Effects of ketoconazole on ovarian response in patients with polycystic ovarian syndrome: A double blind randomized clinical trial

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Abstract

Objectives: The study assessed the efficacy of low dose ketoconazole in addition to clomiphene citrate (CC) and hMG on ovulation induction.

Materials and methods: A double blind, randomized, clinical trial was performed on fifty infertile patients with PCOS who had failed to respond to a daily dose of hMG and 100 mg CC for controlled ovarian hyperstimulation in Vali-e-Asr Reproductive Health Research Center. These patients were randomly divided into two equal groups receiving ketoconazole or placebo. All patients received CC and hMG for controlled ovarian hyperstimulation. Number of mature follicles, estradiol and progesterone levels at the time of hCG administration, endometrial thickness, ovarian hyperstimulation syndrome (OHSS), pregnancy rate, abortion, multiple pregnancies were measured. SPSS 11 software was used for statistical analysis. Statistical significance was defined as \( P < 0.05 \).

Results: No significant statistical differences existed in the number of mature follicles, estradiol and progesterone levels prior to hCG administration, endometrial thickness, OHSS and pregnancy rate between two groups. Estradiol level was lower among those receiving ketoconazole. No abortion and multiple pregnancy were found.

Conclusion: Ketoconazole may suppress steroid production in resistant PCOS patients undergoing CC and hMG induction. But it has no effects on follicular maturation and OHSS prevention.

Key Words: Ketoconazole, polycystic ovarian syndrome, ovulation induction, ovarian hyperstimulation syndrome, ovarian steroid production

Introduction

Polycystic Ovarian Syndrome (PCOS) is the most common (5-10%) endocrine disease among women in reproductive age (1, 2). Sonographic findings show a prevalence of 23% among apparently healthy women (3).

This syndrome is one of the most complicated diseases with multisystem involvement in which various confounding factors affect its diagnosis. One of the most common complications of anovulation in PCOS is infertility. Different drugs are used to induce ovulation in patients with PCOS and infertility. Clomiphene citrate, a nonsteroidal estrogen agonist has been used for the treatment of anovulation in patients with amenorrhea or oligomenorrhea since 1961 (4). About
80% of women with PCOS ovulate after clomiphene citrate therapy and around 60% become pregnant. Relative fertility in females with oligomenorrhea who used clomiphene citrate was 6.8 times more than those who received placebo (5). However, a proportion of patients with PCOS show resistance to ovulation induced by clomiphene citrate; this finding is more common among patients who are obese, have high insulin resistance and hyperandrogenism (6). Another complication reported in ovulation induction in patients with PCOS is ovarian hyperstimulation syndrome (OHSS) (7) which could become life threatening (8). Multiple pregnancy is another problem faced by PCOS patients undergoing ovulation induction and could cause complications and danger for both the mother and the fetus. Ketoconazole is one of the drugs used for PCOS patients under ovulation induction to help improve response of the ovaries and to reduce associated complications. Ketoconazole is a wide spectrum antifungal imidazole, which affects the P450 enzyme in the testes, ovaries, adrenal glands and liver (9). It blocks estrogen production by affecting the enzyme lyase 17-20 and 17-α hydroxylase. Different studies have shown the inhibitory effect of ketoconazole on steroidogenesis (10-15). Low dose ketoconazole only affects gonadal function (16) and higher doses block androgen synthesis by 60% (17). Regarding the inhibitory effect of ketoconazole on androgen synthesis, Ali Hassan et al (6) showed that low dose ketoconazole increases the response of clomiphene citrate in patients with PCOS who are resistant to clomiphene citrate and it can decrease the complications of ovulation induction in these patients. These findings were in accordance to those of Gal et al (18). Tsafri et al(19) also showed that ketoconazole could cause maturation of ovarian follicles. However, in the study performed by Parsanezhad et al (20) low dose ketoconazole was found to have no effect in the prevention of OHSS in patients with PCOS receiving ovulation induction and is only effective in the production of follicles and steroids.

Considering the controversy which exists in this field, we decided to compare the effect of ketoconazole in PCOS patients, who were resistant to ovulation induction produced by clomiphene citrate through a randomized double blind clinical trial and to compare them with patients receiving placebo in respect to level of steroid production, production of follicles, OHSS and pregnancy rate.

Materials and methods

This double blind randomized clinical trial was approved in the ethical committee of Vali-e-Asr Reproductive Health Research Center and performed on fifty infertile patients with PCOS who attended to our infertility clinic of Vali-e-Asr Reproductive Health Research Center, in the year 2002. These patients had failed to respond to a daily dose of 150 mg clomiphene citrate for ovulation induction. Inclusion criteria included: 1) diagnosis of PCOS based on history of oligomenorrhea or amenorrhea along with sonographic findings and hirsutism with a score above seven or presence of hyperandrogenism, 2) age ranges between 18-34 years, 3) absence of pelvic inflammatory disease (PID), 4) normal hysterosalpingography, 5) normal semen analysis according to WHO criteria, 6) 20<Body Mass Index (BMI) <25 kg/m2, 7) history of infertility less than 3 years and, 8) failure of response to ovulation induction produced by 150 mg clomiphene citrate. Exclusion criteria included: 1) indication for Assisted Reproductive Thechnology (ART), 2) failure to obtain patients' consent for enrollment, 3) history of liver diseases. Data was collected and
Effects of ketoconazole on ovulation

compiled by history taking, medical records, laboratory results and physical examination. After selecting the patients according to the inclusion and exclusion criteria, they were divided into two equal groups using permuted block randomization (by randomly picking sealed envelopes). All the patients in both groups received a daily dose of 100 mg clomiphene citrate (Iran Hormone, Iran) started on the third day of the cycle and two ampules of hMG (75IU; Serono, Netherlands) were injected on the 7th day of the menstrual cycles for controlled ovarian hyperstimulation. Patients in group A received 50 mg ketoconazole (Rooz Daru, Iran) every other day while group B received placebo. Number of follicles, level of estradiol (E2) and progesterone, endometrial thickness, signs of ovarian hyperstimulation, abortion and multiple pregnancy were compared between the two groups. At the onset of study, serum E2 was measured and transvaginal sonography by using 6.5 MHz transvaginal probe, GE ultrasound device was performed. In order to assess the response of the ovaries during the treatment period, transvaginal sonography was performed and serum E2 was assayed to control the dose of hMG. Group A received ketoconazole tablets 50 mg every other day along with the start of clomiphene citrate therapy and was continued until the final day of ovulation induction. Because ketoconazole dissolves better in acidic pH, patients were advised to take the dose on an empty stomach two hours before going to bed. Serial transvaginal sonography was performed at intervals of 1-3 days in order to assess follicular growth and endometrial thickness. Measurements were made by determining mean follicular diameter and full endometrial thickness. The number of dominant follicles (diameter>17mm) was recorded accurately and all other small follicles were also recorded for the interest of physicians to look out for OHSS. Serum transaminase levels were checked in both groups before receiving ketoconazole or placebo and once more on the final day of ketoconazole or placebo therapy. Ovulation induction was considered successful if 1-3 dominant follicles were present and if serum estradiol was 1000-1500 pg/ml. Once the dominant follicle reached 17-18mm, 10000 units of hCG (10000IU; Serono, Netherlands) was given intramuscularly. If estradiol level was >2000 pg/ml or if more than five medium sized follicles were present in each ovary, the cycle was halted and hCG was not administered. Two weeks after giving hCG, serum βhCG, was checked and transvaginal sonography was performed after four weeks to observe the fetal sac and heart activity. Transvaginal sonography was repeated after 1 month. Mid luteal (7-9 days after hCG administration) serum progesterone level was estimated to confirm ovulation. Before entering the study, written consent was obtained from all participants and all probable adverse effects and cost of drugs were explained. The researchers paid the cost of the drugs and placebo. After collecting the data, SPSS 11 software was used for statistical analysis. Chi square test, fisher’s exact test, t-test and Mann Whitney test were used to compare the two groups and to analyze the statistical findings. Statistical significance was defined as p < 0.05.

Results

Background variables including age, duration of marriage, infertility and BMI were compared between the two groups. Infertility was primary in 18 (72%) and 16 (64%) of the patients in group A and B, respectively, which was not statistically significant. One patient (4%) receiving ketoconazole and 3 patients (12%) receiving placebo were excluded.
from the study due to their poor cooperation (NS). The number of menstrual cycles that responded to ovulation induction were 21 (87.5%) and 21 (95.5%) in group A and group B, respectively. Mean serum progesterone level prior to hCG was 1.85 ng/ml (0.4-3.0) in group A and 1.9 ng/ml (1.0-4.8) in group B (NS). Mean mid luteal serum progesterone level was 36 ng/ml (13-72) in group B (NS). Results are shown in Table 1. Table 2 shows the results of comparison of the two groups regarding number of mature ovarian follicles, endometrial thickness during hCG therapy, mean number of hMG ampules administered per patient, and mean duration of receiving hMG and clomiphene citrate. Only 3 (13.6%) patients in group B had signs of OHSS (NS). Liver enzymes, including ALT and AST were measured prior to and at the end of therapy and they did not show a statistically significant difference between the two groups (Table 3). Overall, out of the 21 patients in group A and 21 patients in group B who received hCG, 3 (14.3%) and 2 (9.5%) became pregnant in group A and B, respectively and βhCG test was positive two weeks after hCG (NS). Fetal sac was visible on repeated sonography performed 4 and 8 weeks after hCG administration. None showed abortion or multiple pregnancy.

**Table 1: Serum estradiol and progesterone levels in groups under study**

<table>
<thead>
<tr>
<th></th>
<th>Ketoconazole (n=24)</th>
<th>Placebo (n=22)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Estradiol level prior to hCG administration (pg/ml)</td>
<td>597.3 (50-1150)</td>
<td>772.5 (164-1600)</td>
<td>NS</td>
</tr>
<tr>
<td>Progesterone level prior to hCG administration (ng/ml)</td>
<td>1.85 (0.4-3)</td>
<td>1.9 (1-4.8)</td>
<td>NS</td>
</tr>
<tr>
<td>Progesterone level prior to hCG administration (ng/ml)</td>
<td>38 (15-74)</td>
<td>36 (13-72)</td>
<td>NS</td>
</tr>
</tbody>
</table>

*Mann-Whitney test was used for comparison between two groups.*

**Table 2: Number of follicles, endometrial thickness, number and duration of hMG ampoules in two groups.**

<table>
<thead>
<tr>
<th></th>
<th>Ketoconazole (n=24)</th>
<th>Placebo (n=22)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total no. of follicles</td>
<td>5.2±1.5</td>
<td>6.1±2.4</td>
<td>NS</td>
</tr>
<tr>
<td>Endometrial thickness (mm)</td>
<td>8.8±2.3</td>
<td>8.3±1.9</td>
<td>NS</td>
</tr>
<tr>
<td>Mean no. of hMG ampoules administered</td>
<td>8.9±2.9</td>
<td>7.8±2.1</td>
<td>NS</td>
</tr>
<tr>
<td>Mean duration of hMG ampoule administration (days)</td>
<td>5.8±2.8</td>
<td>5.9±3.1</td>
<td>NS</td>
</tr>
</tbody>
</table>

*T test was used for comparison between two groups.*
Table 3: Liver enzyme levels in two groups.

<table>
<thead>
<tr>
<th></th>
<th>*Prior to Intervention</th>
<th>*After Intervention</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean±SD</td>
<td>Mean±SD</td>
<td></td>
</tr>
<tr>
<td>Ketoconazole ALT</td>
<td>29.5±8.4</td>
<td>29.9±7.7</td>
<td>NS</td>
</tr>
<tr>
<td>Ketoconazole AST</td>
<td>26.8±7.3</td>
<td>28.9±7.4</td>
<td>NS</td>
</tr>
<tr>
<td>Placebo ALT</td>
<td>28.4±5.4</td>
<td>28.7±6.6</td>
<td>NS</td>
</tr>
<tr>
<td>Placebo AST</td>
<td>27.3±6.0</td>
<td>28.3±6.4</td>
<td>NS</td>
</tr>
</tbody>
</table>

*Values are presented as U/L.
Paired T test was used for comparison between two groups.

Discussion

Previous reports show the inhibitory effect of ketoconazole on steroid production in diseases such as Cushing's syndrome, hirsutism in women with PCOS, premature puberty, in prostate cancer and ovarian cancer (5-11). The method of study, dose and mode of drug administration varies in each study. The current study assessed the effect of ketoconazole 50 mg every other day on PCOS patients undergoing ovulation induction with clomiphene citrate and hMG. The response of ovaries, in the form of mature follicle production, was not statistically different between the two groups in our study. On the contrary, a study performed by Gal et al (18) on the response of ovaries in PCOS patients using low dose ketoconazole (50 mg daily or every other day) showed that the frequency of mature follicle production and the success rate of induction was significantly higher among patients receiving ketoconazole. This finding was not in accordance with the study performed by Parsanezhad et al (20) in which the effect of ketoconazole administered (50 mg every other day) for controlling OHSS showed that there is no relationship between the frequency of mature follicle production and ketoconazole therapy. Our findings show that ketoconazole has no effect on total number of growing follicles, but rather, it probably decreases medium-sized follicle production. In our study, among patients who responded to ovulation stimulation, the total number of mature follicles in the two groups did not show a statistical difference; this was similar to the findings of Gal et al (18) and Parsanezhad et al (20). Conversely, Hughes et al’s (5) study showed different results because the frequencies of single ovum cycles were significantly higher in the group receiving ketoconazole. Although serum estradiol level, prior to hCG administration was lower among those receiving ketoconazole than those who received placebo, but this difference was not statistically significant. This finding is, to some extent, similar to the results of the study performed by Parsanezhad et al (20) in which serum E2 concentration had significantly decreased on day 9 of the menstrual cycle in patients receiving ketoconazole, whereas a significant statistical difference was not found to exist between the two groups on the day of hCG administration, which is in agreement with to the current study. However, previous studies have shown a significant relationship between ketoconazole therapies and decreased ovarian steroid production (1, 3, 5-11). The effect of ketoconazole on reducing the steroid synthesis is explained by its inhibitory effect on the enzyme 3-β hydroxy steroid dehydrogenase (18, 20). However, this study could only
determine the difference in estradiol level between the two groups by about 50%. In our study, serum progesterone level was not statistically significant prior to administering hCG or in the mid luteal phase. These findings were similar to those of Gal et al (18). However the results of Tsafiri et al (19) differed due to the fact that progesterone synthesis was inhibited by ketoconazole. In addition, another study (10) showed that the level of progesterone synthesis decreased significantly after ketoconazole therapy, which may be due to the accumulation of androgen substrates (17-hydroxy progesterone) as a result of the inhibitory effect of ketoconazole on androgen production. The current study did not show a clear difference in endometrial thickness between the two groups, which resembled the findings of Gal et al (10) and Parsanezhad et al (20), whereas Ali Hassan et al (6) showed that the frequency of thin endometrium (thickness<10mm) was less in those receiving ketoconazole which may be due to the mild effect of ketoconazole on steroid and progesterone production. In our study, the duration of hMG and clomiphene citrate administration did not differ between the two groups that resembled the study performed by Gal et al (18). However, the study performed by Parsanezhad et al indicates that the duration of hMG administration was significantly higher among those receiving ketoconazole as compared to the placebo group. According to our study, ketoconazole therapy was not very effective in the prevalence of OHSS in PCOS patients. This finding was reported in similar studies (18, 20). However, all three patients with OHSS belonged to the placebo group. The power of our study in assessing the relationship between OHSS with ketoconazole administration was 20%. We did not find any complications due to ketoconazole use and a significant difference was not recorded with liver function tests; this resembled the study performed by Gal et al (18) and Parsanezhad et al (20) in that the dose of ketoconazole administered was similar. However, in other studies in which the dose was higher (400 mg daily) complications including pruritis, gastric complaints, mastodynia (7), nausea, dyspepsia, dysfunctional uterine bleeding, hair loss, psychological changes and transient enzyme elevation were reported (5,22). Fertility rate did not differ between the two groups and they resembled some previous studies (1,2). However, Ali Hassan et al (6) showed that fertility was significantly higher among patients who received ketoconazole. The results of this study showed that low dose ketoconazole (50 mg per 48 hours) might, to some extent, inhibit ovarian steroid production. However, it has no effect on the production of follicles, improvement of laboratory test results in patients or in the prevention of OHSS. Meanwhile, a higher statistical power is required to make better judgments in this field.

Acknowledgement

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References

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