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A DOUBLE BLIND, RANDOMIZED, PLACEBO CONTROLLED STUDY TO ASSESS THE EFFICACY OF KETOCONAZOLE FOR REDUCING THE RISK OF OVARIAN HYPERSTIMULATION SYNDROME

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ABSTRACT

Background: In order to evaluate the role of ketoconazole in the prevention of ovarian hyperstimulation syndrome (OHSS) in women with polycystic ovary syndrome (PCOS) undergoing ovarian stimulation with gonadotropins, a prospective, randomized, double-blind, placebo controlled study was done on one-hundred and nine PCOS women that had been referred to be treated by gonadotropins.

Methods: All 109 women were assigned for random allocation. Group A (50 patients) received two ampoules of hMG beginning on day 2 or 3 of the cycle and ketoconazole (50 mg/every 48 hours) starting on the first day of hMG treatment. Group B (51 patients) received the same protocol of hMG combined with one tablet of placebo every 48 hours. Main outcome measures were follicular development, E2 levels, and pregnancy rate.

Results: The total number of hMG ampoules and duration of treatment to attain ovarian stimulation was higher in group A ($p<0.0001$). Serum E2 level and number of patients with dominant follicles on day 9 of the cycle were higher in group B ($p<0.0001$). There was no significant difference between serum E2 level and total number of follicles at the time of hCG administration in the two groups. The cancellation rate and OHSS rate were similar in the two groups.

Conclusion: Ketoconazole has no effect in prevention of OHSS in PCOS patients undergoing ovarian stimulation. It may however reduce the rate of folliculogenesis and steroidogenesis.

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Keywords: Polycystic ovary syndrome, ovarian hyperstimulation syndrome, ketoconazole.

INTRODUCTION

Ovarian hyperstimulation syndrome is a relatively common and potentially life-threatening complication of ovarian stimulation by gonadotropins.[1] In its severe form, the syndrome is characterized by huge ovarian multifollicular enlargement, ascites, hydrothorax, hypovolemia and hemoconcentration.[2] The underlying mechanisms leading to the syndrome still remain elusive, but excessive quantities of

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peptides regulating the growth and permeability of blood vessels are likely to be involved.[3] Some authors have suggested that the augmentation of steroids and peptides (vascular endothelial growth factor) production from the multifollicular hyperstimulated ovaries may be responsible for several features of OHSS.[4]

Vascular endothelial growth factor (VEGF) has been shown to exert a twofold action: it serves as a potent promoter of neovasculogenesis on one hand, and increases vascular permeability on the other.[5,6,7] Early identification of the patients at risk and prevention of OHSS is clinically important. Several methods have been recommended for minimizing the probability of the syndrome in high risk patients including canceling the cycle before administration of hCG or reduction of the ovulatory hCG dose,[8] withholding luteal support with hCG,[9] cryopreservation of all embryos for further use in a non-stimulated cycle,[10] repeated aspiration of ovarian follicles and early corpus luteum cysts,[11] administration of human albumin at the time of hCG injection,[12] use of GnRh analogue to trigger ovulation,[13] and vaginal aspiration of ascites.[14]

Ketoconazole, a broad-spectrum imidazole antimycotic agent, was recently reported to reduce the incidence of OHSS in PCOS women undergoing superovulation program by gonadotropins.[15] Ketoconazole interferes with cytochrome P-450 enzyme systems in several organs (testis, ovary, adrenal gland, liver). Steroidogenesis is inhibited by its action on the C17-20 lyase, the cholesterol side-chain cleavage enzyme and the 17-alpha-hydroxylase. In gonads it inhibits aromatase and adrenocortical steroid biosynthesis is also inhibited at the 11-beta-hydroxylation and 18-hydroxylation steps. Its antiandrogenic effect may be useful in the management of metastatic prostate carcinoma, and hirsutism. Its anticortisolic effect may be useful in most Cushing's syndrome patients.[16,17,18] As the recent report[15] was not a placebo-controlled trial, it was impossible to rule out placebo effects. We designed this double blind, controlled study to assess the role of ketoconazole in prevention of multifollicular development, excess ovarian steroidogenesis, and moderate and severe OHSS in patients with polycystic ovary syndrome (PCOS) undergoing superovulation program by gonadotropins.

MATERIAL AND METHODS

The study was performed in the infertility and reproductive endocrinology division, Department of Obstetrics and Gynecology, Medical School, Shiraz University of Medical Sciences, Shiraz, Iran. From Sep. 2000 to Nov. 2002, a total of 637 women with PCOS from our infertility division enrolled into this prospective, randomized, double blind, placebo controlled study. Patients were interviewed, the study protocol was described and their charts were carefully reviewed. They had undergone a complete infertility evaluation that included hormonal assay, hysterosalpingogram, post-coital test, semen analysis, and endometrial biopsy. The inclusion criteria were infertility, an elevated serum level of luteinizing hormone (LH), normal or elevated follicle stimulating hormone (FSH), LH/FSH>2, elevated testosterone and DHEAS, oligomenorrhea or amenorrhea and at least 10 follicles sized less than 8 mm in diameter in the subcapsular region around hyperechogenic central stroma found by the ultrasound scan.

Only women with PCOS-related anovulatory infertility were enrolled in the study and women with other infertility factors were excluded. A total of 528 patients were excluded: 217 women did not fulfill the inclusion criteria and 311 women refused to participate. One-hundred and nine women who met the full study criteria were allocated for randomization after giving informed consent. Women had been unsuccessfully treated with clomiphene citrate (CC) of up to 200mg/day for 5 days. Women who had clomiphene failure were considered for treatment with human menopausal gonadotropins (hMG). They received allocated intervention from the first menstrual cycle after randomization.

The Ethics Review Committee for Human Research of the university approved the study. Serum concentrations of liver transaminase and bilirubin (LFT) were measured using Kinetic and Colour Anylin method respectively and E2 was assayed by using Coat-a-Count RIA, 2-3 days after the commencement of menstrual bleeding or progesterone induced-bleeding between days 1 and 3 of the menstrual cycle, when transvaginal sonography was performed using a 5-MHz transvaginal transducer (Medison 600). All patients showed the sonographic findings of PCOS. LFT was normal in all subjects. Serum E2 level was normal for the early follicular phase. Prior to receiving allocated intervention and workup, all patients were randomized using a random table. A pharmacist who did not take part in this study performed medication and placebo administration. Neither the patients nor the physician and laboratory staff knew the treatment protocol.

Ketoconazole administration protocol was similar to the one used as alternate protocol by Gal et al.[15] Group A (53 patients) received two ampoules of hMG (75 IU FSH and 75 IU LH per ampule) intramuscularly beginning on day 2 or 3 of the cycle and the minimal dose of ketoconazole (50 mg) starting on the first day of hMG treatment. Ketoconazole was given in a dose of 50 mg every 48 hours and its administration was limited up to the last day of HMG stimulation. Group B (56 patients) received the same protocol of HMG combined with one tablet of placebo every 48 hours. Ovarian stimulation was monitored by ultrasonographic assessment of total numbers of growing follicles, mean diameter of the follicles, and the full endometrial thickness and measurement of E2 levels performed every 2 days.

Ovulation was triggered by IM injection of 10000 IU of

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Table I. Effects of ketoconazole and placebo in the two groups of patients undergoing hMG superovulation.

Variable	Group A	Group B	*p*. Value
No. of cycles	50	51	
Duration of hMG therapy	13.5±0.98	9.6±1.9	$p<0.0001$*
Total No. of hMG ampoules/patient	19.44±1.5	15.18±1.9	$p<0.0001$*
E2 level on day 9 of the cycle	465.27±134.24	1001.54±552.28	$p<0.0001$*
£Patients with D.follicles on day 9	13(25.6%)	30(59.1%)	$p<0.0001$*
† E2 before hCG injection (pg/mL)	1349.58±381.71	1288.65±473.64	$p>0.05$
‡ ET before hCG administration	10.7±1.45	10.4±1.4	$p>0.05$
§ NOP with E2<1500pg/mL	36(72.1%)	38(75%)	$p>0.05$
? NOP who received 5000 IU of HCG	5(10%)	7(13%)	$p>0.05$
¥ No. of patients with 1-3 LF(1-3)	43(86%)	45(88.6%)	$p>0.05$
No. of successful stimulation cycles	36(72.1%)	38(75%)	$p>0.05$

£Number of patients who had dominant follicles (mean diameter >14 mm).

† Peak E2 level at the time of hCG administration.

‡ ET: Endometrial thickness, at the time of hCG administration.

§ NOP= Number of patients with E2 level<1500pg/mL at the time of hCG injection.

? NOP who received 5000 IU of HCG instead of 10000 IU due to the risk of OHSS.

¥ Number of patients with 1-3 lead follicles at the time of hCG injection.

* Significant difference.

hCG when the leading follicle reached a diameter of >16 mm and E2 concentration was >300 pg/mL for each main follicle (>16mm in diameter). LFT was repeated on the last day of ketoconazole and hMG administration. OHSS was predicted using criteria that previously were described by Rabe et al.[19] The syndrome was graded using previously established criteria.[20] If any patient was in the risk of OHSS, the hCG dose would be reduced to 5000 IU or her cycle would be cancelled (administration of hCG was withheld). Patients were monitored during the luteal phase 6±2 days and 12±1 days after the injection of HCG. They were asked about symptoms of OHSS (abdominal pain, nausea, vomiting, diarrhea, weight gain). Transvaginal ultrasonography was performed to measure the peritoneal fluid, ovarian size, a total count of cysts and maximum cyst diameter. Blood samples were taken for E2 and b-hCG level, blood count, plasma proteins and electrolytes measurement. If there was any evidence of moderate OHSS the patients were hospitalized for further management.

Statistical methods

A comparison was made between clinical and laboratory parameters in the treatment and the placebo group. Statistical analysis was performed using the Student t-test and Chi-square test. Correlations were calculated using 95% confidence. Levels of statistical significance was defined as $p<0.05$.

RESULTS

One-hundred and one women completed the study protocol. Three patients of group A and 5 patients of group B were lost to follow up. Comparison of the results of the demographic and infertility characteristics in group A (n = 50) and group B (n = 51) revealed no statistically significant differences in the mean (± SD) age (26.3 ± 3.1 years vs. 28.33 ± 4.0); the mean (±SD) body mass index (27.12 ± 2.1 vs. 28.62 ± 4.8); the mean (±SD) duration of infertility (3.7 ± 1.8 vs. 3.8 ± 1.4); the percent of subjects with primary infertility (81.4% vs. 75%). Ketoconazole showed no side effect with the treatment protocol used.

Duration of treatment with hMG was statistically longer in group A. The difference was significant ($p<0.0001$). Although the total numbers of follicles on day 9 of the cycle were similar in both groups (11.13±3.37 and 10.6±3.63 in group A and B respectively), the number of patients who had dominant follicles (mean diameter >14 mm) was statistically higher in group B. The difference was significant ($p<0.0001$). Serum E2 concentrations on day 9 of the cycle were significantly higher in group B (465.27±134.24 and 1001.54±552.28 in group A and group B respectively) ($p<0.0001$).

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Table II. Outcome of treatment in the two groups of PCOS patients undergoing hMG superovulation with ketoconazole or placebo.

Variable	Group A	Group B	*p*
No of pregnancies	9(18%)	11(21.5%)	$p>0.05$
No.of multifetal pregnancies	0	0	
No.of cancelled cycles	14(27.9%)	16(31%)	$p>0.05$
No.of patients with OHSS	4(7%)	5(%9)	$p>0.05$

The number of hMG ampoules that was needed to attain superovulation was significantly higher in group A ($p<0.0001$)(Table I). As shown in Table I, serum concentrations of E2, endometrial thickness at the time of hCG administration and the number of subjects that received 5000 IU of HCG were similar in the two groups. The number of patients with E2 less than critical levels of<1500pg/mL and the number of leading follicles at the time of hCG administration are shown in Table I. E2 level and number of leading follicles were similar in the two groups. Number of pregnancies, cases of OHSS, and number of cancelled cycles did not differ between ketoconazole and control groups (Table II).

DISCUSSION

The first researchers who suggested the use of ketoconazole for the syndrome were Gal et al.[15] who treated the patients with 50 mg of ketoconazole every 24-48 hours beginning on the first day of hMG administration. They reported a significant reduction in OHSS after treatment with ketoconazole. The regimen that we report on dose not differ from the one used as alternate protocol by Gal et al.[15] in terms of drug, dosage, and follow up. Our results showed a slow rate of E2 production during the follicular phase (on day 9 of the cycle) in the ketoconazole group as compared with control. Similar results were reported when ketoconazole was used in different doses.[21]

On the 9th day of the cycle, there were equal numbers of growing follicles in the two groups. However the number of patients who had dominant follicles (mean diameter >14 mm) was significantly higher in group B. This suggests that the drug had no effect on the total number of growing follicles, but the rate of follicular growth was reduced. Ketoconazole was supposed to prevent multiple follicular growth and excess steroidogenesis in spite of continuing gonadotropins to attain acceptable numbers of dominant follicles and related serum E2 levels and to reach a successful stimulation cycle.

However when the administration of gonadotropins were continued to reach an acceptable number of dominant follicles to trigger ovulation by hCG, some patients developed hyperstimulated ovaries. Zelinski-Wooten et al.[22] and Moudgal et al.[23] also reported similar results. They found out that when trilostane, a 3b-hydroxysteroid dehydrogenase inhibitor and fadrozole, a nonsteroidal aromatase inhibitor was applied throughout the follicular phase in rhesus monkeys undergoing ovarian stimulation with human gonadotropins, despite the profound reduction of E2 levels, the drugs had no effect on the total number of antral follicles, their distribution, the number of retrieved oocytes, and ovulation in the treated animals. In contrast to these reports Gal et al.[15] in their recent study showed a substantial reduction in the number of medium-sized follicles. They also reported a reduction in cancellation rate from 37% to 7%. In our study the incidence of OHSS in both groups are similar to that which Gal et al.[15] observed in their untreated cycles. We also could not find any significant differences in cycles cancellation and hyperstimulated cycles between our two groups. The numbers of successful stimulation cycles were also similar in ketoconazole and control groups. Our results suggest that during ovulation induction with gonadotropins, ketoconazole may lower the rate of folliculogenesis and steroidogenesis.

The total number of follicles including small follicles, medium-sized follicles, lead follicles and also serum levels of E2 are not affected and finally are similar in both groups at the time of hCG administration. Our hypothesis that ketoconazole has no effect on the prevention of OHSS may be explained by these findings. Gal et al.[15] reported that low dose ketoconazole had no effect on progesterone production. Progesterone assay has no critical role in the result of this study, thus we did not consider its values. Zelinski-Wooten et al.[22] reported a reduction of fertilization rate in the trilostane-treated monkeys. Similar results were reported by Moudgal et al.[23] when aromatase inhibitor was administered. Although we did not evaluate the fertilization process, the equal pregnancy rate per ovulatory cycle in our

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groups may support the idea that low dose ketoconazole has no adverse effect on fertilization.

In conclusion, in this randomized, placebo controlled, double blind study, we analyzed the acceptable number of patients. The results show that low dose ketoconazole during stimulation cycles with gonadotropins may reduce the rate of folliculogenesis and steroidogenesis. It has no beneficial effect on the development of multiple follicles, final serum levels of E2 at the time of HCG administration, OHSS, and cycle cancellation. Our study and previous works evaluated the limited parameters regarding the preventive effects of ketoconazole on development of OHSS. A further study to evaluate the effects of various protocols of ketoconazole on vasoactive agents affecting OHSS may be helpful.

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