the effects metformin on the hyperandrogenism and ovarian volume in PCOS.

Methods: The study is an unrandomized clinical trial with before–after design. Twenty eight patients with infertility (male or female factor) meeting the Rotterdam ESHRE/ASRM criteria for PCOS were studied during the 2008-2009. The anthropometric characteristics of the patients, mean bilateral ovarian volume, and morphology by trans vaginal sonography as well as the plasma levels of leutinizing hormone, follicle stimulating hormone, estradiol, testosterone, 17- α -hydroxyprogesterone, and dehydroepiandrosterone sulfate were obtained before and after treatment with metformin (500 mg three times a day) for three months. Paired t, Pearson's Correlation Coefficient, or Partial Correlation test was used to analyze the findings.

Results: The patients had a mean age of 25.67 years. A significant reduction in mean ovarian volume (11.70 ± 4.31 ml vs 8.27 ± 3.71 ml $P=0.001$), body mass index (BMI, 28.11 ± 4.55 kg/m² vs 26.84 ± 4.55 kg/m² $P=0.000$) and serum androgen levels was seen after three months of treatment with metformin. There was positive correlations between the ovarian volume and serum testosterone level ($r=0.589$, $P=0.001$) or BMI ($r=0.663$, $P=0.000$).

Conclusion: Metformin therapy may lead to a reduction in ovarian volume. It is likely that the reduction of ovarian volume reflect a decrease in the mass of androgen producing tissues.

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Keywords • Polycystic ovarian syndrome • metformin • ovarian volume • hyperandrogenism

Introduction

Polycystic ovary syndrome (PCOS) is a common endocrine-metabolic disorder occurring in 5% to 10% of women of reproductive ages.¹ Its clinical manifestations may include menstrual irregularities, signs of androgen excess, obesity and polycystic ovary (PCO) morphology. It is now recognized that women

with regular cycles and hyperandrogenism and/or polycystic ovaries may have the syndrome. It has also been recognized that some women with the syndrome will have PCO without clinical evidence of androgen excess, and will display evidence of ovarian dysfunction. Polycystic ovarian syndrome is a consequence of the loss of ovulation and achievement of the steady state of persistent anovulation.^{2,3}

Although the pathogenesis of the syndrome is still unclear, several authors have suggested that insulin resistance, hyperinsulinemia, and obesity, which affect most PCOS patients, may play a main role. Indeed the increased circulating concentration of insulin seems to contribute to the etiology of hyperandrogenism by acting at several levels of the hypothalamic-pituitary-ovarian axis as well as on the hepatic production of sex hormone-binding globulin (SHBG). At ovarian level, insulin promotes androgen secretion by playing a synergistic role with gonadotropins both directly and by stimulating insulin-like growth factor I (IGF-I) secretion. Moreover, in the liver it decreases serum levels of SHBG.^{1,2,4} In recent years the ultrasound evaluation of PCOS ovaries has received a great deal of attention, focusing on improving its diagnosis.¹ The characteristics of PCO include doubling surface area, an average volume increase of 2.8 times, presence of the same number of primordial follicles, doubling the number of growing and atretic follicles, 50% increase in the thickness of tunica (outermost layer), one-third increase in the cortical stromal thickness due to hyperplasia of theca cells, excessive follicular maturation and atresia, and quadruple increase in ovarian hilus cell nest.⁴

It is well-known that there is a close relationship between the increase in plasma androgen levels and the ultrasound findings of stromal hypertrophy.¹ Insulin-lowering agents, such as metformin, have been shown to improve insulin sensitivity, hyperandrogenism, menstrual pattern and ovulatory function in obese and nonobese women with PCOS.⁵⁻¹³ In the present study, we investigated the possible effects of metformin administration in women with PCOS on the ovarian volume and hyperandrogenism, and the examined likely correlation between the two variables.

Materials and Methods

Ethical Approval and Experimental Design

The study was approved by the Ethics Committee of the Hamedan University of Medical Sciences, Hamedan, Iran, and informed written consent was obtained from each subject. This study is a non randomized clinical trial with before-after

design. Twenty eight patients with infertility (male or female factor) with the criteria of PCOS referring to Infertility Clinic of Fatemie Hospital, Hamedan, Iran during 2008-2009 were studied.

Inclusion Criteria

The patients were selected using Rotterdam ESHRE/ASRM criteria. Pretreatment inclusion criteria were normal prolactin concentration as well as thyroid, renal and hematological indices. None of the participants had received metformin within three months prior to the study.

Exclusion Criteria

Exclusion criteria included concurrent hormone therapy within the previous six weeks, any chronic disease that could interfere with the absorption, distribution, metabolism or excretion of metformin as well as renal or liver diseases. Moreover, patients with significant systemic disease were also excluded. Also, subjects, who were smoking, taking sex hormones or drugs known to affect insulin secretion or clomiphene citrate, those with intense physical activities, and those who had lost three kg of body weight in the previous two months were excluded.

Data Collection

Weight, height and waist and hip circumferences of the participants were measured. Because of the impact of body fat distribution on androgen levels and glucose metabolism, waist-to-hip ratios (WHR) were also measured. Waist circumference was determined as the minimum value between the iliac crest and the lateral costal margin, whereas hip circumference was determined as the maximum value over the buttocks. Cut-off point for high WHR for women was set at 0.80. Body weight was measured using analogue scales in light clothing and height was measured barefoot using stadiometre. Body mass index (BMI, kg/m²) was calculated to assess obesity, and waist to hip ratio was used to assess body fat distribution. Obesity was defined as a BMI of ≥ 30 and overweight as a BMI more than 25 but less than 29.9. Ovarian morphology was assessed in all subjects by the same operator using a 6.5-MHz endovaginal probe. The ultrasound examination was performed on the same day that the blood samples were obtained.

The calculation of ovarian volume was performed for each ovary using the simplified formula for a prolate ellipsoid as follows:

$$\text{Ovary volume} = (\pi/6 \times (D1 \times D2 \times D3))$$

where D1, D2 and D3 were the maximum diameter in the transverse, anteroposterior, and longitudinal axes, respectively.¹ The mean

ovarian volume for each patient was calculated as the sum of their volume divided by two. No patient showed a dominant follicle (over 12-mm mean diameter) or a cyst (over 30-mm mean diameter) in his ovaries.

Non-amenorrhoeic women were studied during the early follicular phase of their menstrual cycle, and amenorrhoeic women were studied after progesterone withdrawal. Physical examination of each patient was performed under the supervision of a physician.

Biochemical Assays

Venous blood samples were collected from all patients after 12 hours overnight fasting. They were centrifuged immediately, and their serums were separated and stored at -20 °C until assayed for total testosterone, estradiol, 17- α -hydroxy progesterone (17 OHP), Luteinizing hormone (LH), follicle stimulation hormone (FSH) and dehydroepiandrosterone sulphate (DHEAS).

All patients were given 1500 mg metformin per day (500 mg three times a day) for three months. All women were urged to maintain the same diet as before the treatment. The patients were examined monthly, and no severe side effects were reported during the study. After three months of treatment, they were re-evaluated clinically, biochemically and hormonally. All measurements including total testosterone (ng/dl), 17OHP (ng/ml), Estradiol (pg/ml), LH (mlu/ml), FSH (mlu/ml) and DHEAS (μ g/ml) were performed using the ChemWell® Analyzer, unless otherwise stated.

Statistics

The findings at before and after the treatment were compared using paired t test. Correlation between mean ovarian volume and androgen levels or BMI was examined using Pearson's Correlation Coefficient. Partial Correlation was used to eliminate age bias. The analysis was performed using Statistical Package for Social Sciences (SPSS, version 16.0). A p value of <0.05 was considered statistically significant.

Results

Twenty eight patients with PCOS with an age range of 19 to 37 years and a mean of 25.67 years were recruited in this study. No serious

metformin-related adverse event was observed. None of the recruited women suffered from hypertension. Mean systolic and diastolic blood pressures remained stable during the treatment period.

Twenty one (75%) of the patients had hirsutism and half of them had acne, seven patients (25%) had regular menstruation, 18 patients (64.3%) had oligomenorrhea and three patients (10.7%) had amenorrhea. After treatment, 17 patients (65.38%) had regular menstrual cycles. Two patients became pregnant during the treatment, and were exclude from the study.

Twenty one patients (75%) had sonographic characteristics of polycystic ovary. Seventeen women (60.71%) had mean ovarian volume greater than 10 ml. The mean ovarian volume was 11.70 ± 4.31 ml (mean \pm SD) before treatment. After three months of treatment the mean ovarian volume declined to 8.27 ± 3.71 ml, representing a decrease of $29.31 \pm 13.92\%$ ($P=0.001$).

Seven patients (25%) were obese, 15 patients (53.57%) were overweight, and the BMIs of six women (21.42%) were in the normal range. After treatment, there were positive correlations between mean ovarian volume and serum levels of testosterone ($r=0.589$, $P=0.001$) or BMI ($r=0.663$, $P=0.000$).

Anthropometric characteristics of patients with PCOS before and after treatment are listed in table 1. There were significant differences between weight or BMI before and after the treatment. They decreased by $3.82 \pm 2.35\%$ and 4.51% , respectively. However, there was no significant difference between waist to hip ratio or blood pressure before and after the treatment. Serum levels of FSH increased significantly after the treatment, but those of LH, DHEAS, 17-OHP, estradiol and testosterone decreased significantly.

Twenty one PCOS women (75%) had serum testosterone levels more than 95 percentile. Main hormonal and androgenic profile before and after metformin treatment in patients with PCOS are listed in table 2.

To eliminate age bias, we used partial correlation between mean ovarian volume and anthropometric or androgenic profiles before and after treatment with metformin. There was a significant positive correlation between the mean ovarian volume and weight, BMI or serum testosterone levels before and after treatment with metformin. The results are shown in table 3.

Table 1: The characteristics (mean \pm SD) of patients with polycystic ovary syndrome before and after treatment with metformin.

	Before treatment	After treatment	P value
Weight (kg)	70.26 \pm 13.60	67.57 \pm 13.28	0.000
Waist to hip ratio	0.80 \pm 0.02	0.79 \pm 0.03	0.137
Blood pressure (mmHg)	88.73 \pm 9.26	86.19 \pm 5.77	0.176
BMI (kg/m ²)	28.11 \pm 4.55	26.84 \pm 4.55	0.000

Table 2: Main hormonal and metabolic profile (mean±SD) of patients with polycystic ovary syndrome before and after treatment with metformin

	Before treatment	After treatment	Percent of change	P value
LH (mIU/ml)	10.46 ± 1.11	7.25 ± 1.01	-30.68 ± 9%	0.000
FSH (mIU/ml)	4.26 ± 0.70	5.67 ± 0.79	24.86 ± 11.39%	0.000
Estradiol (pg/ml)	60.92 ± 7.68	54.69 ± 7.25	-10.22 ± 5.59%	0.000
Testosterone (ng/ml)	1.33 ± 0.33	1.26 ± 0.32	-5.26 ± 3.03%	0.000
DHEAS (µg/ml)	2.97 ± 0.43	2.87 ± 0.31	-3.36 ± 27.90%	0.027
17OHP (ng/ml)	0.57 ± 0.23	0.44 ± 0.18	-22.80 ± 21.73%	0.006

LH; leutinizing hormone, FSH; follicle stimulation hormone, DHEAS; dehydroepiandrosterone, 17OHP; 17-hydroxyprogesterone

Table 3: Partial correlation between the mean ovarian volume and anthropometric or androgenic profile in patients with polycystic ovary syndrome before and after treatment with metformin

	Before treatment		After treatment	
	Before treatment	P value	After treatment	P value
Weight (kg)	0.234	0.00	0.345	0.001
Waist to hip ratio	0.145	0.134	0.156	0.127
BMI (kg/m ²)	0.532	0.001	0.234	0.002
LH (mIU/ml)	0.034	0.121	0.012	0.132
FSH (mIU/ml)	0.041	0.061	0.011	0.081
Estradiol (pg/ml)	0.032	0.069	0.023	0.066
Testosterone (ng/ml)	0.424	0.002	0.456	0.003
DHEAS (µg/ml)	0.021	0.052	0.023	0.145
17OHP (ng/ml)	0.031	0.123	0.032	0.127

BMI; body mass index, LH; leutinizing hormone, FSH; follicle stimulation hormone, DHEAS; dehydroepiandrosterone, 17OHP; 17-hydroxyprogesterone

Discussion

Pelvic ultrasound scans have assumed an increasing importance in the diagnosis and management of ovulatory disorders. Assessment of ovarian morphology by the use of ultrasound has become a substitute for histologic examination in diagnosing PCOS.¹⁴ Although, increased stromal volume is a feature of PCO, it has been shown that the measurement of the ovarian volume is a good surrogate for the quantification of stromal volume in clinical practice. This definition does not apply to women taking oral contraceptive pills, since their use modify ovarian morphology in normal women as well as in women with PCOS. Polycystic ovary may sometimes be absent in women who presents other classic clinical characteristics of PCOS. Hyperinsulinemia stimulates the development of antral follicles, increasing the sensitivity of granulosa cells to FSH, thus increasing the numbers of follicles as well as ovarian volume.^{2,3} Although Morin-Papunen et al reported that mean volumes of the right and left ovary did not change after two and four to six months of metformin therapy, respectively,⁹ the mean ovarian volume significantly decreased after three months of metformin administration. Similarly, Bayrak et al,¹⁰ showed that one week of metformine therapy (850 mg/day) was associated with a significant improvement in PCO morphology. Although a significant reduction in mean ovarian volume was observed after three months of metformin therapy in the present study. The sample size of the present study was small, however, and larger scale studies are needed

to confirm the findings.

It has been demonstrated that metformin directly inhibited the androgen production in human ovarian theca-like androgen producing tumor cells in culture.¹⁵ The results of the present study, which is also in agreement with those of Genazzani et al,¹¹ Zeyneloglu et al,¹² and Banaszewska et al,¹⁶ may be taken as an evidence for the efficacy of metformin in modulating the ovarian activity. Therefore, it may be possible to conclude that metformin therapy, even in a relatively short time such as three months, in patients with PCOS may cause a decrease in the ovarian volume by decreasing intraovarian stromal androgens.

The ovarian volume correlated to BMI, thus suggesting a possible relationship between ultrasound findings and anthropometric characteristics. Furthermore, our finding that the prevalence of obesity and high androgen levels within the patients with larger ovarian volume is higher than that seen within the patients with PCOS with normal ovarian volume seems to confirm the possibility of an interaction between ovarian morphology and anthropometric characteristics. It could be hypothesized that the patients with PCOS are much more insulin resistant. This would explain the higher BMI and androgen values.

Hyperandrogenism is a key feature of PCOS. Although the adrenal gland may contribute, hyperandrogenism is principally ovarian in origin among women with a primary diagnosis of PCOS. In various populations around the world, it has been found that most women with

PCOS have elevated levels of serum androgens; however, normal levels may be found in some women. Serum testosterone level is the best marker for ovarian hyperandrogenism, and DHEAS is the best adrenal marker. It is recommended that these variables be measured.^{2,3} In our study, most of the PCOS cases had testosterone level more than 95 percentile. After treatment, there was a significant reduction in serum testosterone concentrations, 17OHP and DHEAS similar to those reported by Nestler et al,¹⁰ Kolodziejczyk et al,¹³ and Bayrak et al,¹⁷ studies. There was an important change in the menstrual pattern during metformin therapy. In agreement with the study by Morin-Papunen et al,⁹ up to 65% of the women with menstrual disturbances achieved more regular menstruation with metformin and two patients became pregnant. In our study, patients with PCOS had both male and female factors of infertility, and such a low rate of pregnancy may be due to these two factors. Unfortunately, we could not assess how many of the cycles were ovulatory during therapy. However, spontaneous menstruation is psychologically important for the patient because it implies better ovarian function. In addition, the more frequent occurrence of menses in these patients may alleviate the known risks of endometrial hyperplasia and carcinoma in obese patients with PCOS.

Conclusion

The findings of the present study indicate that metformin therapy leads to comparable reduction of ovarian volume in a manner, which correlates with the degree of reduction of hyperandrogenemia. It is likely that the reduction of ovarian volume reflect a decrease in the mass of androgen producing tissues. Larger-scale studies are needed not only to confirm our findings, but also to define the lowest dose of metformin that could achieve biochemically and clinically significant outcomes.

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Conflict of Interest: None declared

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