Effects of Metformin on Menstrual Cyclicity in Women with Polycystic Ovary Syndrome

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OBJECTIVE: To evaluate effects of metformin on menstrual cyclicity in women with polycystic ovary syndrome.
STUDY DESIGN: The study was designed as pre-, post prospective clinical trial. To enter the study, patients had to have well-documented PCOS, be oligo-(six cycles or less in the preceding year) or amenorrheic (absence of menstrual cycles for 1 year), and not have exclusionary diseases or drugs. Metformin 500 mg orally twice daily was administered for 6 months. Serum fasting insulin, glucose, FSH, LH, estradiol, progesterone, prolactin, free testosterone, DHEAS, total cholesterol, triglyceride, AST, ALT, HDL, LDL, VLDL were measured. Quantitative insulin sensitivity check index (QUICKI) was used to measure the insulin resistance. Blood samples were collected at the initiation of therapy and 6 months after. Follow-up 6 months was scheduled with interval history, review of menstrual status, assessment of any metformin-related side effects, brief physical and laboratory examination.
RESULTS: Total 13/23(39.1%) of the patients with metformin treatment resumed normal menses 6 months after. Nine of 19 oligomenoreic women (47.4%) resumed regular normal menses; 4 of 4 (100%) amenoreic women resumed oligomenoric menses. The fasting blood glucose, fasting blood insulin, free testesteron, VLDL levels were decreased after 6 months of treatment (p=0.005; p= 0.002; p= 0.008; p=0.006 respectively). First and sixth months measurements of QUICKI were found significantly different (p=0.000).
CONCLUSION: Metformin therapy is well tolerated by the majority of patients and may be clinically useful, in nonobese patients with PCOS and menstrual disturbances.
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Key Words: PCOS, Metformin, Insulin resistance, Menstrual cycles

Polycystic ovary syndrome is a heterogeneous disease effecting 4- 6% of women of reproductive age[1] It characterized by chronic anovulation, elevated androgen level, enlarged cystic ovaries, and obesity. Many PCOS patients also have some risk factors for development of diabetes mellitus, cardiovascular disease and endometrial cancer.[2,3,4] Insulin resistance is the one of the most important aspect of PCOS.[5,6] Insulin resistance leads to an increase of ovarian androgen production, exaggerates the evolution of the PCOS.[5] Both lean and obese women with PCOS display insulin resistance.[7]

Many treatments have been used to improve the clinical symptoms of PCOS, but most of them, such as oral contraceptives and sequential progestins, do not eliminate the basic problem, that is, insulin resistance. Metformin is a biguanide antihyperglycemic drug used to treat NIDDM. Metformin increases the intestinal use of glucose, enhances peripheral glucose uptake and inhibits hepatic glucose production. Additionally, it enhances insulin sensitivity at postreceptor levels and stimulates insulin-mediated glucose disposal.[8] Velazquez et al. suggested that most of the metabolic disturbances of PCOS can be reversed by metformin, with the additional advantage of allowing regular menstrual cycles, reversal of infertility and spontaneous pregnancy.[9] Recent studies have commonly focused on metformin to treat infertility[5,9] menstrual pattern[10-14] and as a chronic therapy to prevent long-term consequences of PCOS.[15]

The aim of our study was to assess in more detail the effects of long-term metformin therapy on menstral cyclicity in lean patients with PCOS.

Material and Methods

The study was designed as pre-, post prospective clinical trial. During the study period of 2002-2004, thirty nonobese patients with PCOS who attended the outpatient clinics of the Gynecology Unit of the Zonguldak Karaelmas University Hospital were recruited to the study. Seven of the women were lost during the follow-up period, 23 were completed the study. Study was approved by Local Ethics Committee. Informed consent was obtained from each patient.

To be eligible for this study, women must have had a history of[1] polycystic ovaries;[2] oligo-/amenorrhea (less than six

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menstrual periods in the last year);[3] clinical or laboratory evidence of hyperandrogenism;[4] no secondary causes of anovulation[5] and no present use of lipid-lowering drugs, antidiabetic medications, or hormonal contraception (in the last 3 months). Ultrasonographic criteria for polycystic ovaries were the presence of ≥8 subcapsular follicles of 3-8 mm diameter in one plane in one ovary and increased stroma.[16] Ultrasonographic measurements were performed using (LOGIQ 7 Scanner, GE Medical Systems, USA). The exclusion criteria included the presence of[1] Cushing's syndrome,[2] late-onset 21-hydroxylase deficiency,[3] thyroid dysfunction,[4] hyperprolactinemia,[5] androgen-secreting tumors,[6] diabetes mellitus,[7] evidence of chronic renal or hepatic disease and[8] Body mass index (BMI)>25 kg/m^2. Height, weight, was measured following a standardized protocol. Body mass index (BMI, weight [kg]/height^2[m^2]) was used as an estimate of overall adiposity.

Metformin 500 mg orally twice daily was administered for 6 months. After 12h fasting blood samples were collected at the initiation of therapy and 6 months after. Serum fasting insulin, glucose, FSH, LH, estradiol, progesterone, prolactin, free testosterone, DHEAS, total cholesterol, triglyceride, AST, ALT, HDL, LDL, VLDL were measured after at least 20 min rest. After measurement of parameters all blood samples were centrifuged and stored at -40° throughout the study. We used the quantitative insulin sensitivity check index (QUICKI=1/[log(fasting insulin{mIU/ml})+log (fasting glucose{mg/dl}]) to measure the insulin resistance.[17] All patients were instructed to remain on their usual diet during the study. To reduce diarrhea and/or nausea, which were occasionally experienced in the first week with metformin, all patients were instructed to start with 500 mg metformin with the evening meal for 2 days, and thereafter 500 mg two times a day with meals. After 6 months patients were evaluated with review of menstrual status, assessment of any metformin-related side effects, brief physical examination. All subjects were instructed to use barrier contraception if they were sexually active and to report immediately to us if they became pregnant.

Assays

Plasma glucose, total cholesterol, triglyceride, AST, ALT, HDL, LDL, VLDL levels was measured using the colorimetric technique on an autoanalyzer (Roche Cobas Integra 800, Mannheim, Germany). Insulin, progesterone, free testosterone, FSH, LH, E2, Prolactin, DHEAS were measured by electrochemiluminescence technique on an immunoanalyser (Roche Elecsys 2010, Mannheim, Germany).

Analytical sensitivities (AS), Intra- and inter-assay coefficients of variations (CV) of glucose, total cholesterol, triglyceride, AST, ALT, HDL, LDL, VLDL, insulin, free testosterone, LH, Prolactin, FSH, E2 and progesterone were as follows: Glucose (AS: 0.59 mg/dl, intra-assay CV: 1.22%, inter-assay CV: 1.30%), Total cholesterol (AS: 0.35 mg/dl, intra-assay CV: 3.25%, inter-assay CV: 3.53%), Triglyceride (AS: 3.50 mg/dl, intra-assay and inter-assay CV: 4.50%), AST (AS: 1.5 U/L, intra-assay CV: 2.57%, inter-assay CV: 2.19%), ALT (AS: 1.0 U/L, intra-assay CV: 1.99%, inter-assay CV: 2.11%), HDL (AS: 0.39 mg/dl, intra-assay CV: 3.90%, inter-assay CV: 2.89%), LDL cholesterol was calculated according to the Friedwald formula (LDL = Total cholesterol-HDL- Trig/5), Trig/5 is an estimate of VLDL concentration (VLDL=Trig/5), insulin (AS: 0.13 mIU/ml, intra-assay CV: 1.15%, inter-assay CV: 2.13%), Free Testosterone (AS: 0.01 ng/ml, intra-assay CV: 1.10%, inter-assay CV: 1.19%), DHEAS, (AS: 0.10 mg/dl, intra-assay CV: 2.29%, inter-assay CV: 2.71%), LH (AS: 0.10 mIU/ml, intra-assay CV: 1.60%, inter-assay CV: 2.21%), Prolactin (AS: 0.47 ng/ml, intra-assay CV: 1.80%, inter-assay CV: 4.11%), FSH (AS: 0.1 mIU/ml, intra-assay CV: 1.90%, inter-assay CV: 2.11%), E2 (AS: 15 pg/ml, intra-assay CV: 2.50%, inter-assay CV: 3.23%), Progesterone (AS: 0.1 ng/ml, intra-assay CV: 1.13%, inter-assay CV: 1.72%).

Statistical Analyses

All patients followed prospectively by recording variables and contact information were collected with a custom form and variables recorded in SPSS database for analysis (SPSS for Windows 11.5, SPSS Inc.). Variable pairs recorded before and after treatment were compared with paired-samples T test and Wilcoxon signed rank sum test for parametric and nonparametric data, respectively. In all calculations statistical significance was defined as $p<0.05$.

Results

Ages of patients were 23±5.9 (ranging from 18 to 37). Body mass index of the patients were 23.4±0.9 (ranging from 21.5 to 24.8). With metformin therapy, menstrual cyclicity was determined if women had three or more sequential regular menstrual cycles encompassing 21-38 days. While taking metformin, 9 of 19 oligomenoreic women (47.4%) resumed regular normal menses; 4 of 4 (100%) amenoreic women resumed oligomenoric menses. Total 13/23 (39.1%) of the patients resumed normal menses. After taking metformin, fasting blood glucose, fasting blood insulin, free testesteron, VLDL levels fell ($p=0.005$; $p=0.002$; $p=0.008$; $p=0.006$ respectively). After six months of metformin treatment, QUICKI increased from 0.313±0.016 to 0.337±0.018 ($p=0.000$). Pre- and posttreatment variables are summarized in Table 1.

No patients developed lactic acidosis, and there were no untoward changes in any of the laboratory tests performed to monitor safety. Nausea and/or diarrhea occurred in 10 % in the first 1-2 weeks after women started metformin, these symptoms subsequently resolved. No women got pregnant during the study period.

Table 1. Characteristics of women with PCOS before and 6 months after metformin treatment.

	Initial values	Sixth month values	P
FSH (mUI/L)	5.4 ±1.5 (2.9-8.9)	5.3 ±1.8 (1.4-10.0)	0.72
LH (mUI/L)	10.5 ± 4.6 (5.3-23.6)	9.2 ±3.6 (2.6-16.0)	0.12
Fasting blood glucose mg/dL	94.2±9.8 (77.0-118.0)	84.6±9.1(67-110)	0.005*
Fasting blood insulin µIU/mL	18.4 ± 9.8 (10-59)	11.9 ± 4.5 (5.3-24.0)	0.002*
Free testosteron (ng/mL)	4.9 ± 5.7 (2.1-27)	2.4 ± 0.8 (1.0-3.4)	0.08*
DHEAS (µg/dL)	281.6 ± 171.2 (85-690)	300.5 ± 142.1 (100-605)	0.70
Kolesterol (mg/dL)	183.3 ± 33.5 (126-255)	172.4 ± 36.4 (28.0-172.0)	0.29
Trigliserid (mg/dL)	109.0 ± 73,5 (30-360)	81.0 ± 36.4 (28-172)	0.17
VLDL (mg/dL)	38.6 ± 19.0 (6.0-68.0)	21.8 ± 15.9 (6-85)	0.006*
LDL (mg/dL)	115.4 ± 27.7 (62-164)	114.0 ± 31.0 (58-180)	0.926
QUICKI**	0.313 ± 0.016 (0.270-0.345)	0.337 ± 0.018 (0.302-0.377)	0.001*

Notes: Values were mean± standard deviation

*Statistically significant ($p<0.05$)

**OUICKI: Quantitative insulin sensitivity check index

Discussion

Metformin can be used to treat a number of features associated with PCOS, including hyperandrogenism, menstrual irregularities, insulin resistance, decreased ovulation rates, and infertility.[5,9,15] Several studies have examined the effect of metformin on menstrual cyclicity in women with PCOS and the improvement in menstrual cyclicity using metformin ranged from 25% to almost 96%, with a mean around 40%.[10,12,14] Spontaneous menstruation is psychologically important for the patient because it means better ovarian function. Also, regular menses in these patients may lessen the known risks of endometrial hyperplasia and carcinoma in patients with PCOS.[18] Velasquez et al reported for the first time that metformin caused clinical improvement such as blood pressure and menstrual cyclicity, in 7 PCOS patients.[6] In our study, 39.1% of the women with menstrual disturbances achieved more regular menstruation after 6 months of metformin treatment.

Insulin resistance and hyperinsulinemia may be central to many of the pathophysiologic aspects of PCOS.[5,6,12,14,19,20] It has been shown that insulin stimulate androgen production by thecal cells.[21,22] It appears that women with PCOS there are an intrinsic ovarian dysfunction associated with excessive androgen production. It also appears that hyperinsulinemia could play a function in exaggerating this intrinsic ovarian dysfunction rather than being a primary cause of PCOS. Additionally, insulin decreases SHBG production by the liver, and increases levels of free testosterone.[23] Hyperinsulinemia may also potentiate ACTH-stimulated adrenal androgen production.[24] Hyperinsulinism and resultant hyperandrogenism in PCOS chronically effect gonadotropin secretion, increasing LH,[5,12,14,19] disrupting the pituitary-ovarian axis, and causing to oligoamenorrhea and infertility. Hyperinsulinemia in combination with hyperandrogenemia also may lead to morbid obesity, hirsutism, acne, frequent hypertension, hyperlipidemia and also increasing risk for myocardial infarction and stroke later in life.[5,6,13,14,19,20]

Despite lack of the exact mechanisms of insulin in increased androgen production in the ovary, the use of metformin for the treatment of PCOS has become more frequent in clinical practice. The supposed mechanism of action of metformin in aiding return of normal menses in PCOS is that reduction of metformin in insulin resistance causes to a reduction in serum androgens, reduction of androgen-mediated inhibition of normal LH and FSH release, and consequent ovulation with more normal estradiol and progesterone production.[6,14,19] Consequently, metformin causes to lower androgen production both directly at the level of the ovary and indirectly through a reduction in insulin levels. Within a duration of two menstrual cycles, the results have included quick metabolic improvements[25,26] increases in spontaneous ovulation rates of between 30% and 40%.[5,27,28]

Weight loss may be an efficient to decrease insulin resistance, improve PCOS symptoms, restore menstrual cyclicity, and improve ovuation rates[5] For the 10 % to 30 % of women with PCOS who are nonobese, weight loss is not a treatment option.[29] Maciel et al. demonstrated that nonobese women with PCOS respond better than obese women to metformin treatment for 6 months.[30] Also they showed that, nonobese women showed a statistically significant decrease in serum androgens level, fasting insulin level, and an improvement in menstrual cyclicity. Our study also demonstrates that; nonobese PCOS women may benefit from metformin for menstrual irregularities.

Our findings showed that fasting blood glucose, fasting blood insulin, decreased after the metformin treatment. After 6 months of treatment, there was a significant decrease in the levels of fasting insulin and glucose as reported previously in some,[6,17,19] but not in all studies.[31,32] We used QUICKI to measure the insulin resistance.[17] It is an index of

insulin sensitivity and obtained from a fasting blood sample. It is reported that results of QUICKI were totally independent obese and nonobese subjects.[17] In our study insulin sensitivity increased significantly after the 6 months of metformin treatment. With these apparently durable clinical and laboratory effects, the serum testosterone level also decreased after 6 months of treatment. But in some studies, the serum testosterone level returned close to the starting value after 6 months of treatment, after being transiently decreased at 2 months of therapy.[12,33] Hence they concluded that, the metformin effect may be to some extent transitional and some adaptation may occur during more prolonged therapy.

Reducing hyperinsulinemia with metformin treatment may have long-term health benefits for lipid disorders, coronary artery disease, and hypertension.[20] Metformin therapy was associated with significant reductions in total plasma cholesterol after weight loss in teenagers with PCOS.[34] Some studies reported that abnormal lipid values in some patients normalized during the treatment.[6,13] This finding is in contrast to an earlier report of diabetic and nondiabetic men, in whom long-term therapy with metformin resulted in a moderate lessening in plasma triglyceride and total cholesterol levels and in a small increase in plasma HDL concentrations.[35] Metformin treatment had little effect on blood lipids in this study. Only serum VLDL levels decreased with metformin treatment.

Our results support the fact that, metformin therapy is well tolerated by the majority of nonobese PCOS patients. Metformin may be a therapeutic option for PCOS patients based on our data showing improvement in laboratory and clinical parameters. The most important changes were seen in the menstrual pattern and insulin resistance during metformin therapy. Up to 39.1% of the women with menstrual disturbances achieved more regular menstruation with metformin. But the absence of a control group receiving a placebo is the limitation of our study. Also we must keep in mind the potentially side effects (i.e., nausea, diarrhea, and bloating) and the cost of the medication.

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