Evaluation of low-dose letrozole addition to ovulation induction in IVF

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Summary

Purpose: The aim was to investigate the impact of low-dose letrozole usage along with gonadotropin treatment in vitro fertilization (IVF) cycles in comparison to gonadotropin treatment alone. *Materials and Methods:* Fifty patients were prospectively included in this randomized study and were divided into two groups. Age, demographic features, causes, and period of infertility were adjusted and matched for both groups. Group 1 included 25 patients who received gonadotropin treatment and letrozole along with gonadotropin-releasing hormone (GnRH) antagonist protocol; group 2 included 25 patients who received gonadotropin treatment along with GnRH antagonist protocol. *Results:* Total follicle-stimulating hormone (FSH) and daily FSH doses were lower in group 1, although not statistically significant ($p > 0.05$). The period of ovulation induction was significantly shorter in group 2. While numbers of retrieved oocytes and transferred embryos were lower in group 1, they were not statistically significant ($p > 0.05$). Number of clinical pregnancies per embryo transfer, number of clinical pregnancies per cycle, and number of ongoing pregnancies (> 16 gestational weeks) were similar in both groups ($p > 0.05$). *Conclusions:* Addition of low-dose letrozole to gonadotropin treatment in GnRH antagonist protocols may result in a lower dose of gonadotropin administration. However, routine clinical practice remains questionable due to no evident positive effect on pregnancy rates.

Key words: Aromatase inhibitor; Gonadotropin; GnRH antagonist; In vitro fertilization: Letrozole; Ovulation induction.

Introduction

Letrozole is an aromatase inhibitor (AI) that was first used in the treatment of advanced breast cancer in 1997 [1]. Then, it was soon realized that letrozole had potential for the treatment of other estrogen-dependent conditions, particularly in the field of gynecology. One application area of interest is the use of letrozole for induction of ovulation in women with World Health Organization (WHO) type II anovulation. The first report in this area was by Mitwally and Casper [2]. They argued that letrozole was effective in inducing ovulation in women with polycystic ovarian syndrome (PCOS). The use of letrozole for superovulation prior to in vitro fertilization (IVF) was also explored, especially its usage in women who had responded poorly to conventional treatment with gonadotropins [3-5]. Recently, some studies showed that addition of letrozole in IVF treatment may reduce the amount of gonadotropin administration that is required for ovulation induction [3, 6-7]. Less gonadotropin exposure may have a positive impact on oocyte quality and consequently pregnancy rates. In addition, reduced gonadotropin administration may have a beneficial effect on treatment cost.

The aim of this study was to investigate the impact of low-dose letrozole usage along with gonadotropin treatment in IVF cycles, in comparison to gonadotropin treatment alone, and the subsequent effect on IVF outcome.

Materials and Methods

Fifty patients administered to the Infertility Clinic of Istanbul University School of Medicine were prospectively included in this randomized study. Approval of the ethics committee and informed consent from all participants were obtained prior to the treatment.

Patients were divided into two groups. Age, demographic features, causes, and period of infertility were adjusted and matched for both groups. Group 1 included 25 patients who received gonadotropin treatment and letrozole along with gonadotropin-releasing hormone (GnRH) antagonist protocol, while group 2 included 25 patients who received gonadotropin treatment along with GnRH antagonist protocol without letrozole during IVF.

All patients included in the study received a thorough physical examination and were assessed with transvaginal ultrasonography (TVUS). Patients who were clinically infertile for at least two years and who were attempting IVF for the first time were included in the study. Reasons for infertility were male factor, ovarian factor, tubal-peritoneal factor, or unexplained factor. Male factor infertility was evaluated with spermiogram that was repeated twice in which WHO global reference values for human semen characteristics were utilized. Hysterosalpingography was also used as a diagnostic tool to identify potential tubal and peritoneal pathology in all patients. Patients were assessed by day-3 follicle-stimulating hormone (FSH), luteinizing hormone (LH), and estradiol (E2) levels as well. Thyroid function test and prolactin values were reviewed and patients who had lower or higher than normal results were excluded from the study. TVUS was performed on the day-2 of the menstrual cycle and patients who had ovarian cysts were also excluded from the study.

Exclusion criteria were: age above 40 years, FSH levels of more than 15 IU/l, antral follicle count (AFC) less than 5, body mass index (BMI) greater than 30, any abnormal ultrasound results (i.e. cyst, endometrioma, endometrial polyp, etc.), and previous IVF attempt(s).

Either Puregon® (Schering-Plough, NJ, USA) or Gonal-f® (EMD Serono, MA, USA) was used in controlled ovarian stimulation (COH) which was first administered on day-3 of the

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menstrual cycle. Initial gonadotropin dosage was decided according to the patient's age, AFC, BMI, FSH, E2, and ovarian reserve, and was regulated with a range from 150 IU to 225 IU. It was then adjusted according to the response of ovarian follicles, which were followed-up via TVUS. The total dosage administered to every patient was recorded. In addition, patients in group 1 also received 2.5 mg letrozole orally starting on the day-2 of the menstrual cycle and continued the usage until day 6. In both groups, when the dominant follicle size was ≥ 14 mm, GnRH antagonist protocol was initiated by administration of 0.25 mg cetrorelix acetate, which was continued until the human chorionic gonadotropin (hCG) injection day. Ovarian follicular development was observed via TVUS at a one- to three-day frequency. When at least three follicles ≥ 16 mm in size were found, 10,000 IU hCG were injected to achieve follicular maturation. Oocyte retrieval (OCT) took place 36 hours after hCG injection. All follicles ≥ 14 mm in size were retrieved. The number of retrieved MII oocytes was recorded. Three days after retrieval, one to three embryos were transferred to the uterine cavity according to number of good quality embryos (grade 1). The number of transferred grade 1 embryos was recorded. The first morning-after oocyte retrieval, all patients received 3 x 200 mg micronized progesterone vaginally as luteal phase support. If pregnancy occurred, vaginal luteal phase support continued until the 12th week of gestation. Twelve days after embryo transfer, β-hCG level in the blood was measured and recorded. If β-hCG level was > 5 mIU/ml in either measurement, it was considered positive β-hCG and patients with such levels were considered as pregnant. At the sixth week of gestation, continuation of pregnancy was confirmed by TVUS. Any complications that occurred during the entire process were recorded.

All statistical calculations were performed using the Statistical Package for Social Sciences 18.0 (SPSS Inc., Chicago, IL, USA). After using the Kolmogorov-Smirnov's distribution function, parametric variables were evaluated by chi-square test and Student's t-test, while non-parametric variables were evaluated by the Mann-Whitney U-test. A $p < 0.05$ was considered statistically significant.

Results

Demographics of the study population are presented in Table 1. The mean age of participants, etiology of infertility, basal FSH, and E2 concentrations were matched for both groups.

When gonadotropin treatment was compared, total FSH (2,668 ± 1,027.11 vs 2,919 ± 1,126.02) and daily FSH (203.35 ± 101.43 vs 245.29 ± 142.61) doses were lower in group 1, although not statistically significant ($p > 0.05$). The period of ovulation induction was significantly shorter in group 2 (13.12 ± 2.02 vs 11.9 ± 2.03; $p < 0.05$) (Table 2). The mean number of follicles > 16 mm (5.28 ± 4.48 vs 4.76 ± 3.32) and endometrial thickness (8.89 ± 1.50 vs 9.45 ± 2.05) on the day of hCG injection were similar in both groups ($p > 0.05$) (Table 2). However, the mean concentration of serum E2 on the day of hCG administration was significantly higher in group 2 than in group 1 (1,666 ± 337.34 vs 2,848 ± 623.47; $p < 0.001$) (Table 2).

Although the number of retrieved oocytes (8.76 ± 7.35 vs 10.44 ± 6.12) and transferred embryos (1.92 ± 1.11 vs 2.44 ± 0.91) was lower in group 1, it was not statistically significant ($p > 0.05$) (Table 3). The number of clinical pregnancies per embryo transfer, number of clinical pregnancies per cycle, and number of ongoing pregnancies (> 16 gestational weeks) were similar in both groups ($p > 0.05$) (Table 3).

As for complications, one patient had an ectopic pregnancy in group 1, while one patient developed ovarian hyperstimulation syndrome (OHSS) in group 2.

Table 1. — *Baseline characteristics of the patient groups*.

Variable	Group 1 (n = 25)	Group 2 (n = 25)	*p*
Female age, years	32.6 ± 5.97	32.5 ± 5.01	0.96
Causes of Infertility			
Unexplained	10 (40%)	10 (40%)	
Ovarian factor	2 (8%)	1 (4%)	NS
Tuboperitoneal factor	6 (24%)	6 (24%)	
Male factor	7 (28%)	8 (32%)	
Basal FSH (mIU/ml)	8.03 ± 4.20	6.24 ± 3.61	0.11
Basal E2 (pg/ml)	43.41 ± 25.58	49.79 ± 42.38	0.52
Infertility period, years	4.44 ± 2.20	5 ± 2.14	0.36

Values are expressed as mean ± SD unless otherwise indicated. NS = not significant.

Table 2. — *Response to ovulation induction*.

Variable	Group 1 (n = 25)	Group 2 (n = 25)	*p*
Total dose of FSH used, IU	2,668 ± 1,027.11	2,919 ± 1,126.02	0.41
Daily dose of FSH used, IU	203.35 ± 101.43	245.29 ± 142.61	0.23
Duration of induction with FSH, days	13.12 ± 2.02	11.9 ± 2.03	0.03
Number of follicles > 16 mm on the day of hCG administration, mm	5.28 ± 4.48	4.76 ± 3.32	0.64
E2 level on the day of hCG administration, pg/ml	1,666 ± 337.34	2,848 ± 623.47	< 0.001
Endometrial thickness on the day of hCG administration, mm	8.89 ± 1.50	9.45 ± 2.05	0.27

Values are expressed as mean ± SD unless otherwise indicated.

Table 3. — *Embryological data and pregnancy outcome*.

Variable	Group 1 (n = 25)	Group 2 (n = 25)	*p*
Number of retrieved oocytes	10.44 ± 6.12	8.76 ± 7.35	0.38
Number of transferred embryos	1.92 ± 1.11	2.44 ± 0.91	0.07
Number of clinical transfers per embryo transfer, also in %	7 (31%)	7 (30%)	NS
Number of clinical transfers per cycle attempt, also in %	7 (28%)	7 (28%)	NS
Number of ongoing pregnancy (> 16 weeks), also in %	5 (20%)	5 (20%)	NS

Values are expressed as mean ± SD unless otherwise indicated. NS = not significant.

Discussion

In this study, the authors sought to determine whether the use of low-dose letrozole in ovulation induction along with gonadotropin treatment could have a positive impact on IVF cycles and subsequently, clinical pregnancy rates.

Goswami *et al*. carried out the first randomized clinical trial (RCT) with the use of letrozole in IVF treatment cycles published in 2004 [3]. The study consisted of 38

patients, and showed a significantly lower dosage of total FSH in the letrozole group with a comparable pregnancy rate. Oktay *et al*. in a randomized study found a decrease of 44% in the total FSH dosage again in the letrozole group [6]. A RCT by Ozmen *et al*. also confirmed the use of a lower dose of FSH in the letrozole group [5]. An observational study, consisting of 147 poor-responding patients, of Garcia-Velasco *et al*. revealed a better implantation rate in letrozole group but no significant difference in the total FSH dosage [4]. The findings in this study revealed a lower total FSH dosage in the letrozole group, although the difference was not statistically significant.

Prospective trial of Schoolcraft *et al*. did not find a difference in the ovulation induction period between the two groups in a prospective study consisting of 534 patients [7]. A retrospective case-controlled study of Yarali *et al*., which consisted of 885 patients, revealed a shorter period of ovulation induction in the letrozole group [8]. In contrast, the authors found that the period of ovulation induction was significantly longer in the letrozole group. On the other hand, although statistically insignificant, total and daily FSH doses were lower, while number of follicles was higher. Therefore, it can be argued that low-dose letrozole may have a positive impact on the initial period of ovulation induction when follicles mature.

Verpoest *et al*. carried out a prospective study with a small study group, consisting of 20 patients [9]. Their results indicated an increased number of retrieved ooctyes in the letrozole group that was not statistically significant.

Schoolcraft *et al*. showed a non-significant increase in the ongoing pregnancy rate in the letrozole group [7], whereas the study of Yarali *et al*. revealed a significantly higher implantation rate in the letrozole group [8]. Ozmen *et al*. did not find a statistically significant difference in pregnancy rate per cycle, pregnancy rate per transfer, and ongoing pregnancy rate between the two groups [5]. A study by Lee *et al*., consisting of 53 patients who were poor responders, revealed comparable live birth rates [10]. In a very recent study, Miller *et al*. evaluated the impact of letrozole usage in patients with suspected endometrial receptivity defects [11]. They concluded that the usage of letrozole resulted in higher conception rates in patients with endometrial receptivity defects. Our findings revealed no significant differences between the groups in regard to clinical pregnancy.

Fouda and Sayed carried out a RCT to compare the low-dose letrozole regimen (2.5 mg/day) to the high dose letrozole regimen (5 mg/day) [12]. They found no significant differences between the two groups with regards to the number of oocytes retrieved and clinical pregnancy rate. The authors opted for low-dose letrozole regimen (of 2.5 mg/day from cycle days 2-6 in this study.

Conclusion

The addition of low-dose letrozole to gonadotropin treatment in GnRH antagonist protocols may result in a lower dose of gonadotropin administration. However, routine clinical practice remains questionable due to no evident positive effect on pregnancy rates. Consequently, further research in carefully controlled clinical trials is needed to determine whether higher-dose letrozole may be effective and whether the benefit is worth the cost of the treatment.

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