The effect of metformin plus clomiphene citrate on ovulation and pregnancy rates in clomiphene-resistant women with polycystic ovary syndrome

Hasan Y. Malkawi, MD, Hussein S. Qublan, MD.

ABSTRACT

Objective: To study the effect of metformin in combination with clomiphene citrate, as compared with placebo plus clomiphene citrate, on the ovulation and pregnancy rates in clomiphene citrate-resistant women with polycystic ovary syndrome.

Methods: This study was carried out at King Hussein Medical Center, Amman, Jordan, during the period January 2001 through to July 2001. Twenty-eight clomiphene citrate-resistant polycystic ovary syndrome women were evaluated prospectively for 6 treatment cycles by receiving metformin, 850mg twice daily throughout the cycle along with 50 mg clomiphene citrate, starting on day 5-9 of the same cycle (N=16), or by taking placebo with clomiphene citrate (N=12). During cycles 2-6, clomiphene citrate was added with increments of 50mg (up to 200 mg/day) for both groups. Progesterone level on day 21 and 28 >5ng/dl was indicative of ovulation.

Results: A statistically significant increase in the rates of ovulation (68.6% versus 25%, $p<0.05$) and pregnancy (56.3% versus 16.6%, $p<0.05$) were observed in the metformin-clomiphene citrate group as compared with the placebo-clomiphene citrate controls. Insignificant increase in the rate of ovarian hyperstimulation was noted in the placebo-clomiphene citrate group.

Conclusion: Metformin-clomiphene citrate regimen in resistant-clomiphene citrate polycystic ovary syndrome women significantly increases the ovulation and pregnancy rates, and decreases the occurrence of ovarian hyperstimulation syndrome.

Keywords: Polycystic ovary syndrome, clomiphene citrate, metformin, ovulation rate, pregnancy rate.

Saudi Med J 2002; Vol. 23 (6): 663-666

Polycystic ovary syndrome (PCOS) is a heterogeneous disorder often associated with obesity, oligomenorrhea, elevated luteinizing hormone (LH) and hyperandrogenemia.[1] Insulin resistance is most commonly found in obese PCOS women (65%), but can also be demonstrated in nearly 20% of lean PCOS women.[2] It has been suggested that the resulting hyperinsulinemia augments the LH-driven production of androgens from the ovarian theca cells in these women.[3] The association between PCOS related hyperandrogenemia and insulin resistance is well documented.[4,5] Several studies of women with PCOS have shown that when insulin secretion is decreased with insulin sensitizing drugs, such as metformin, troglitazone or d-chiro-inositol, the rates of spontaneous ovulation and ovulation in response to clomiphene citrate (CC) increases.[6-8] Despite these studies, data is limited in CC-resistant women on combined use of oral insulin sensitizing agents and

From the Department of Obstetrics and Gynecology, King Hussein Medical Center, Amman, Jordan.

Received 30th September 2001. Accepted for publication in final form 15th December 2001.

Address correspondence and reprint request to: Dr. Hussein S. Qublan, PO Box 97, Irbid-Aidun, Jordan. Tel. +962 (7) 7410465. Fax. +962 (2) 7103268. E-mail: hqublan@hotmail.com

CC with respect to ovulation rates, number of ovulating cycles produced per woman, and pregnancy rates. We conducted this study to determine the effect of metformin in combination with CC versus placebo plus CC on the ovulation and pregnancy rates in CC-resistant PCOS women.

Methods. Twenty-eight CC-resistant PCOS women were studied prospectively at King Hussein Medical Center (KHMC), Amman, Jordan during the period January 2001 through to July 2001. Diagnosis of PCOS was based on the presence of polycystic ovaries on vaginal ultrasound examination combined with 3 or more of the following criteria: Oligomenorrhea (<6 menstrual periods in the preceding year), hirsutism (when Ferriman-Gallwey score >7), hyperandrogenemia (elevated free testosterone, androstenedione, dehydroepiandrosterone sulfate, (DHEAS), and elevated concentrations (LH) or LH: follicle stimulating hormone (FSH) ratio>2. Congenital adrenal hyperplasia, Cushing's syndrome, hypperprolactinemia and thyroid disease were excluded by appropriate tests. Clomiphene citrate resistance was defined as failure to ovulate or to conceive after CC treatment up to a daily dose of 150 mg from cycle day 5-9 for at least 3 consecutive cycles. Progesterone level on day 21 and 28 >5 ng/ mL was indicative of ovulation. Radioimmunoassay was used for free testosterone, DHEAS, androstenedione, and 17-hydroxyprogesterone (OPH) (Diagnostic Product Corporation, United States of America, (USA)). Fluoroimmunoassay was used for LH and FSH (DPC, USA), and serum insulin (Abbott, USA). Serum glucose level was determined by glucose oxidase method (Randox, United Kingdom). All patients were required to have normal uterine cavity and tubal patency on hysterosalpingography. All male partners had normal semen parameters according to the World Health Organization (WHO) criteria. All subjects were informed clearly regarding the nature and purposes of the study and with regards to the potential benefits and risks of metformin. A written informed consent was obtained from each woman participating in the study.

Sixteen women were randomly assigned to take 850 mg of metformin (Glucophage: Lipha Sante, Lyon, France) twice daily throughout the cycle along with 50mg CC, starting on day 5-9 of the same cycle. Twelve women were assigned to take placebo with CC. During cycles 2-6, CC was added with increments of 50mg (up to 200mg/day) for both groups. Serum progesterone was assayed on day 21 and 28 of each cycle. With ovulation, the daily dose of CC was unchanged, but with anovulation, it was increased by 50mg for the next cycle. Women, who did not menstruate within one week of a serum progesterone level indicative of ovulation, had urine pregnancy testing or serum β-human chorionic gonadotropin (HCG) measurement. If the test was positive or β-hCG> 25 mIU/ ml, metformin or placebo treatment was discontinued. Clinical pregnancy was considered when a gestational sac was detected by ultrasonography. Student t-test and Fisher's exact test were used to analyze differences between the 2 groups. Differences were statistically significant when $p<0.05$.

Results. **Table 1** shows the demographic data of 28 CC-resistant women with PCOS. Sixteen women received metformin and CC, while the other 12 received placebo and CC. There were no significant differences in terms of age, weight, body mass index (BMI), and duration of infertility between the 2 groups. No significant differences were also detected in the fasting insulin and glucose levels, hormonal levels (testosterone, androstenedione, Estradiol, 17a-OHP, progesterone, DHEAS, LH and FSH), and LH: FSH ratio, as shown in **Table 2. Table 3** shows the clinical results following the treatment with metformin-CC versus placebo-CC. Women in metformin-CC group underwent 63 cycles in which ovulation occurred in 43 cycles, while women in the placebo-CC group underwent 66 cycles in which ovulation occurred in 16 cycles. The difference in the total number of cycles between the 2 groups was statistically significant ($p<0.01$). A statistically significant increase in the rates of ovulation (68.8% versus 25%, $p<0.05$), and pregnancy (56.3% versus 16.6%, $p<0.05$) was observed among women of the metformin-CC group as compared with the placebo-CC group. Ovarian hyperstimulation syndrome (moderate type) was detected in 2 patients of the placebo-CC group, and in none of the metformin–CC group. This difference was not statistically significant.

Discussion. Anovulatory women with PCOS who remained anovulatory with CC show greater insulin resistance accompanied by compensatory hyperinsulinemia than women with PCOS who ovulated successfully in response to CC treatment.[9] Insulin may affect androgen production in several ways. One proposed mechanism is that insulin increases the activity of ovarian cytochrome P450c-17a in patients with PCOS, which is the key enzyme in the synthesis of androgens.[6] In concert with LH, insulin may produce androgens from theca cells effected either by insulin growth factors (IGF) or preferably by its own receptors.[10] Additionally, androgen production may be enhanced by an increase in the biological activity of IGF-I/-II as insulin is shown to reduce concentrations of IGF binding protein-1 (IGFBP-1).[11] Another mechanism of action could be inhibition of liver secreted sex hormone

Table 1 - Clinical data for 28 clomiphene citrate resistant women with polycystic ovary syndrome.

Demographic Data	Metformin-CC N=16	Placebo-CC N=12
Age (year)	29 ± 3.1	29 ± 7.3
Weight (kg)	90 ± 6.7	89 ± 7.2
Body mass index (kg/m^2)	27.5 ± 4.1	27.8 ± 3.3
Duration of infertility	3.2 ± 1.1	3.0 ± 1.3

CC - clomiphene citrate, N - number
Values are means ± standard deviation

Table 2 - Hormonal data for 28 clomiphene citrate resistant women with polycystic ovary syndrome.

Hormone Data	Metformin-CC N=16	Placebo-CC N=12
Fasting insulin level (µU/mL)	20.5 ± 4.2	21.2 ± 5.3
Fasting glucose level (mg/dL)	80.6 ± 2.3	79 ± 3.6
Serum testosterone level (ng/dL)	330 ± 48	310 ± 52
Serum androstenedione level (ng/mL)	2.5 ± 0.9	2.4 ± 1.1
Serum estradiol level (pg/mL)	49 ± 7.7	50.2 ± 5.6
Serum DHEAS level (µg/dL)	234 ± 23	225 ± 20.6
Serum 17-OHP level (µg/mL)	1.3 ± 0.2	1.3 ± 0.3
Serum progesterone level (ng/mL)	1.0 ± 0.2	0.9 ± 0.4
Serum LH level (mIU/mL)	10.9 ± 1.7	10.7 ± 1.8
Serum FSH (mIU/mL)	5.3 ± 1.6	5.4 ± 1.3
LH: FSH ratio	2.3 ± 0.2	2.3 ± 0.3

CC - clomiphene citrate, N - number,
DHEAS - dehydroepiandrosterone sulfate,
17-OHP - hydroxyprogesterone, LH - luteinizing hormone,
FSH - follicle stimulating hormone
Values are means ± standard deviation

Table 3 - Clinical results following treatment with metformin plus clomiphene citrate versus placebo plus clomiphene citrate.

Clinical Outcome	Metformin-CC N=16 (%)	Placebo-CC N=12 (%)
N of cycles	63 (65.6)**	66 (91.6)
Ovulation rate	11/16 (68.8)*	3/12 (25)
Pregnancy rate	9/16 (56.3)*	2/12 (16.6)
Ovarian hyperstimulation rate	0	2 (16.6)

CC - clomiphene citrate, N - number
* - P<0.05 metformin-CC versus placebo-CC, ** - p<0.01

binding globulin (SHBG), increasing the availability of androgens.[12] Thus, the androgen dominant intraovarian milieu mediated by hyperinsulinemia could adversely affect folliculogenesis and ovulation by altering gonadotropin secretion, or directly affecting follicle development.[13] The rational basis for its use in the treatment of PCOS, metformin, a biguanide antihyperglycemic drug that used to treat non insulin independant diabetes mellitus, reduces hyperinsulinism and ovarian levels of androgens, and it is classified as a category B drug which means that no teratogenic effect has been demonstrated in vitro.[7]

In the present study, we have shown that metformin in addition to clomiphene citrate increased significantly the ovulation and pregnancy rates. Furthermore, significant reduction in the number of the treatment cycles to produce ovulation and to achieve pregnancy was found in the metformin-CC group when compared with the placebo. During the 6 months treatment, 11 of 16 (68.8%) ovulated in the metformin-CC group and 9 (56.3%) became pregnant, while only 3 of 12 (25%) ovulated and 2 (16.6%) achieved pregnancy in the placebo-CC group. Our results are in agreement with those reported in previous studies, which detected an increased rated of ovulation and pregnancy in CC-resistant women with PCOS when treated with metformin.[7,14,15] Vandermolen et al[14] reported a 3-fold increase in the number of ovulatory cycles (75% versus 27%), and an 8-fold in the pregnancy rate (55% versus 7%) in the meformin-CC treated group as compared with the group of women who received placebo-CC. A 7-fold (89% versus 12% in the placebo group) increase in the ovulatory rate was also reported by Nestler and his colleagues.[7] The ovulation rate (75% and 89%) reported in these studies was higher than that found in our series (68.8%) although the BMI of their patients was higher than ours. Possible explanation for this discrepancy is that metformin was administered 1-2 months before initiating their studies. It seems that prolonged treatment with metformin, the insulin-sensitizing drug, ameliorates the insulin resistance. This hypothesis supports the suggestion that the common mechanism of action for this drug in PCOS is directly related to improvement of insulin sensitivity or reduction of circulating insulin levels.[16] Marca et al,[17] reported that the main effect of metformin was on the liver with increased secretion of SHBG, and the reduction of androgens is a secondary effect. They suggested that at one month after metformin treatment, ovulation was produced in 14%, another observation that supports the benefit of prolonged treatment with metformin. One serious complication of ovulation drugs, mainly in patients with PCOS is ovarian hyperstimulation syndrome (OHSS). This complication occurred in 2 cases (16.6%) of the placebo-CC group, and in none of the

metformin-CC group. It has been reported that treatment with metformin lead to a reduction in E2 levels on the day of HCG administration, and in the number of follicles >15 in diameter, resulting in a lower incidence of ovarian overstimulation.[18]

In conclusion, women with PCOS and documented failure of CC therapy are especially insulin-resistant. With this eskeptism, the prolonged treatment with insulin-sensitizing drugs can ameliorate their insulin resistance. The co-administration of metformin to CC in CC-resistant women with PCOS increases significantly the ovulation and pregnancy rates, and decreases the incidence of ovarian overstimulation.

References

1. Karlo BN, Loucks TL, Berga SL. Neuromodulation in polycystic ovary syndrome. *Obstet Gynecol Clin North Am* 2001; 28: 35-62.
2. Franks S. Polycystic ovary syndrome. *N Engl J Med* 1995; 333: 853-861.
3. Tiitinen AE, Laatikainen TJ, Seppala MT. Serum levels of insulin-like growth factor binding protein-1 and ovulatory responses to clomiphene citrate in women with polycystic ovary syndrome. *Fertil Steril* 1993; 60: 58-62.
4. Dunaif A. Molecular mechanism of insulin resistance in the polycystic ovary syndrome. *Semin Reprod Endocrinol* 1994; 12: 15-20.
5. Burghen GA, Givens JR, Kibatchi AE. Correlation of hyperandrogenism with hyperinsulinism in polycystic ovary syndrome. *J Clin Endocrinol Metab* 1980; 50: 113-116.
6. Nestler JE, Jakubowicz DJ, Reamer P, Gunn RD, Allan G. Ovulatory and metabolic effects of d-chiro-inositol in the polycystic ovary syndrome. *N Engl J Med* 1999; 340: 1314-1320.
7. Nestler JE, Jakubowicz DJ, Evans WS, Pasquali R. Effects of metformin on spontaneous and clomiphene-induced ovulation in the polycystic ovary syndrome. *N Engl J Med* 1998; 338: 1876-1880.
8. Hasegawa I, Murakawa H, Suzuki M, Yamamoto Y, Kurabayashi T, Tanaka K. Effects of troglitazone on endocrine and ovulatory performance in women with insulin-resistance-related polycystic ovary syndrome. *Fertil Steril* 1999; 71: 323-327.
9. Murafawa H, Hasegawa I, Kurabayashi T, Tanaka K. Polycystic ovary syndrome. Insulin resistance and ovulatory response to clomiphene citrate. *J Reprod Med* 1999; 44: 23-27.
10. Willis D, Mason H, Gilling-Smith C Franks S. Modulation by insulin of follicle stimulating hormone and luteinizing hormone action in human granulosa cells of normal and polycystic ovaries. *J Clin Endocrinol Metab* 1996; 81: 302-309.
11. Rosenfeld RG, Lamson GL, Oh Y. Insulin-like binding proteins. *Recent Program and Hormonal Research* 1990; 46: 99-163.
12. Nestler JE, Powers LP, Matt DW, Steingold KA, Plymate SR, Rittmaster RS. A direct effect of hyperinsulinaemia on sex- hormone binding globulin levels in obese women with the polycystic ovary syndrome. *J Clin Endocrinol Metab* 1991; 72: 83-89.
13. Dunaif A. Insulin resistance and the polycystic ovary syndrome: mechanism and implications for pathogenesis. *Endocr Rev* 1997; 18: 774-800.
14. Vandermolen DT, Ratts VS, Evans WS, Stoval DW, Karma SW, Nestler JE. Metformin increases the ovulatory rate and pregnancy rate from clomiphene citrate in patients with polycystic ovary syndrome who are resistant to clomiphene citrate alone. *Fertil Steril* 2001; 75: 310-315.
15. Velazquez E, Acosta A, Mendoza SG. Menstrual cyclicity after metformin therapy in polycystic ovary syndrome. *Obstet Gynecol* 1997; 90: 392-395.
16. Nestler JE, Jakubowicz DJ. Decreases in ovarian cytochrome P 450c-17a activity and serum free testosterone after reduction of insulin secretion in polycystic ovary syndrome. *N Engl J Med* 1996; 335: 617-623.
17. De Leo V, laMarca A, Ditto A, Morgante G, Cianci A. Effects of metformin on adrenal steroidogenesis in women with polycystic ovary syndrome. *Fertil Steril* 1999; 72: 985-989.
18. De Leo V, laMarca A, Ditto A, Morgante G, Cianci A. Effects of metformin on gonadotropin-induced ovulation in women with polycystic ovary syndrome. *Fertil Steril* 1999; 72: 282-285.