Improved Responsiveness of PCOS Patients to Clomiphene After CYP17a Inhibitor

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Purpose: To study the effect of CYP17a inhibitor, "ketoconazole," on clomiphene responsiveness in PCOS patients.

Methods: Prospective analysis was employed with the setup at Alexandria IVF/ICSI center. Ninety-seven insulin-resistant PCOS patients undergoing ovulation induction using clomiphene citrate were randomly divided, by random number table, into two groups. The first group (n = 49) received ketoconazole (400 mg daily) till correction of metabolic syndrome followed by clomiphene (100 mg/day); the second group (n = 48) receiving clomiphene without ketoconazole pretreatment. Main outcome measures were incidence of clomiphene resistance, monofollicular response, fasting insulin/glucose ratio, serum testosterone, and pregnancy rates.

Results: The ketoconazole group showed significantly ($p < 0.05$) higher incidence of monofollicular response (38%), higher pregnancy rates, and significantly less marked antiestrogenic manifestations than did the control group. They also had significantly lower incidence of clomiphene resistance (11.6%), lower serum testosterone levels, less hyperinsulinaemia, than did the control group.

Conclusion: Ketoconazole improved clomiphene responsiveness in PCOS patients and attenuated its untoward biological effects.

KEY WORDS: clomiphene resistance; CYP17a inhibitor; hyperandrogenism; insulin resistance; ketoconazole; PCOS.

INTRODUCTION

In polycystic ovarian syndrome (PCOS), excessive autophosphorylation of serine residues, together with reduction of tyrosine autophosphorylation, result in an upregulation of the 17,20 lyase component of ovarian and adrenal cytochrome P450 C17 alpha, leading to the characteristic hyperandrogenaemia (1). Patients most likely not to respond to clomiphene citrate are those who are most hyperandrogenic, overweight, and insulin resistant (2).

The hyperandrogenic intrafollicular milieu leads to inhibition of aromatase activity through 5-alpha reduced androgenic metabolites (3,4), favoring a premature shift of granulosa cells from the proliferating to the steroidogenic stage (5-7). Hyperinsulinaemia and insulin resistance further disturb folliculogenesis and granulosa cell function (8,9). They mainly act through IGF-1 receptor (10) that stimulates FSH and LH receptor formation (10). This, in addition, stimulates granulosa cell proliferation, steroidogenesis, and oocyte maturation (11-13). Hyperinsulinaemia also augments the deleterious effects of androgens on folliculogenesis through inhibition of sex hormone binding globulin (SHBG) synthesis (14), an upregulated IGF system (15,16), and an augmented thecal androgen production in response to LH (17).

Ketoconazole, a broad-spectrum antifungal drug, has shown to be a nonspecific inhibitor of cytochrome P450 hydroxylase-lyase enzyme (18). At low doses, it affects gonadal 17 lyase activity only (19). In high doses it reduces basal adrenal androgen by 60% (20). Low-dose ketoconazole, administered to PCOS women, decreased serum values of androstenedione, testosterone, and free testosterone. Because ketoconazole reportedly decreases serum androgen it could play a role in improving responsiveness to clomiphene. In this study, the effect of

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ketoconazole on clomiphene-induced ovulation in PCOS is evaluated.

MATERIAL AND METHODS

The study included 97 infertile females having PCOS and insulin resistance, undergoing ovulation induction over 20 months duration. Their male partners had normal semen analysis.

After counseling about protocol design and duration of treatment, the patients were randomly divided into two groups, using random-number table:

Group A ($n = 48$). Induction of ovulation was done using clomiphene citrate for 3–6 cycles. Eleven women dropped out after 1–3 cycles and were excluded from the study. The remaining 37 patients had a total of 136 cycles over a period of 9 months. Only patients who persistently failed to respond to clomiphene 150mg daily (clomiphene resistant) were shifted to hMG.

Group B ($n = 49$). They received ketoconazole therapy (400 mg/day) for 85 days as a pretreatment before induction of ovulation. They then received clomiphene citrate 100–150 mg daily. After six patients had dropped out, the remaining 43 received treatment over a period of 9 months during which 180 cycles of stimulation were performed. Only patients who persistently failed to respond to 150 mg clomiphene citrate were shifted to hMG.

Assessment and Follow Up

Initial assessment included Body Mass Index (BMI), waist-to-hip ratio, Ferriman-Gallway score, *Acanthosis nigricans*, serum total testosterone level, insulin response to 75 g oral glucose load (Insulin curve), as well as serum FSH, LH, and prolactin. Follow up for Group B included also pattern of menstrual cycle, weight, serum transaminases, ovulation scanning, serum progesterone, testosterone and insulin levels, as well as any side effects noticed by the patient.

Methods of Hormonal Assay

Insulin Assay. It (Medgenix-Ins-EASIA Biosource, Belgium) is a solid phase enzyme amplified sensitivity immunoassay performed on microtitre plate. Performance characteristics include minimum detectable concentration (MDC) estimated to be $0.15 \mu\text{IU/mL}$, intraassay CV (5.3–3.0%), and interassay CV (5.6–9.8%), for low and high concentration, respectively.

Testosterone Assay. Direct solid phase enzyme immunoassay for the quantitative measurements of testosterone was performed using EIA testosterone kit (Bio CHEM IMMUNOSYSTEMS, Bologna, Italy). For serum samples with testosterone concentration ranging from 1 to 10 ng/mL, within and between assay coefficients of variation are 4–9%. The lower limit of detection was assessed to be approximately 0.1 ng/mL, conversion factor ($1 \text{ ng/mL} = 3.467 \text{ nmol/L}$).

The Statistical Analysis

The statistical analysis was performed using a statistics computer program (SPSS for windows, package release 5.0; SPSS Inc. Chicago, IL, U.S.A.). Clinical characteristics of the two groups were analyzed by using the unpaired student *t* test or the Mann-Whitney rank-sum test. All other analyses were performed by using χ^2 analysis. *P* value <0.05 was considered statistically significant.

RESULTS

Before treatment the mean value of serum testosterone and insulin were not different between the two groups (testosterone $1.8 \pm 0.5 \text{ ng/mL}$ fasting insulin $14.5 \pm 3 \mu\text{IU/mL}$, and insulin after 30, 60, and 120 min were $129 \pm 9 \mu\text{IU/mL}$, $134 \pm 12 \mu\text{IU/mL}$, and $119 \pm 11 \mu\text{IU/mL}$. In Group B, 85 days of ketoconazole resulted in a significant reduction in testosterone and insulin values (testosterone $1.1 \pm 0.4 \text{ ng/mL}$ fasting insulin $12 \pm 3 \mu\text{IU/mL}$ and insulin after 30, 60, and 120, were $84 \pm 8 \mu\text{IU/mL}$, $72 \pm 5 \mu\text{IU/mL}$, and $51 \pm 7 \mu\text{IU/mL}$). The incidence of clomiphene resistance was significantly ($p < 0.05$) less in Group B (receiving ketoconazole pretreatment) as compared to Group A (11.6 vs. 32.4%). In the clomiphene-responsive women, Group B (who received ketoconazole pretreatment) showed significantly higher incidence of monofollicular response (38%) and lower incidence of triple follicular response (13%) as compared to Group A (23 and 27%, respectively) (Table I).

In clomiphene, responsive women ($n = 63$) in Group B the incidence of poor cervical mucus (10.6%), thin endometrium (3.9%), and low serum progesterone level (8.9%), were significantly lower ($p < 0.05$) as compared to Group A (30.2, 25.7, 30.2%, respectively) (Table II).

In the clomiphene-resistant patients in both groups, serum testosterone and insulin values before

Table I. Comparison Between Groups A and B as Regards Pattern of Follicular Response

Follicular response	Group A ^a	Group B ^a
One follicle	31 cycles (23%)*	68 cycles (38%)*
Two follicles	68 cycles (50%)	89 cycles (49%)
Three follicles	37 cycles (27%)*	23 cycles (13%)*

^a Group A: clomiphene without ketoconazole, Group B: ketoconazole followed by clomiphene.

* Significantly different ($p < 0.05$).

randomization were significantly higher than the clomiphene-responsive group (Table III). These metabolic abnormalities, before randomization, were more marked in the subgroup who failed to respond despite ketoconazole pretreatment (Group B), indicating a more profound metabolic syndrome (Table III).

Lastly, in Group A the pregnancy rate/patient was 21.6% and the incidence of cardiac pulsation/patient was 13.5%. For Group B, pregnancy and cardiac pulsation rates were 39.5 and 37.2%, respectively. The incidence of singleton twins and triplets as well as abortion after cardiac pulsation are shown in Table IV.

DISCUSSION

Clomiphene citrate antagonizes the action of estrogen at the level of the hypothalamus and pituitary by blocking the replenishment system (21) and exhibiting a negative cooperative effect on ligand-receptor complex (22), releasing the hypothalamus and pituitary from the inhibitory negative feedback of estrogen (23).

The improved response to clomiphene after correction of the hyperandrogenic state possibly reflects a less androgen-dominant intrafollicular environment (24). Also, lower systemic androgen levels seem to check the estrone-induced sensitized state of the pituitary and hypothalamus to the negative feedback effect of estradiol (25), augmenting the action of clomiphene. The recovered SHBG synthesis, no longer suppressed by androgens, leads to a

Table II. Antiestrogenic Manifestations in Groups A and B

Parameter	Group A	Group B
% poor mucous	41/136 (30.2)*	18/180 (10.0)*
% Thin endometrium	35/136 (25.7)*	7/180 (3.9)*
% progesterone <10 ng/mL	41/136 (30.2)	16/180 (8.9)

* Significantly different ($p < 0.05$).

Table III. Metabolic Syndrome and Clomiphene Resistance in Groups A and B

Parameter	Group A		Group B	
	CC responsive	CC resistant	CC responsive	CC resistant
No. of patients	25	12	38	5
No. of cycles	94	42	164	16
Testosterone (ng/mL)	1.2 ± 0.4*	1.6 ± 0.3*	1.1 ± 0.3*	1.8 ± 0.6*
FSH (mIU/mL)	6.7 ± 1.2*	3.9 ± 1.0*	4.9 ± 1.1	5.2 ± 2.1
LH (mIU/mL)	10 ± 2	9.6 ± 1.6	8.7 ± 1.1	7.8 ± 2.0
Fasting insulin (mu/mL)	12 ± 6*	21 ± 4*	10 ± 3*	29 ± 7*
Fasting glucose/insulin	4.1 ± 0.6*	3.4 ± 0.5*	3.8 ± 0.3*	2.9 ± 0.7*

Note. Assessment is before randomization.

* Significantly different ($p < 0.05$).

lower free plasma estradiol level (26), keeping the feedback inhibition to the minimum. Estradiol has been proved to act at the level of GnRH gene regulation (27), to modulate the amplitude and frequency of gonadotrophin release from the hypothalamus (28) and to alter gonadotrophin activity by affecting the sialylation pattern of the molecule during posttranscriptional modification (29).

The marked improvement in the peripheral antiestrogenic parameters shown after ketoconazole treatment points to the hyperandrogenic state as a major contributor to blame, beside clomiphene. Androgens are documented to antagonize estrogen by inhibiting estrogen-induced mRNA activity in the cytoplasm (30).

The higher progesterone levels, in the ketoconazole pretreated group, indicate a more adequate corpus luteum function, a possible consequence to a more optimized LH receptor expression in granulosa and theca cells during the follicular phase (31).

In conclusion, ketoconazole, through correction of the metabolic syndrome, improved clomiphene responsiveness in PCOS and attenuated its untoward biological effects. Because of its clinical implications,

Table IV. Pregnancy Outcomes In Groups A and B

Pregnancy outcome	Group A	Group B
% of clinical pregnancy	8/37 (21.6)	17/43 (39.5)
% of cardiac pulsation	5/37 (13.5)	16/43 (37.2)
Singleton pregnancy (%)	2/8 (25)	9/17 (52.9)
Twins (%)	5/8 (62.5)	5/17 (29.4)
Triplets (%)	1/8 (12.5)	3/17 (17.6)
Abortion after cord pulse (%)	1/5 (20)	0

clomiphene resistance in PCOS women possibly needs to be redefined.

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