The effects of metformin on insulin resistance, clomiphene-induced ovulation and pregnancy rates in women with polycystic ovary syndrome

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Abstract

Objective(s): To evaluate the effects of metformin on insulin resistance, ovarian androgen production, and clomiphene-induced ovulation and pregnancy rates in infertile women with polycystic ovary syndrome (PCOS).

Study design: Twenty-one infertile women with PCOS were selected in this prospective randomized clinical study. Basal steroid and gonadotropin levels were measured, and oral glucose tolerance test (OGTT) was performed. The patients were divided randomly into group 1 (n=11) and group 2 (n=10). Group 1 patients were treated with 1700 mg per day of metformin for 3 months. The basal tests and OGTT were repeated after metformin therapy. Group 2 patients did not receive metformin. The patients in both groups received 100 mg of clomiphene citrate (CC) daily for 5 days until either a pregnancy occurred, or six CC cycles were reached. Metformin administration continued during CC therapy until the day of hCG in group 1. Serum progesterone (P) level ≥5 ng/ml was considered as confirmatory of ovulation. Ovulation and pregnancy rates after six cycles were determined.

Results: Serum androgens and insulin response to OGTT decreased significantly after metformin therapy. Midluteal serum P level was significantly higher in cycles treated with metformin plus CC (P < 0.05). The ovulation (38 of 51 cycles, 74.4% versus 34 of 55 cycles, 61.8%) and pregnancy rates (5 of 11 women, 45.5% versus 3 of 10 women, 30%) were higher, but not significantly, in the metformin plus CC group than in the CC alone group. All the patients who conceived had insulin resistance in group 1 whereas non-insulin resistance in group 2.

Conclusion(s): Metformin improves insulin resistance and reduces androgen levels. Metformin did not increase significantly the ovulation and pregnancy rates.

Keywords: Polycystic ovary syndrome; Metformin; Insulin resistance; Clomiphene; Ovulation; Pregnancy

1. Introduction

Polycystic ovary syndrome (PCOS) is a heterogeneous disorder characterized by elevated androgen levels, signs of hyperandrogenism, obesity and chronic anovulation [1,2]. PCOS affects approximately 5–10% of all reproductive age women and is a major cause of infertility [1]. Insulin resistance and compensatory hyperinsulinemia are common findings in women with PCOS [3,4]. Insulin plays an important role in the regulation of ovarian function and the pathogenesis of PCOS. Hyperinsulinism has been shown to contribute to ovarian over-production of androgens and it appears to adversely affect ovulation after both clomiphene and pulsatile GnRH administration [5,6].

The anti-estrogen clomiphene citrate (CC) is widely accepted as a first line drug for ovulation induction in PCOS. For women who do not become pregnant with clomiphene treatment gonadotropins, ovarian surgery or IVF is commonly recommended. But these approaches are expensive and associated with potentially serious side effects [7].

Several clinical studies have demonstrated that agents ameliorate insulin resistance and reduce circulating insulin levels, such as metformin and troglitazone result in a reduction of hyperandrogenism in PCOS women [8–12]. Metformin which is a biguanide antyhyperglycemic drug, has been extensively used in non-insulin-dependent diabetes. It lowers blood glucose mainly by inhibiting hepatic glucose production and by increasing peripheral glucose uptake. Therefore, metformin can reduce peripheral insulin concentrations and improve glucose tolerance and metabolism [13]. Hyperinsulinemia may contribute to hyperandrogenism and infertility in women with PCOS. For this reason, insulin-
sensitizing agents, including metformin, may provide a new therapeutic modality in women with PCOS and infertility. Recent several studies suggest that metformin can reverse regular menstrual cycles and increase the number of ovulatory cycles in women with PCOS [14].

The number of studies that evaluate the effect of metformin on insulin resistance, ovulation and pregnancy rates among infertile women with PCOS are limited and have the short term treatment period. Also, their results are conflicting. While metformin improved the fasting insulin or the insulin response to an oral glucose challenge in some studies [9,12,14–16], it did not alter the baseline or oral glucose challenged levels of insulin in women with PCOS in some other studies [17–21]. The aim of the present study was to evaluate the effect of metformin on insulin resistance, ovarian androgen production, and clomiphene-induced ovulation and pregnancy rates in infertile women with PCOS for a relatively long treatment period.

2. Patients and methods

Twenty-one infertile patients with PCOS were included in the study. The study was approved by the Ethical Committee of Erciyes University Medical School and informed consent was obtained from each woman. All patients had primary infertility. Male factor and tubal-uterine factor infertility were excluded with semen analyses and hysterosalpingogram and/or laparoscopy.

The diagnosis of PCOS was made on the basis of three or more of the following criteria: polycystic ovaries on pelvic ultrasound examination, oligo/amenorrhea, hirsutism, hyperandrogenemia (total testosterone > 80 ng/dl and/or free testosterone > 3.18 pg/ml) and elevated serum LH:FSH ratio (LH:FSH > 2). Pelvic ultrasound examinations were performed by the same investigator (Y. Ş) using a 6.5 MHz vaginal endoprobe (Hitachi, EMB 450, Japan) in all women. The ultrasound diagnosis of polycystic ovary was made by the presence of 10 or more cysts, 2–10 mm in diameter, arranged around a dense stroma or scattered throughout an increased amount of stroma [22]. The patients were divided randomly into group 1 (n=11) and group 2 (n=10). Of the patients, 91% versus 90% were oligomenorrheic (intermenstrual interval > 35 days) and 9% versus 10% were amenorrheic (no menstrual period for >6 months) in metformin plus CC and CC only groups, respectively. Of the patients, 45 and 50% had hirsutism (modified Ferriman–Gallway score ≥ 8) in metformin plus CC and CC only groups, respectively. Age and duration of infertility were recorded and body mass index (BMI) was measured.

Of the patients, 73 and 60% had obese (BMI ≥ 25 kg/m²) in metformin plus CC and CC only groups, respectively. A complete clinical and laboratory evaluation were performed to exclude the patients with androgen secreting tumors of ovarian or adrenal origin, Cushing’s syndrome, thyroid dysfunctions, nonclassic adrenal hyperplasia and hyperprolactinemia. For at least 12 weeks before the study, none of the subjects in both groups had received any medication known to affect pituitary–gonadal function or carbohydrate metabolism. They did not have a history of diabetes mellitus. All patients in the study groups had normal renal and liver function tests. The tests were conducted during the first 10 days after the onset of vaginal bleeding or after medroxyprogesterone-induced vaginal bleeding.

After an overnight fasting, blood samples were obtained for the determination of fasting blood glucose (FBG), insulin, FSH, LH, oestradiol (E₂), total and free testosterone (T), androstenedione (A), DHEAS, PRL, and sex hormone-binding globulin (SHBG) levels in all women. Before the treatment, the patients were given a 3-day 2000 cal standardized diet (300 g carbohydrate per day) before an oral glucose tolerance test (OGTT) which was performed after ingestion of a 75 g glucose load, and blood samples were obtained at 30 min intervals for 2 h for the measurement of glucose and insulin after 10–12 h of fasting between 8:00 and 10:00 a.m. in both groups. After baseline studies were completed, metformin was given at a dose of 850 mg two times a day for 3 months, at that time the pretreatment studies including OGTT were repeated in metformin plus CC group. The glucose and insulin responses to OGTT were also expressed as area under the curve (AUC) estimated by the trapezoidal rule. The ratio of fasting glucose to insulin was calculated. Glucose tolerance was assayed by the WHO criteria [23]. The serum samples were stored at −20°C until they were assayed.

As shown in Table 1, there were no statistically significant differences in age, duration of infertility, body mass index, and in hormonal data between the two groups. Serum insulin, glucose, AUC glucose, AUC insulin, AUC glucose:AUC insulin ratio, and fasting glucose (G):fasting insulin (I) ratio values were also similar in both groups.

Spontaneous ovulation was checked by blood samples taken on day 21, if no spontaneous menstruation occurred they were taken at every 2 weeks for 3 months during the metformin therapy. A progesterone (P) level ≥ 5.0 ng/ml was considered as confirmatory of ovulation. No subject ovulated in the response to metformin only. PCOS women were instructed not to alter their usual eating habits and lifestyle during the study. Serum β-hCG test was negative before starting the protocol. Group 2 patients did not receive metformin.

Clomiphene citrate was given at a dose of 100 mg a day for 5 days on day 5 of each cycle in both groups after a withdrawal bleeding induced with medroxyprogesterone acetate. Metformin administration continued during induction of ovulation and terminated the day of hCG administration in group 1. The same treatment regimen was repeated until either a pregnancy occurred, or a maximum of six clomiphene citrate cycles were reached. Follicular development was monitored by serial ultrasound scanning. Ovulation was induced by i.m. injection of 10.000 IU hCG.
Table 1
Clinical, hormonal, and metabolic characteristics of patients in both groups\(^a\)

<table>
<thead>
<tr>
<th>Variable</th>
<th>Metformin plus clomiphene (n = 11)</th>
<th>Clomiphene (n = 10)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>27 (21–31)</td>
<td>24.5 (19–28)</td>
</tr>
<tr>
<td>Duration of infertility (years)</td>
<td>5 (2–10)</td>
<td>3.5 (1–8)</td>
</tr>
<tr>
<td>FGS</td>
<td>7 (1–12)</td>
<td>8 (1–16)</td>
</tr>
<tr>
<td>BMI (kg/m(^2))</td>
<td>30.4 (24.6–33.9)</td>
<td>25.7 (23.1–35.7)</td>
</tr>
<tr>
<td>FSH (2.5–12.5 mIU/ml)</td>
<td>5.1 (3.0–14.3)</td>
<td>5.8 (4.0–15.2)</td>
</tr>
<tr>
<td>LH (1.9–12.5 mIU/ml)</td>
<td>11.3 (3.1–18.5)</td>
<td>10.5 (7.0–17.5)</td>
</tr>
<tr>
<td>Oestradiol (11–69 pg/ml)</td>
<td>39.0 (20–54)</td>
<td>33.0 (19–147)</td>
</tr>
<tr>
<td>Total testosterone (10–80 ng/dl)</td>
<td>114 (79–170)</td>
<td>108 (63–131)</td>
</tr>
<tr>
<td>Free testosterone (0.29–3.18 mg/ml)</td>
<td>3.9 (1.2–5.1)</td>
<td>2.37 (1.7–3.9)</td>
</tr>
<tr>
<td>Androstenedione (0.21–3.08 ng/ml)</td>
<td>3.6 (1.2–5.9)</td>
<td>4.6 (0.5–6.1)</td>
</tr>
<tr>
<td>SHBG (20–40 nmol/l)</td>
<td>24.3 (14.6–41.6)</td>
<td>22.9 (13.0–121.0)</td>
</tr>
<tr>
<td>DHEAS (30–333 pg/dl)</td>
<td>237 (135–348)</td>
<td>206 (111–377)</td>
</tr>
<tr>
<td>Prolactin (2.8–29 ng/ml)</td>
<td>9.3 (2.4–28.1)</td>
<td>9.8 (6.6–14.0)</td>
</tr>
<tr>
<td>Fasting glucose (70–110 mg/dl)</td>
<td>84 (68–110)</td>
<td>87 (76–97)</td>
</tr>
<tr>
<td>Fasting insulin (2–25 μU/ml)</td>
<td>24.8 (13.4–30.1)</td>
<td>19.5 (12.6–27)</td>
</tr>
<tr>
<td>AUC insulin (mg min/ml)</td>
<td>18030 (11640–20145)</td>
<td>15788 (12240–18810)</td>
</tr>
<tr>
<td>AUC insulin (μU min/ml)</td>
<td>11865 (2730–17925)</td>
<td>11562 (3500–23085)</td>
</tr>
<tr>
<td>Fasting glucose:insulin ratio</td>
<td>3.7 (3.0–5.7)</td>
<td>4.49 (2.7–6.8)</td>
</tr>
<tr>
<td>AUC glucose:AUC insulin ratio</td>
<td>1.31 (1.03–5.36)</td>
<td>1.39 (0.6–3.79)</td>
</tr>
</tbody>
</table>

FGS: Ferriman–Gallwey score; BMI: body mass index; FSH: follicle-stimulating hormone; LH: luteinizing hormone; SHBG: sex hormone-binding globulin; DHEAS: dehydroepiandrosterone sulfate; AUC: area under the curve.

\(^a\) Values are median and range.

(Pregnyl 5,000 IU, Organon, Oss, Holland). The criteria for hCG administration were: fewer than four follicles of diameter ≥15 mm on ultrasound with a leading follicle of ≥18 mm in diameter. Otherwise the injection of hCG was withheld and the cycle was cancelled. The number of follicles ≥15 mm in diameter and endometrial thickness were obtained on the day of hCC treatment. Serum P level was measured after 7 days of hCG administration in all cycles. Pregnancy was defined by ultrasound evidence of a gestational sac and the presence of fetal heart motion.

2.1. Hormone assays

Plasma glucose level was determined by a glucose oxidase method (Konelab, Espoo, Finland). Serum insulin, total testosterone and free testosterone, progesterone, androstenedione, SHBG, DHEAS, and PRL levels were determined by RIA, using commercial kits (insulin, DPC, Los Angeles, USA; total testosterone and free testosterone, DSL Inc., Texas, USA; SHBG, Euro-Diagnostica AB, Malmö, Sweden; P, DHEAS and A, Immunootech, Marseille, France; PRL, ICN Biomedicals Inc., Costa Mesa, CA). Serum FSH, LH (Bayer C, Tarrytown, NY, USA), and E\(_2\) (Chiron Diagnostics C, East Walpole, USA) levels were measured with specific automated chemiluminescence system, using commercial kits. The intra-assay and inter-assay precision coefficients of variation were 9.3 and 10.0% for FSH; 8.1 and 9.1% for total testosterone; 3.7 and 7.9% for free testosterone; 4.7 and 7.6% for androstenedione; 3.1 and 3.1% for progesterone; 4.1 and 6.5–8.3% for SHBG; 3.2–7.4 and 4.1–10.6% for DHEAS; 14.8 and 15.3% for E\(_2\); 4.8 and 8.2% for PRL, respectively. The intra-assay coefficients of variation were 2.8 and 5% for FSH and LH, respectively. All samples from the same patients were assayed in the same assay.

2.2. Statistical analysis

The results were reported as means ± S.E.M. or median and range. Wilcoxon-signed rank test was used to evaluate the effect of metformin on hormones and glucose levels. Chi-square test was used to evaluate ordinal variables, and Mann–Whitney U-test and the unpaired Student’s t-test were used to evaluate continuous numeric variables. \(P\) value of <0.05 was considered as statistically significant.

3. Results

Table 2 shows clinical, hormonal, and metabolic parameters of patients before and after metformin therapy in group 1. There were no significant changes in the hirsutism score or BMI during metformin therapy. Mean LH, total testosterone, free testosterone, androstenedione, DHEAS, fasting insulin levels, AUC insulin and fasting glucose:fasting insulin ratio values decreased significantly after metformin therapy (\(P < 0.05\)). SHBG level increased significantly (\(P < 0.05\)). The mean basal serum FSH, E\(_2\), PRL and fasting glucose levels, AUC glucose and AUC glucose:AUC insulin ratio values did not change significantly.

Mean serum P level and endometrial thickness were significantly higher in cycles treated with metformin plus CC (35.2 ± 24.3 ng/ml, 8.3 ± 1.4 mm) than in those treated with CC alone (24.3 ± 15.9 ng/ml, 7.7 ± 1.2 mm), respectively (\(P < 0.05\)).
No patients discontinued their treatment because of adverse drug effects. Table 3 shows the results of induction of ovulation with metformin plus CC and CC alone. A total of 51 cycles with metformin plus CC and 55 with CC were performed. Only one patient had one cycle cancelled due to the development of a large follicle cyst in the CC only group. The patients were followed up for a total of 51 cycles with metformin plus CC and 55 with CC were performed. Only one patient had one cycle cancelled due to the development of a large follicle cyst in the CC only group. The patients were followed up for a total of 51 cycles in the metformin plus CC group and 55 in the CC group. The day of hCG administration and the number of follicles >15 mm in diameter on day of hCG administration were similar in both treatment groups.

The ovulation and pregnancy rates in cycles were higher, but not significantly, in the women given metformin plus CC than in those given CC alone. The pregnancy rate in ovulatory cycles was also higher though not significantly so, in the metformin plus CC group (5/38, 13.5% versus 3/34, 8.8%). Five pregnancies (45.5%) occurred in 11 women treated with metformin plus CC and three pregnancies (30%) occurred in those treated with CC alone. One pregnancy in the metformin plus CC group ended in first trimester spontaneous abortion and one preterm delivery, the other three have delivered full-term healthy babies. Three women have delivered full-term healthy babies in the CC only group. All the patients who conceived had insulin resistance (fasting $G:I$ ratio $<4.5$) in the metformin plus CC group. Three women who conceived were non-insulin resistance in the CC group (Table 4).

4. Discussion

In the present study, we examined the effects of metformin on insulin resistance and ovarian androgen production and the effect of the reduction in insulin secretion on the induction of ovulation by clomiphene. We have shown that metformin significantly reduces the hyperinsulinemia and hyperandrogenemia in women with PCOS.

Metformin is considered as a category B drug, which means that sufficient human data are available. Mouse embryos exposed to metformin have shown no major malformations in the offspring [24]. Although, metformin has been used by some clinicians to determine whether metformin therapy reduced development of gestational diabetes in women with PCOS [25] or to treat diabetes [26] in a small number of pregnant women throughout their pregnancies, and no adverse effects were seen in infant. There are no adequate and well-controlled studies on its use in pregnant women. For this reason we discontinued the administration of metformin on the day of hCG injection.

Several studies examined changes in ovulatory function on metformin therapy. These studies suggest that metformin may increase the number of ovulatory cycles in infertile women with PCOS, especially when used combined with...
clomiphene. Nestler et al. [14] randomized 61 obese women with PCOS to treatment with metformin or placebo. Women in both groups who did not ovulate were added clomiphene. Thirty-four percent of women in the metformin group ovulated spontaneously during treatment with metformin alone, as compared only 4% in the placebo group. Ninety percent of women who received combined metformin and clomiphene ovulated, in contrast to only 8% of women in the group given placebo and clomiphene. The treatment with metformin significantly decreased the serum insulin response to oral glucose challenge and serum androgen concentrations did not change significantly [14]. In contrast, in another study performed by the same authors on clomiphene-resistant infertile patients with PCOS who were on clomiphene, metformin markedly increased the ovulation and pregnancy rates despite a lack of improvement in serum levels of androgens and the insulin response to oral glucose challenge [20]. Insulin-sensitizing agent troglitazone alone and the combination of troglitazone plus clomiphene is associated with increased rates of ovulation and pregnancy in insulin-resistant women with PCOS [27].

De Leo et al. [28] evaluated whether pretreatment with metformin improves FSH-induced ovulation in women with clomiphene-resistant PCOS. Twenty infertile women with clomiphene-resistant PCOS were randomized into treatment with FSH alone or FSH plus metformin. The number of dominant follicles, cycles cancellation rate and peak estradiol level were significantly lower in cycles treated with FSH and metformin than in those treated with FSH alone. That study shows that metformin leads to an orderly FSH-induced ovulation in patients with PCOS.

In the present study, the day of hCG administration and the number of follicles >15 mm in diameter on day of hCG administration were similar in both treatment groups. The ovulation and pregnancy rates in cycles were higher, but not significantly, in the women given metformin plus CC than in those given CC alone. The pregnancy rate in ovulatory cycles was also higher though not significantly so, in the metformin plus CC group. Five pregnancies (45.5%) occurred in 11 women treated with metformin plus CC and three pregnancies (30%) occurred in those treated with CC alone. Three women have delivered full-term healthy babies in each group. The mild increased frequency of ovulation may be accompanied by an increased pregnancy rate or birth rate.

We found that the mean serum progesterone level and endometrial thickness were significantly higher in cycles treated with metformin plus CC than in those treated with CC alone. There is only limited data about the effects of metformin on progesterone production of corpus luteum in PCOS. Attia et al. [29] reported that a higher dose of metformin directly increased progesterone production in human ovarian theca-like tumor cells. Metformin might increase the endometrial thickness by reducing the insulin and androgen levels and by modulating the insulin-like growth factor (IGF) level. IGF-I may play an important role in the regulation of ovarian follicular maturation and steroidogenesis [30], and in endometrial proliferation [31]. The in vivo action of IGF-I is modulated by IGF-binding protein I (IGFBP-I), because bound IGFs are not biologically active. IGFBP-I synthesis is inhibited by insulin and IGF-I. In PCOS, the decreased IGFBP-I levels lead to elevated free IGF-I levels [30]. De Leo et al. [32] have demonstrated that metformin-induced insulin reduction is associated with an increase in IGFBP-I and a reduced IGF-I/IGFBP-I ratio. Hyperinsulinism and free IGF-I levels in PCOS women may inhibit normal endometrial maturation and it seems that the treatment of hyperinsulinism with metformin would increase the ovulation and pregnancy rates.

Dale et al. [33] examined the possible correlation between insulin resistance and outcome of gonadotropin stimulation in infertile clomiphene-resistant women with PCOS. In that study the insulin-resistant PCOS women required more gonadotropin and a longer time to achieve follicular maturation. Although ovulation rate in completed cycles was similar between the groups, the conception rate was significantly better in the non-insulin-resistant PCOS women. In contrast to that study, Yarah et al. [21] suggested that

<table>
<thead>
<tr>
<th>Patient</th>
<th>Metformin plus clomiphene</th>
<th>Ovulatory cycle</th>
<th>Pregnancy</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Fasting G/I</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Before metformin</td>
<td>After metformin</td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>3.1</td>
<td>8.8</td>
<td>2</td>
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<td>2</td>
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</tr>
<tr>
<td>11</td>
<td>3.0</td>
<td>6.6</td>
<td>2</td>
</tr>
</tbody>
</table>

Table 4

Fasting glucose: insulin ratio values and pregnancy response to treatment with metformin plus CC and CC alone. G, glucose; I, insulin
metformin did not improve insulin resistance, ovulation rate and pregnancy rate during low-dose step-up protocol using rFSH.

Legro et al. [34] suggested that the fasting $G:I$ ratio (cut-off value, \(<4.5\)) may be a useful test identifying PCOS women with insulin resistance and has both high sensitivity and specificity for detecting insulin-resistant women. These women may be more likely to benefit from therapies that lower circulating insulin levels.

In the present study, 8 of 11 patients had insulin resistance (fasting $G:I$ ratio value \(<4.5\)) in the metformin plus CC group and 5 of 10 patients in the CC only group. All the women may be more likely to benefit from therapies that lower circulating insulin levels.

In the present study, 8 of 11 patients had insulin resistance (fasting $G:I$ ratio value \(<4.5\)) in the metformin plus CC group and 5 of 10 patients in the CC only group. All the women may be more likely to benefit from therapies that lower circulating insulin levels.

In conclusion, metformin therapy was effective in reducing insulin resistance and hyperandrogenism in women with PCOS. Metformin did not increase significantly the ovulation and pregnancy rates. More randomized, controlled studies in a larger population are needed to determine the effect of metformin on ovulation induction with CC in women with PCOS.

References


[34] Legro RS, Finegood D, Dunaif A. A fasting glucose to insulin ratio is a useful measure of insulin sensitivity in women with polycystic ovary syndrome. J Clin Endocrin Metab 1998;83:2694–8.