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Metformin-therapy Effects in 50 Clomiphene Citrate Resistant PCOS Patients

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Polycystic Ovary Syndrome (PCOS) is the association of hyperandrogenism with chronic anovulation in women without specific underlying diseases of the adrenal or pituitary glands. We performed a randomized, placebo-controlled trial over 50 Clomiphene Citrate (CC) resistant PCOS women to determine metformin-therapy effects on metabolic disorders in patients with polycystic ovary syndrome who are resistant to CC. Participants were randomized in two groups with 25 cases in each group. One group received metformin, 500 mg three times daily, for 3 months and another one (Placebo group) received vitamin B1, 300 mg daily, for 3 months. Information on screening tests (2nd day of menstrual cycle) was obtained at baseline and after treatment (if there was no pregnancy after 3 months of treatment). In both groups, with anovulation after 3 months of treatment, CC treatment was begun at 100 mg daily for 5 days. Serum progesterone (P) levels were obtained in monthly visits and serum P level≥16 nmol mL^{-1} was considered to indicate ovulation. Main Outcome Measures were ovulation and pregnancy rates and correction of metabolic disorders. There was no ovulation in this study, but some metabolic disorders were improved. In anovulatory women with PCOS who were resistant to CC, metformin use improved some metabolic disorders due to PCOS, but there was no increased ovulation rate and further investigations are needed.

Key words: Metformin, ovulation, metabolic disorders

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INTRODUCTION

Polycystic ovary syndrome (PCOS) is a common and heterogeneous disorder of women of reproductive age, characterized by hyperandrogenism and chronic anovulation (Kamini, 2001). The PCOS diagnostic criteria suggested by NIH conference have been shown in Table 1 (David and Guzick, 2004).

Anovulation is responsible for approximately 40% of woman's infertility, many of them being due to the PCOS (Vadermolen *et al*., 2001). Although the fundamental pathophysiologic defect in PCOS is unknown, these women have several interrelated characteristic, including insulin resistance, hyperandrogenism and altered gonadotropin dynamics. Insulin resistance can be defined as a subnormal biological response to insulin (David and Guzick, 2004). There is a strong correlation between insulin resistance and hyperandrogenism. So that, decreasing insulin secretion by diazoxide or somatostatin in these subjects has resulted in concurrent reductions of serum androgens (Norman *et al*., 2004; Speroff *et al*., 1999; Carmina *et al*., 1992).

Another key pathophysiologic feature of PCOS is altered gonadotropin-releasing hormone dynamics (David and Guzick, 2004). There is increased GnRH pulse frequency and amplitude because of hypothalamus dysfunction, leading to gonadotropin release and sustained increased LH/FSH (Azziz and Zacur, 1995). Increased androgen production in women with PCOS is augmented by increased LH and is associated with anovulation but the proximate cause of the anovulation may be insufficient FSH. Follicles in the ovaries of women POCS do not mature fully and the granulose cells in these arrested follicles are low in number and in aromatase activity (David and Guzick, 2004).

Given a history of chronic anovulation and androgen excess, the only condition that needs to be excluded to secure the diagnosis of PCOS is non-classical congenital adrenal hyperplasia, which is very uncommon. This diagnostic pathway has shown in bold in Fig. 1 (David and Guzick, 2004).

Table 1: Polycystic ovary syndrome research diagnostic criteria (National Institutes of Health, April, 1990)

Definite or probable	Possible
Hyperandrogenemia, 64%	Insulin resistance, 69%
Exclusion of other etiologies, 60%	Perimenarchal onset, 62%
	Elevated LH/FSH, 55%
Exclusion of CAH, 59%	Polycystic ovary syndrome by ultrasound, 52%
Menstrual dysfunction, 52%	
Clinical hyperandrogenism 48%	Clinical hyperandrogenism, 52%
	Menstrual dysfunction, 45%

LH = Luteinizing Hormone; FSH = Follicle-stimulating Hormone. Percentage refers to the fraction of participants who endorsed that criterion (n = 58)

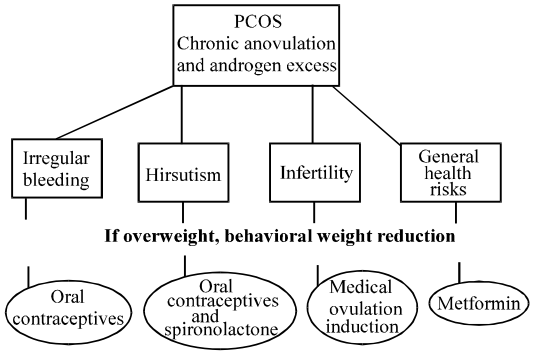

Fig. 1: Initial diagnostic evaluation of polycystic ovarian syndrome (PCOS). The pathway in bold is the most common scenario↑. Testosterone is defined as more than 60 ng dL^{-1}. ↑17-Hydroxyprogesterone (17-HP) is more than 2 ng mL^{-1}. Guzick. Polycystic Ovary Syndrome. Obstet Gynecol 2004

The finding of total testosterone concentrations more than 60 ng dL^{-1} is consistent with PCOS. High 17- hydroxy progesterone is defined as its concentrations of >2 ng mL^{-1}. A LH to FSH ratio of greater that 2:1 is certainly consistent with PCOS (Wilshire and Santoro, 1994). Testosterone is also helpful in evaluation of women with chronic anovulation who does not have clinical evidence of hirsutism or other sings of androgen excess (Fig. 1) (David and Guzick, 2004). The typical ultrasonographic view in women with PCOS is the large ovaries with follicles in different sizes (2-8 mm) and hyperechogenic stroma reflecting PCOS (Balen *et al*., 2003).

The most effective approach in treatment of PCOS women is weight loss. It can reduce both hyperandrogenism and hyperinsulinemia (Brindsen, 1999; Speroff *et al*., 1999).

In anovulatory or irregular ovulatory women with PCOS, ovulation inducing drugs are effective as an alone or associated with weight loss. In ovulation induction, clomiphene Citrate (CC) provides an excellent initial pharmacologic strategy (Balen *et al*., 2003). Small group of women with PCOS who have had high androgen levels and insulin resistance, had failed ovulated in response to CC. In such patients, insulin resistance results in failing of treatment and low fertility rate (Kamini, 2001).

In the last few years some studies assessed the effects of attenuation of hyperinsulinemia and insulin resistance, obtained by insulin sensitizing agents, in women with polycystic ovary syndrome (PCOS), suggesting potential scope for these drugs in treating the whole spectrum of reproductive, endocrine and

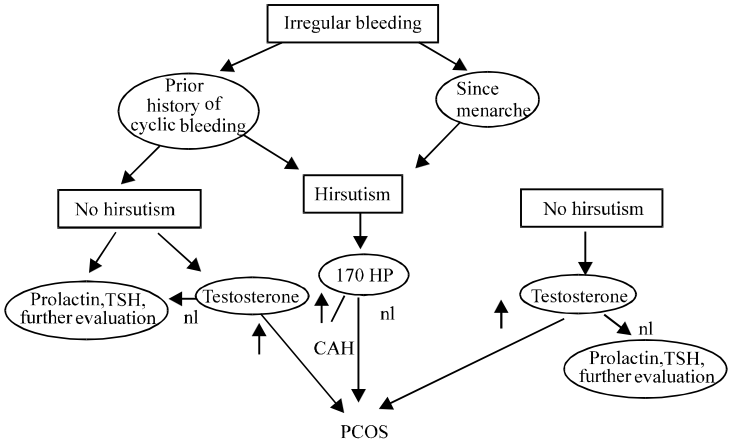

Fig. 2: Treatment algorithm for women with polycystic ovarian syndrome (PCOS). OCs=oral contraceptives. Guzick. Polycystic Ovary Syndrome. Obstet Gynecol 2004

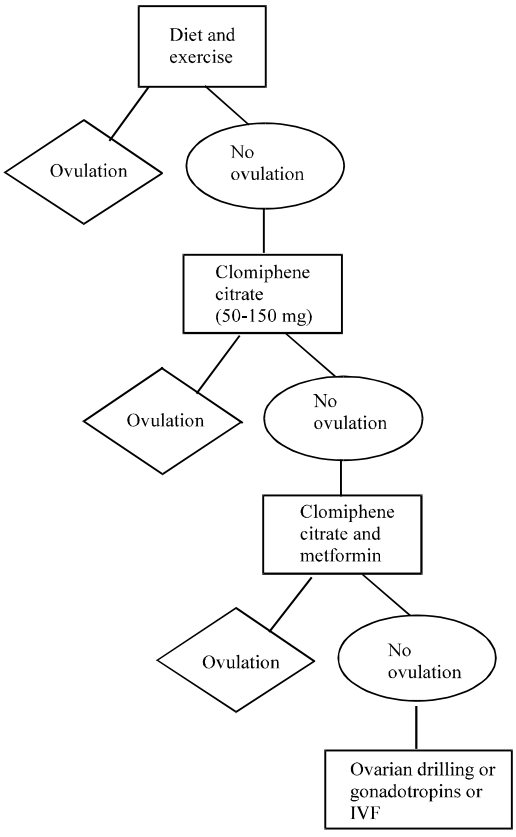

Fig. 3: Approach to ovulation induction in women with polycystic ovarian syndrome. IVF: *In vitro* fertilization. Guzick. Polycystic Ovary Syndrome. Obstet Gynecol. 2004

metabolic abnormalities found in such subjects (Moghetti *et al*., 2000). It seems that insulin sensitizers to be less invasive compared with other alternative treatments including HMG and ovarian surgery (Kim *et al*., 2000; Palomba *et al*., 2004).

Insulin sensitizing agents, such as metformin or troglitazone, have been tested for the treatment of PCOS. These drugs improve insulin sensitivity by different mechanisms, thus determining a subsequent reduction in plasma insulin levels (Moghetti *et al*., 2000). Metformin has been used extensively in the treatment of non-insulin-dependent diabetes mellitus. It helps with glycemic control by reducing hepatic glucose output and to a lesser extent, by increasing peripheral uptake of glucose (Speroff *et al*., 1999). When metformin was given for PCOS over-weight patients, the sensitivity to insulin and hyperinsulinemia were improved probably secondary to serum androgen reduction and SHBG level elevation (Hung and Mingsun, 2001). Treatment algorithm and approach to ovulation in women with PCOS are shown in Fig. 2 and 3 (David and Guzick, 2004).

Some short-term studies report significant reductions of serum androgens in women with PCOS given either metformin or troglitazone. Interestingly, improvements in reproductive abnormalities of these patients have also been reported in some of these studies (Moghetti *et al*., 2000). On the other hand, other authors failed to observe any clinical or biochemical changes after metformin. These discrepancies are not easily explained. In addition, controlled long-term studies assessing the clinical effects of these treatments are still lacking. The present study was designed to assess the effects on menstrual abnormalities of a 3 months course of metformin in 50 subjects with PCOS with normal glucose tolerance, divided in 2 case and control groups (Moghetti *et al*., 2000).

MATERIALS AND METHODS

Among patients being visited in Infertility Clinic of Al-Zahra Hospital and Specialized Clinic of Tabriz University of Medical Sciences during 2002-2003, we randomized 50 patients. Twenty five were categorized as a case group, treated with metformin for 3 months and 25 were categorized as a control group, received placebo for 3 months. Inclusion criteria were as a following:

- Age < 40 years old
- Women who were anovulatory in response to a 5 day course of cc, 100 mg day^{-1} during 3 cycles. Anovulation was documented by a mid-luteal P level <16 mmol mL^{-1}.
- Presence of more than 10 follicles in a size of 2-10 mm and increased ovarian stroma density in transvaginal ultrasound suggesting PCOS.
- Bilateral tubal patency in laparoscopy.
- Normal spermogram in their partner.
- Loss of endocrine disorders including congenial adrenal hyperplasia, hyperprolactinemia and thyroid and renal dysfunction in laboratory study.

On day 2 of each spontaneous or post-progesterone administration bleeding, we performed screening tests including LH, FSH Androstenedione, DHEAS, SHBG, fasting insulin, fasting leptin. Fasting glucose and 2 h post prandial (2 hpp) glucose, fasting cholesterol, fasting triglyceride, HDL and LDL. Transvaginal ultrasound was performed and three dimensions of each ovary measured in sagittal and coronal planes; then the ovarian mass was calculated using length, width and height. Progesterone levels measured monthly and ovulation was documented by a mid-luteal P level >16 mmol mL^{-1}. Body weight, menstrual pattern and drug side effects including nausea, Vomiting, diarrhea were registered in monthly visits.

In patients with ovulation, pregnancy test was performed and if the test result was positive, treatment was discontinued and the patient exclude form sturdy. When pregnancy didn't occur after 3 months treatment, screening test was performed again. Women who did not have ovulation after 3 months treatment, cc, 100 mg for 5 days initiated. All of screening tests were performed in Infertility department laboratory of Al-Zahra hospital and results registered in related forms.

Finally, in both groups, the results of tests were compared before and after treatment. Data were analyzed using SPSS software by t-test.

RESULTS

In this study 50 anovulatory patients with PCOS who resistant to CC, were randomized in two groups including Case and Control.

No significant changes in age, systolic and diastolic blood pressures, BMI, menstrual cycle and mid-luteal serum progesterone were seen in the case group compared with the controls, but there is significant difference in infertility duration (Table 2).

There is no significant difference in ovarian sizes. All of women in both groups had oligomenorrhea before treatment. Frequency of infertility type in case and control groups were 19 (primary) and 6 (secondary) and 17

Table 2: Characteristic of clomiphene citrate-resistant anovulatory women with PCOS

Characteristic	Metformin group (n = 25)	Placebo group (n = 25)	p-value
Age (year)	24.9±3.1	23.7±1.6	0.1
Systolic blood pressure	115.5±13	115.0±11.8	0.9
Diastolic blood pressure	71.3±9.6	75.0±10.8	0.3
Body mass index (kg m^{-2})	28.7±4	29.4±4.6	0.6
Infertility duration (Y)	5.3±2.6	3.4±1.9	0.02
Menstrual cycle	7.0±4	5.4±3.2	0.2
Midluteal serum progesterone (mg mL^{-1})	1.6±0.65	2.7±1.7	0.15

Table 3: Metformin effects on tests before and after intervention in both groups

	Case group		Control group		
Tests	Change mean	Standard deviation	Change mean	Standard deviation	p-value
LH	-0.25	2.40	-0.025	0.20	0.60
FSH	+0.76	1.00	-0.36	0.87	0.004
Testosterone	-0.12	0.32	-0.14	-0.14	0.80
DHEAS	-9.15	27.80	-0.80	3.00	0.13
SHBG	22.60	26.50	-0.50	1.60	0.0005
INS	-6.60	7.70	-0.40	0.30	0.0005
Leptin	1.56	19.00	0.13	0.60	0.70
FBS	-4.96	7.40	0.80	1.40	0.001
2hrppG	-14.70	16.50	0.60	2.10	0.0005
Cholesterol	-21.00	17.80	0.90	7.00	0.0005
TG	-24.80	23.20	-15.20	48.50	0.60
LDL	-22.50	14.10	1.10	2.40	0.0005
HDL	23.30	14.10	0.32	1.00	0.0005

(primary) and 8 (secondary), respectively. There is no significant difference in infertility between both groups (p = 0.18). Spermogram was normal in partners of women in both groups. Frequency of sonographic evidence was 80 and 84% in case group and control group, respectively. There is no significant difference in sonographic evidence in both groups (p = 0.7).

Creatinine, prolactin and 17-hydroxyprogesterone concentrations were normal in both groups. There were no significant difference in FSH, SH1BG, insulin, FBS, 2 hr pp glucose, cholesterol, LDL and HDL after treatment in both groups. There is, also, no significant difference in LH, testosterone, DHEAS, leptin and triglyceride in both groups (Table 3).

DISCUSSION

Insulin resistance and hyperinsulinemia play a pathogenic role in PCOS. It had been repeatedly shown that there is a strong correlation between insulin resistance and hyperandrogenism. Hyperinsulinemia induces clinical manifestation of PCOS by several ways; increases ovarian androgen production directly, decreases IGF-BP1 production and increases IGF1 activity. Finally, increased insulin and IGF1 level affects gonadotropin and adrenal androgen secretion and often result in lipids and lipoproteins disorders (Norman, 2004; Carmina *et al*., 1992).

Weight loss and insulin sensitizers, which also lead to a reduction in insulin, similarly are associated with a reduction in androgens, particularly testosterone and androstenedione. However, administration of a gonadotropin-releasing hormone analog, which reduces androgen secretion from the ovary by suppressing gonadotropins, does not result in a reduction in insulin (Dunaif *et al*., 1992). Development of menstrual dysfunction and hyperandrogenism is more likely in women with hyperinsulinemia than women with normal levels of insulin (Brindsen, 1999). Moreover, insulin inhibits hepatic production of SHBG and decreased SHBG results in increased androgen (Speroff *et al*., 1999).

There is now a large body of data documenting the clinical efficacy of metformin in the treatment of PCOS-associated insulin resistance. Metformin is an "old" drug mainly used to lower blood sugar in NIDDM. Its mechanism of action is still not entirely understood. Metformin and phenformin were introduced in 1957. Phenformin was withdrawn from clinical use in many countries in the late 1970s, when an association with lactic acidosis was recognized. Metformin became available for use in the United States in 1995. It is administered orally and improves insulin sensitivity that is impaired in NIDDM. Its efficacy is considered similar to that of sulfanilylurea (De Leo *et al*., 2003).

Metformin, a biguanide class drug used in obese patients with type 2 DM, has become the most widely used drug to treat women with insulin resistance (IR) and PCOS (Ortega *et al*., 2005).

Metformin reduces P450C17 activity by lowering increased insulin levels, then, resulting in reduction of plasma and ovarian androgen (Carmina *et al*., 1992). It appears that metformin capability in regulation of sensitivity to insulin is associated with body weight. BMI reduction is necessary in order to improved sensitivity to insulin, especially when BMI > 30 (Hung and Mingsun, 2001).

Velazquez *et al*. (1994) first reported, in an uncontrolled study, a reduction of serum free testosterone in 29 nondiabetic women with PCOS, mostly overweight, given metformin for 8 weeks. This protocol was designed to evaluate the effects of attenuation of insulin resistance and hyperinsulinemia on the metabolic and endocrine features of PCOS. Unexpectedly, 3 of these subjects became pregnant and menstrual cycles were normalized in another 7, who continued the treatment indefinitely. These findings prompted the testing of insulin sensitizing agents, such as metformin or troglitazone, for the treatment of both the metabolic and the endocrine abnormalities of women with PCOS.

The initial use of metformin in the treatment of PCOS was initially received with some skepticism but is now accepted to be a valuable and inexpensive therapeutic modality (Norman, 2004). Metformin has become an established treatment for women with polycystic ovary syndrome, although controversy remains as to how effective it is and in which populations it should be used. Moreover, metformin should always be used as an adjuvant to general lifestyle improvements and not as a replacement for increased exercise and improved diet (Lord and Wilkin, 2004).

Most studies have shown that metformin causes improved hyperinsulinemia and reduced free androgen levels, specially free T, increased SHBG, decreased LH and finally, improved ovulation in patients with PCO by decreased resistance to insulin and serum insulin levels reduction (Christine *et al*., 2003).

However, the results of studies are in contrary; for examples, in a study (Crave *et al*., 1995) there was no change in metabolic and hormonal profiles in metformin therapy group compared with placebo group. On the other hand, the reports about metformin effects on obese and thin women are in controversy. Some researchers suggested weight loss as a reason of benefit effects of metformin (Crave *et al*., 1995).

Most of available information is related to a few studies performed in obese women and it has been reported that metformin improves ovulation rate in obese women with PCOS significantly (Nestler *et al*. (1999, 1998) showed increased ovulation rate and elevated SHBG concentration by using metformin in obese women with PCOS. In another study, Moghett *et al*. (2000) have reported increased ovulation rate, decreased serum T, decreased fasting insulin and increased HDL after metformin administration.

Vandermolen *et al*. (2001) have reported increased ovulation rate after metformin administration in PCOS women who are resistant to CC.

This study was performed on women that did not have ovulation after 3 months treatment with 100 mg CC for 5 days and metabolic and hormonal profiles were compared before and after treatment in both groups.

The present study shows that metformin improves some of endocrine disorders in PCOS patients who are resistant to clomiphen citrate and restores regular menstrual cycles; however, its definite role in increased ovulation rate is unknown, because not only it is difficult to demonstrate its role, but also it is not possible to serial measure of patients serum progesterone levels as a weekly because they could not present regularly to perform tests.

In this study, most of patients had regular menstrual cycles after metformin administration but we did not detect any ovulation (Table 5). Pregnancy occurred after metformin therapy course completed in one case both in metformin group and placebo group.

There was significant weight loss in most of patients in the end of treatment course (Table 4). It appears that metformin capability in regulation of sensitivity to insulin is associated with body weight. In other words, metformin improves endocrine disorders by weight loss and it is not related to obesity and thinness of patients because in this study, mean BMI was less than 30. In summery, in present study, metformin therapy for 3 months with 500 mg, TDS, in PCO patients who are resistant to CC, improved factors including FSH (increased), SHBG (increased), fasting insulin (decreased), FBS and 2 hpp (decreased), cholesterol (decreased), LDL (decreased) and HDL (increased). There was no significant change in androgen levels. Because laboratory did not present androstenedione, it is only possible to measurement of T and DHEAS levels. In similar studies, DHEAS levels increased only in 50% of patients, but in our study it did not increase. Also, there was no significant change in T levels.

Table 4: Weight changes after treatment

Wight loss month	Case group (kg)	Control group (kg)	p-value
First	0.59	0	0.06
Second	1.4	0	0.06
Third	4.4	0	0.05

Table 5: Menstrual function and drug side effects after treatment

Frequency month	Regular menses	Irregular menses	Menstrual loss	Drugs side effects
First	15	3	7	13
Second	17	4	4	13
Third	18	5	2	13

There was appropriate change in lipid levels after therapy, perhaps secondary to hyperinsulinemia correction. There was significant difference after metformin therapy in levels of LDL, HDL and fasting cholesterol except triglyceride. Given that both hyperandrogenemia and hyperinsulinemia result in lipid profile disorders in PCOS patients independently, it appears that improved lipid profiles is secondary to improvement of hyperinsulinemia and hyperandrogenemia due to metformin therapy.

CONCLUSIONS

Despite of benefit effects of metformin therapy demonstrated in this study, we cannot prove its role in ovulation rate, but further studies will be required to evaluate its effects on ovulation and pregnancy.

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