

Low Dose of Gonadotropin with Letrozole Versus High Dose of Gonadotropin in Patients with Poor Ovarian Response Undergoing Ivf: A Randomised, Single-Blind, Prospective Trial

Poor Responder Hasta Grubunda Yüksek Doz Gonadotropine Karşı
Letrozol ile Düşük Doz Gonadotropinin Karşılaştırılması: Randomize, Tek Kör, Prospektif Çalışma

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ABSTRACT

Objective: The ideal controlled ovarian stimulation protocol for patients with poor ovarian response (POR) is not clear yet, and is the subject of many studies. Aromatase inhibitors have been introduced as a new treatment modality in controlled ovarian stimulation as they were found to elevate follicular sensitivity to gonadotropins (Gn). The aim of this study was to evaluate whether it is possible to reduce the required Gn dose by adding letrozole to the treatment without compromising success.

Material and Methods: Patients who underwent in vitro fertilization treatment between 2014 and 2015 in our department and who were classified as poor responder patients according to Bologna criteria were recruited and randomized. In the first group, 33 patients were treated with 150 IU Gn in combination with letrozole 5 mg /day for the first five days of the stimulation. In the second group, 27 patients were treated with 300 IU Gn.

Results: Among the groups there were no statistically significant difference in duration of ovulation stimulation, duration of antagonist use, number of retrieved oocytes, number of transferred embryos, implantation, cycle cancellation, chemical, clinical and ongoing pregnancy rates (all $p > 0.05$). Gn use was significantly higher in 300 IU Gn alone group compared to 150 IU Gn in combination with letrozole group (1354 ± 468 IU versus 2555 ± 725 IU, $p < 0.05$).

Conclusion: The addition of letrozole yields comparable pregnancy outcomes with significantly low doses of Gn, so may be regarded as an effective adjuvant agent in POR patients.

Keywords: gonadotropin, letrozole, poor ovarian response

ÖZET

Amaç: Kontrollü over stimülasyonuna zayıf cevap veren hastalar için ideal protokol henüz net değildir ve birçok çalışmanın konusudur. Aromataz inhibitörleri ile foliküllerde gonadotropin(Gn) duyarlılığının arttığı tespit edilmiştir. Bu çalışmanın amacı, başarıdan ödün vermeden tedaviye letrozol ekleyerek gerekli olan Gn dozunu azaltmanın mümkün olup olmadığını değerlendirmektir.

Gereç ve Yöntemler: Kliniğimizde 2014-2015 yılları arasında infertilite tedavisi gören ve Bologna kriterlerine göre zayıf cevap veren hasta olarak sınıflandırılan hastalar randomize edildi. Birinci hasta grubuna ($n=33$), stimülasyonun ilk beş günü için 5 mg/gün letrozol ile birlikte 150 IU/gün Gn uygulandı. İkinci gruba ($n=27$) ise sadece 300 IU/gün Gn uygulandı.

Bulgular: Gruplar arasında ovulasyon stimülasyonu süresi, antagonist kullanım süresi, elde edilen oosit sayısı, transfer edilen embriyo sayısı, implantasyon, siklus iptali, kimyasal, klinik ve devam eden gebelik oranlarında istatistiksel olarak anlamlı bir fark yoktu ($p > 0.05$). Ancak Gn kullanımı, sadece 300 IU Gn grubunda, letrozol grubu ile birlikte 150 IU Gn'e kıyasla daha yüksekti (1354 ± 468 IU, 2555 ± 725 IU, $p < 0.05$).

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Sonuç: Letrozol sayesinde önemli ölçüde düşük Gn dozları ile karşılaştırılabilir gebelik sonuçları elde edilmiştir; bu nedenle POR hastalarında etkili bir adjuvan ajan olarak kabul edilebilir.

Anahtar Kelimeler: gonadotropin, letrozol, zayıf over yanıtı

INTRODUCTION

The insufficient follicle development as a result of the poor ovarian response to the controlled ovarian hyperstimulation (COH) is an important problem in the IVF. The term "poor responder", which describes this group of patients, is first used in 1983 (1). In these patients, the poor follicular response and low estradiol (E2) levels are the cause of the insufficient number of retrieved oocytes. Therefore, embryo transfer and pregnancy rates remain low while the rate of the cycle cancellation is high. The generally accepted rate of the poor responders among the patients underwent COH is between 10% and 25% (2, 3). The different criteria used by the investigators to define these patients caused some conflicting results in the studies. The aim of the Bologna criteria (published in 2011) was to become a universal definition. These criteria are the following: (i) advanced maternal age (>40 years) or any risk factor related to a poor ovarian response, (ii) previous poor responses (collection of insufficient number of oocytes (≤ 3) with the traditional stimulation protocol), (iii) abnormal ovarian reserve test (antral follicle count (AFC) $< 5-7$ or anti-Müllerian hormone (AMH) $< 0.5-1.1$ ng/ml). For the diagnosis, at least two of these three criteria should be present. In addition, two poor ovarian responses in spite of the maximum stimulation are enough for the diagnosis of a poor responder (4).

Several protocols and drugs, which were tried in these patients for an optimal COH, did not deliver a definitive conclusion. High dose Gn are the most common strategy to induce the follicular response. However, studies had shown that this treatment provided a limited benefit and increased the cost significantly (5-7). Following studies demonstrated that the addition of letrozole to the treatment increased the ovarian response to follicle stimulating hormone (FSH) and the number of the retrieved oocytes and decreased the Gn dose needed for COH (8, 9).

Regarding the ovulation induction, letrozole has a couple of different mechanisms of action. The main mechanism of action is to inhibit the hypophyseal negative feedback and to increase the Gn release as a result of the decline of the estrogen levels de-

pending on the selective inhibition of the estrogen synthesis (10). The secondary mechanism of action is to boost the ovarian response to FSH as a result of the increase of the FSH receptor expression in the follicles due to the increased androgen concentration in the ovarian tissue (11). Moreover, the increased androgen level may cause a synergistic effect with FSH on the folliculogenesis depending on the synthesis of some endocrine factors such as IGF-1 (12). These effects of letrozole are particularly useful in poor responders. It was also demonstrated that letrozole increased the integrin expression, which plays an important role in the endometrial receptivity and consequently in the success of IVF (13).

The objective of this prospective, randomized and controlled study was to demonstrate whether the amount of the high-dose Gn could be decreased with the addition of letrozole to the gonadotropin releasing hormone (GnRH) antagonist protocol without decreasing the treatment effectiveness in poor responders, who will undergo IVF.

MATERIAL AND METHOD

This study was designed as a prospective randomized controlled trial. This study is approved by the Ethics Committee of Istanbul University, Istanbul Faculty of Medicine. Patients, who had applied to the Infertility Clinic of the Department of Gynecology and Obstetrics in the Medical Faculty of Istanbul University with the complaint of infertility between 2014 and 2015, were investigated with the help of the physical examination, ultrasonography (USG), hormone analysis, and hysterosalpingography/hysteroscopy and semen analysis.

Patients, who were classified as poor responder patients according to Bologna criteria were recruited and who were at age of between 18-42 years and had regular menstrual cycles (between 25-34 days), had normal body mass index (BMI) (between 19.3-28.9 kg/m²), did not have any endocrine disorders, had normal hormone levels, had no pathological finding in the USG and hysterosalpingography or hysteroscopy and diagnosed with ovulatory and/or unspecified infertility were included in the study, if they gave their informed consent. Exclusion criteria consisted of history of chemotherapy and/or radiotherapy, history of ovarian surgery, history of dehydroepiandrosterone and/or any other testosterone supplement use, patients undergoing natural IVF cycle.

In total of 60 patients met the inclusion criteria between study period. They are randomly assigned to one of the two study groups. The randomization list was a computer generated sequence and took place on the first day of ovarian stimulation treatment. The infertility specialist and embryologist were blinded; the infertility nurse provided the appropriate instructions about the treatment protocol to the patients. The infertility specialist observed follicular development with two-dimensional transvaginal ultrasound, retrieved oocytes and made embryo transfers. The first group consisted of 33 patients and the second group of 27 patients. On the 3rd day of their cycle the serum levels of E2 and FSH are

evaluated and transvaginal ultrasound of the uterus and ovaries is also done on the same day. The patients in the first group received letrozole (2x2.5 mg oral tablet) and they were asked to continue the treatment for 5 days and were administered 150 IU/day contemporaneously (75 IU human menopausal Gn (hMG); Menogon; Ferring Pharmaceuticals, Saint-Prex, Switzerland and 75 IU recombinant FSH (rFSH); follitropin alpha; Gonal-f; Merck KGaA, Darmstadt, Germany and letrozole; Femara; Novartis, Basel, Switzerland). The second group received only high-dose (300 IU/day) Gn without letrozole (150 IU hMG; Menogon; Ferring Pharmaceuticals, Saint-Prex, Switzerland and 150 IU rFSH; follitropin alpha; Gonal-f; Merck KGaA, Darmstadt, Germany). Gn preparations were administered as a subcutaneous injection. Both groups were monitored with transvaginal USG in 3-4 day intervals and the treatment was continued with increased doses if the administered dose was considered as insufficient. After the size of the dominant follicle reached ≥ 13 mm, a subcutaneous GnRH antagonist (Cetrotide, 0.25mg/day; Merck KGaA, Darmstadt, Germany) was added to the treatment until the initiation of human chorionic gonadotropin (hCG) in order to prevent a premature ovulation. After the dimension of the dominant follicle became ≥ 18 mm, 250 μ g subcutaneous hCG was administered to induce the ovulation (Ovitrelle, Merck KGaA, Darmstadt, Germany). 36 hours after the hCG administration, oocyte pick-up (OPU) was performed with a transvaginal OPU needle under the general anesthesia. Cycles are cancelled when no follicle development is achieved (no follicle ≥ 11 mm in size) on transvaginal ultrasound on the day 8 of the stimulation. Intracytoplasmic sperm injection (ICSI) were carried out. Gamete and the embryos were cultured in a G medium and incubation was performed under a gas mixture consisting of 6% carbon dioxide, 5% oxygen and 89% nitrogen. In the 3rd day, the embryos were transferred into the uterus with a soft catheter with the help of USG. Always one embryo was transferred to the patients who were younger than 35 years in their first two IVF cycles. When patients younger than 35 years had two previous failed IVF attempts, two embryos were transferred. In patients who were aged ≥ 35 years always two embryos were transferred (National Legislation of Elective Single Embryo Transfer). Luteal phase support is achieved with vaginal progesterone gel (8% Crinone, Actavis, Parsippany, NJ, USA) starting on the evening of oocyte retrieval continued for 10th gestational week, or a negative pregnancy test. Blood level of β hCG is measured 14 day after embryo transfer. If the beta hCG was ≥ 5 mIU/mL, it was considered positive. When fetal heartbeat is seen on 6th gestational week, it was named clinical pregnancy.

The primary outcome was defined as the number of oocytes retrieved as there is a strong association between the number of retrieved oocytes and live birth rate. The secondary outcome measures were defined as total dose of Gn used for ovarian stimulation, duration of stimulation, number of cycles cancelled, number of cycles reaching embryo transfer and chemical and clinical pregnancy rates. This study was registered with ClinicalTrials.gov (NCT02158689).

STATISTICAL ANALYSIS

Statistical analysis was done with SPSS (Statistics Package for Social Sciences; SPSS Inc., Chicago, IL, USA) version 16.0 software package. Mann-Whitney U, Wilcoxon W, and χ^2 tests were used for the analysis. A p value of <0.05 was considered statistically significant. Variables were presented with descriptive statistics (mean \pm standard deviation for continuous variables and number and percentage for categorical variables). The study results are given using parametric tests because all continuous variables and number of retrieved oocytes were normally distributed.

RESULTS

43 of the 60 participating patients (71.7%) had primary infertility and 17 (28.3%) secondary infertility. Considering the causes of infertility, 8 patients (13.3%) had unexplained infertility, 34 (56.7%) had an ovulatory factor, 1 (1.7%) had ovulatory and tubal factors and 17 (28.3%) had male factor along with the ovulatory factor. There were no statistically significant differences between the age, BMI, duration of infertility, number of previous IVF attempts, day-3 E2, FSH, prolactin serum levels, baseline AMH levels and AFC among two groups (all $p > 0.05$) (Table 1).

Table 1: Demographics of patients.

	150 IU Gn+ Letrosole (n=33)	300 IU Gn (n=27)	p	
Age, years	35.48 \pm 3.63	36.11 \pm 3.73	NS	
BMI, kg/m ²	24.79 \pm 4.59	25.33 \pm 3.07	NS	
Infertility period, months	105.31 \pm 64.75	107.33 \pm 39.88	NS	
Day-3 E2, pg/mL	77.27 \pm 20.87	80.69 \pm 25.13	NS	
Day-3 FSH, mIU/mL	15.83 \pm 6.80	16.34 \pm 7.42	NS	
Baseline AMH, ng/mL	0.64 \pm 0.31	0.70 \pm 0.23	NS	
Baseline AFC	Right	1.79 \pm 0.70	1.81 \pm 1.04	NS
	Left	1.85 \pm 1.25	1.70 \pm 1.20	NS

Note: Data are mean \pm SD, AFC: antral follicle count, AMH: anti-Müllerian hormone, BMI: body mass index, E2: estradiol, FSH: follicle stimulating hormone.

Both groups had a similar distribution in respect of the infertility etiology. The first group, which received letrozole (5mg/daily) along with Gn (150 IU/day; 75IU hMG and 75IU rFSH), consisted of 33 patients and the second group, which received only high-dose Gn (300 IU/day;150IU hMG and 150IU rFSH) consisted of 27 patients.

Cycle cancellation rates were high in both groups and there were no statistically significant difference; OPU and embryo transfer could not be performed in 20 out of 33 patients (60.6%) of the first group and in 17 out of 27 patients (62.9%) of the second group as no follicle development was observed.

The previous number of IVF implementations, the number of antral follicles and the duration of the antagonist drug usage were similar in both groups. Although the duration of the ovulation induction and Gn treatment were similar in both groups, the

total dose of Gn was significantly lower in the letrozole group (1355 \pm 468 IU versus 2556 \pm 725 IU, $p < 0.001$). However, the number of the retrieved oocytes was similar in both groups (3.45 versus 3.75, $p > 0.05$). Also the mean number of the transferred embryos was similar in both groups (1.31 vs 1.40, $p > 0.05$). Finally, there was no statistically significant difference in biochemical and clinical pregnancy rates between the groups (Table 2).

Table 2: Comparison of COH and outcome parameters.

	150 IU Gonadotropins + Letrosole (n=33)	300 IU Gonadotropins (n=27)	p
Duration of ovulation stimulation, days	7.76 \pm 1.79	8.19 \pm 2.00	NS
Gonadotropin dosage, IU	1354.55 \pm 468.35	2555.56 \pm 725.34	<0.001
Endometrial thickness on hCG injection day, mm	8.38 \pm 1.55	9.74 \pm 2.19	<0.05
# of retrieved oocytes	3.45 \pm 1.54	3.75 \pm 1.47	NS
# of transferred embryos	1.31 \pm 0.48	1.40 \pm 0.52	NS
Cancellation rate, %	60.61 (20/33)	62.96 (17/27)	NS
Chemical pregnancy rate, %			
per started cycle	3.03 (1/33)	7.41 (2/27)	NS
per embryo transferred cycle	7.69 (1/13)	20 (2/10)	NS
Clinical pregnancy rate, %			
per started cycle	3.03 (1/33)	3.70 (1/27)	NS
per embryo transferred cycle	7.69 (1/13)	10 (1/10)	NS

Note: Data are mean \pm SD unless otherwise specified
hCG: human chorionic gonadotropin.

DISCUSSION

In our study, we compared letrozole + low-dose Gn with high-dose Gn monotherapy in poor responders, who will undergo ICSI with GnRH antagonist protocol. According to the results of our study, the comparison of the combination therapy consisting of letrozole and low-dose Gn with high-dose Gn monotherapy revealed that the oocyte collection, cycle cancellation rate, implantation and pregnancy rates were comparable in both groups. However, the administered Gn dose was significantly lower in the letrozole group.

In the recently published two reviews, the authors concluded that the addition of Gn to the letrozole treatment in the GnRH agonist/antagonist IVF protocols did not affect the live birth and pregnancy rates in normal and poor responders. They determined a decrease in the Gn dose and in the number of the retrieved oocytes with letrozole and an increase in the rates of the cycle cancellation (14, 15). They suggested that premature luteinizing hormone peaks and poor follicular development were responsible for the cycle cancellation. Mitwally and Casper had reported that aromatase inhibition with letrozole

decreased the FSH dose needed for the COH in women with unexplained infertility and had also improved the ovarian response in the poor responders. (10, 16).

In 2005, a pilot study conducted by Garcia-Velasco et al., it was shown that the addition of letrozole to the antagonist gonadotropin protocol during the treatment of the poor responders increased significantly the number of the retrieved oocytes and implantation rates. However, the increase in the pregnancy rate was not statistically significant. This success rate was considered as depending on the increase of the intraovarian androstenedione and testosterone concentrations due to the letrozole, which improved the IVF cycle in the poor responders (9). As it is well known, there are androgen receptors in the ovaries and androgens play an important role in the follicular development (17). It was shown that testosterone treatment increased the FSH sensitivity in the ovaries in patients with poor response to IVF and thus improved the follicular response (18). In contrary, Lossl et al. added letrozole to the COH protocol and showed that the aromatase inhibition increased the androgens due to the change of the testosterone/E2 ratio in the preovulatory follicular fluid and they assumed that in spite of the increase of the FSH response by testosterone, it decreased eventually the survival of the oocytes as a result of the deterioration of the nature of the follicles (19). In other words, it was believed that androgens decreased the oocyte quality even though they promoted an increase in the number of the oocytes (20). In our study, probably because of this mechanism, in the letrozole group, the results were relatively weaker even though with no statistically significant difference.

There are only a limited number of studies focused on the comparison of the letrozole + low-dose Gn and high-dose Gn treatment with the antagonist protocol. Ozmen et al. conducted a randomized controlled study on a total of 70 patients with a similar design to our study (21). Although we administered initially 150 IU/day Gn in the letrozole group and 300 IU/day in the second group and increased the dose if needed, Ozmen et al. administered a fixed FSH dose of 450 IU/day in both groups. Although they demonstrated that lower doses of FSH were needed in the letrozole group compared to the control group, we used much lower Gn doses in both letrozole and control groups compared to it. Several studies showed that increasing the Gn dose over 450 IU/day did not provide better results (5, 22). It is believed that this phenomenon depends on the small number of antral follicles. As this number does not increase, higher doses do not improve the response (23, 24). In the study by Ozmen et al., the cycle cancellation rate was lower in the letrozole group (8.6% vs 28.6%; $p < 0.05$).

Another study which evaluates whether letrozole incorporation to Gn in antagonist protocol improves IVF cycles results belongs to Lee et al. (25). They included 103 patients with POR to their retrospective study. Their patients underwent to ovarian stimulation with either 2.5 mg/day letrozole and 225 IU rFSH or 225 IU rFSH alone. The letro-

zole group had significantly shorter duration of Gn use (9.5 ± 1.0 versus 10.6 ± 1.5 , $p < 0.001$), required significantly less doses of Gn (2549 ± 393 versus 3012 ± 431 , $p < 0.001$) and the number of oocytes retrieved was significantly higher in letrozole group (5.3 ± 2 versus 4.3 ± 1.9 , $p: 0.02$). However, pregnancy rates and live birth rate per cycle were not statistically different. They concluded that the addition of letrozole in GnRH antagonist protocol resulted in similar pregnancy outcome compared to standard GnRH antagonist protocol but fewer dose and days of Gn administration were required in letrozole group. Although they used similar and flexible doses of FSH, unlike our study, they initially administered the same FSH dose in both groups. The limiting factor of this study was its retrospective design. In the retrospective study of Lazer et al., which had a very similar design to our study, they compared letrozole + low-dose Gn (150-225 IU/day) and letrozole + high-dose Gn (>300 IU/day) in poor responding IVF patients. In the letrozole + low-dose Gn group, the Gn dose was significantly lower and pregnancy and live birth rates were significantly higher. The retrieved oocytes, the number of the fertilization and transferred embryos were comparable in both groups (26). However, in this study, the cycle cancellation rate (4.2-5%) was optimistic regarding the poor responders and it might depend on the biases in the inclusion criteria.

Although the prospective, randomized and controlled design of our study was an advantage, the most important limiting factor was the decreased power due to the high number of the cycle cancellation in both groups. The second limitation is that Gn were not administered with a equal dose in both study and control group; letrozole groups underwent 150 IU Gn in combination with letrozole 5 mg /day whereas the second group underwent 300 IU Gn alone. When the doses of Gn are not equal, the pure effect of letrozole addition can not be retrieved. On the other hand, one of our study's strength is using Bologna criteria; thus exact definition of POR was disposed.

In our study, we determined adding letrozole to Gn treatment in the GnRH antagonist protocol provided lower treatment cost. However, we would have shown the efficacy of letrozole much better, if we would have added letrozole + high-dose Gn (300 IU/day) protocol as a third group to our study. Furthermore, the small number of the enrolled patients was another limiting factor. Further prospective, randomized controlled studies with larger sample size are needed to clarify the abovementioned issues.

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