Research Article

Ovarian Response to Letrozole Combined with Gonadotropin Regimen in Patients with Diminished Ovarian Reserve Candidate for IVF

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Abstract

Background: Diminished ovarian reserve (DOR) is characterized by poor fertility outcomes even by using assisted reproductive techniques (ART). Recent data suggests that co-treatment by letrozole may improve ovarian response to FSH in poor responders and reduce gonadotropin dose required for ovarian stimulation. This study aimed to investigate the effect of letrozole administration combined with gonadotropin in patient with decreased ovarian reserve candidate for IVF. **Material and Methods:** A clinical trial was conducted in the Infertility clinic, Shahid Beheshti University of Medical Sciences, Tehran, Iran. Sixty-nine infertile women with DOR who have poor response to ovarian stimulation by gonadotropin-only regimen were recruited. The patients were treated by letrozole at 7.5 mg/day and FSH at 225 mg/day, started on the 2nd - 3rd day of the menstrual cycle and continued for 5 days. Ovitrell was given (at 250 mg) when at least 2-3 follicle reached 16-18mm (as matured follicle). AMH level, BMI and duration of infertility was recorded. The main outcomes measured as number of retrieved oocytes, number of oocyte II, endometrial thickness and pregnancy rate. **Result:** Mean number of retrieved oocytes and oocyte II was 3.95 and 3.09 respectively. Mean endometrial thickening was 5.74 mm and rate of pregnancy was 19.7%. There was a statistically significant positive correlation between number of retrieved oocytes and AMH level (r=+0.27, P-value=0.028). There was a statistically significant negative correlation between number of retrieved oocytes and age (P value=0.04 ،r= -0.253). Although, there was no significant correlation between number of retrieved oocytes and BMI (P value=0.2). **Conclusion:** This study demonstrated that using letrozole combined with gonadotropin regimen in patient with DOR and poor response to gonadotropin-only protocol, improve response to ovarian stimulation. Number of retrieved oocytes is correlated with younger in age and higher level of AMH.

Keywords: Infertility; Diminished ovarian reserve; Poor ovarian response; Letrozole; Gonadotropin

Introduction

Diminished ovarian reserve (DOR) characterized by decreased number and quality of oocytes, accounted for approximately 10% of women who undergo in vitro fertilization (IVF). [1,2] DOR is highly associated with infertility and poor response to ovarian hyperstimulation and lower oocyte retrieval and a lower rate of pregnancy. [3] It has been suggested that bologna criteria used to define poor ovarian response (POR) could be used as an appropriate definition for DOR, except the criteria of previous stimulation results and advance maternal age (Over 40). [2]

Bologna criteria including: (1) advanced maternal age (>40 years) and/or any other risk factor for POR; (2) previous history of POR (retrieval of ≤3 oocytes during conventional stimulation protocol); and (3) an abnormal ovarian reserve test (i.e., AFC<5–7 follicles or AMH <0.5–1.1 ng/ml). [4] Several studies were performed about the best stimulation protocol in POR women [2,5] but it still remains a challenge in reproductive medicine.

Combination of oral letrozole with low dose gonadotropin is an alternative to high-dose gonadotropins protocols to increase ovarian response and improve oocyte retrieval in women with POR. [1,6]

Letrozole is a highly potent inhibitor of aromatase which inhibits the intracellular aromatase enzyme activity at the major sites that originally approved for treatment of postmenopausal breast cancer. [7] Strong blocking of estrogen synthesis inducing secretion of endogenous gonadotropin by negative feedbacks and increasing ovarian response to follicle stimulation hormone (FSH), make letrozole as an alternative to common stimulators in IVF cycle, with lower costs. [8]

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Some studies, reported that using letrozole as an adjuvant therapy could reduce gonadotropin consumption in addition to increase number of retrieved oocytes and implantation rate. [9-12] This study aimed to determine ovarian response to induction ovulation by minimally-stimulation protocol of letrozole+gonadotropin in women with DOR candidate for IVF.

Materials and Methods

Study design

This study was performed as a clinical trial in the Infertility clinic, Mahdieh Hospital, Shahid Beheshti University of Medical Sciences, Tehran, Iran between March 2017 and January 2019. Women who attended the infertility clinic of Mahdieh Hospital were recruited.

Subjects had to have all of the following criteria:

- Age 18-42 years,
- Diminished ovarian reserve defined by the Bologna criteria; [4] the patients have to be met at least two of the following three bologna criteria: (1) advanced maternal age (>40 years) and/or any other risk factor for POR; (2) previous history of POR (retrieval of ≤3 oocytes during conventional stimulation protocol); and (3) an abnormal ovarian reserve test (i.e., AFC<5–7 follicles or AMH <0.5–1.1 ng/ml).

The study was approved by Ethics committee of Shahid Beheshti University of Medical Sciences for the use of letrozole for ovarian stimulation.

All patients who met the criteria were informed about the potential benefits and risks of protocol and ethical consent was obtained.

Patients and stimulation protocol

Seventy-two patients met our inclusion criteria and enrolled in this study. Serum levels of anti-mullerian hormone (AMH) was measured in all patients and recorded before starting stimulation protocol. Patients who did not receive any hormonal treatment in the last two months started the treatment protocol.

Ovarian hyper stimulation was initiated with Letrozole 7.5 mg/day (Famaroz 2.5, SOHA pharmaceutical company, Tehran) and gonadotropin 225 mg/day (Gonal-F; Merck KGaA, Darmstadt, Germany) on day 3 of menstrual cycle and continued for the next 5 days.

Ovarian follicular development, follicular count and endometrial thickening were monitored by fellowship of infertility using transvaginal ultrasonography with high-frequency endo-vaginal probe (7.5 MHz) before drug administration on cycle day 2 or 3. After completing 5-day stimulation protocol, ovarian follicular development was observed for matured follicles every 2-3 days using transvaginal ultrasound according to previous condition. To trigger ovulation, subcutaneous human chorionic gonadotropin (hCG) 250 microgram (Ovitrelle, Merck KGaA, Darmstadt, Germany) was injected when at least two follicles reached 16-18 mm in mean diameter. Ovarian puncture was carried out and oocytes were retrieved in 36 h after hCG injection. Number and quality of retrieved oocytes were recorded by laboratory of embryology as well. If the infertility specialist observed no follicular growth on ultrasonography after 5days following complete stimulation protocol, cycle was recorded as cancelled.

In vitro fertilization was performed and embryo transferred 72h after fertilization. 2 weeks after embryo transferring, βhCG serum levels were measured by ELISA test and recorded. Chemical pregnancy was considered in such patients with βhCG serum levels ≥30 mIU/mL. Ultrasound was carried out in 3 weeks after positive βhCG to confirm clinical pregnancy by presence of FHR.

All patients' data included age, body mass index (BMI), AMH level, and endometrial thickness, total number of oocytes, number of Metaphase II oocytes, βhCG and FHR were recorded in prepared checklists and was available for the analysis.

Statistical analysis

Statistical analysis was performed using Statistical Package for the Social Sciences, version 22.0 (SPSS, Chicago, USA). Descriptive statistics for variables were reported as mean ± standard deviation (SD). The Kolmogorov–Smirnov test was used to test the normal distribution of variables. Spearman correlation test was performed to consider correlation of variables and outcomes. The two-tailed P-value <0.05 was considered statistically significant.

Results

From seventy-two patients who met the inclusion criteria, three patients were excluded because of poor compliance for taking medication or not attending to clinic at follow-up time and sixty-nine patients continued to the end of the protocol. Patients were in the mean age of 35.3 years, majority of whom (58.8%) were estimated as overweight considering to their BMI. Characteristic data, AMH serum level and duration of infertility are summarized in Table 1.

4.34% of patients presented no response to stimulation regimen and reported as cancelled cycle (n=3).

Mean number of retrieved oocytes following letrozole+gonadotropin stimulation was 3.95 [Table 2]. Mean number of oocytes M II and endometrial thickness were summarized in Table 2 as well.

Of total 66 patients, chemical pregnancy rate was 19.7% (n=13), and clinical pregnancy rate was 16.7% (n=11). Miscarriage took place in 2 patients (rate=3.03%).

Table 1: Characteristic data.

	Mean ± SD	Median	Min	Max
Age	35.39 ± 4.68	36.00	21.00	42.00
BMI	25.99 ± 3.88	25.00	19.70	37.80
AMH	88.72 ± 1.04	65.00	0.05	6.00

Table 2: Treatment outcomes in patients treated with letrozole + gonadotropin protocol.

	N	Minimum	Maximum	Mean	Std. Deviation
Total oocyte count	66	0.00	17.00	3.9545	3.38
Oocyte II count	66	0.00	15.00	3.0909	3.00
endometrial thickness (mm)	65	3.00	9.40	5.7431	1.63

Nonparametric correlation test revealed a significant negative statistical correlation between patient's age and number of retrieved oocytes following treatment regimen (P=0.04, correlation coefficient r= -0.253). Furthermore, there was no significant correlation between BMI and number of retrieved oocytes (P >0.05). We found a significant positive statistical correlation between AMH level and number of retrieved oocytes (P=0.028, correlation coefficient r= +0.27), however data analysis showed no significant correlation between AMH level and number of oocyte M II (P>0.05) (data not shown).

Discussion

Recent data have confirmed the potential beneficial effect of letrozole administration in reduction of gonadotropin dosage used to induce ovulation in poor ovarian responders. [9,12,13] Since aromatase inhibitors lead to increase FSH secretion by suppressing estradiol synthesis, it is expected that combination use of letrozole could improve number of retrieved oocytes. [7,8] This was justified by such literature demonstrated a significant increase in number of retrieved oocytes using letrozole combined with FSH/hMG antagonist when compared with letrozole-free protocol (6.1 vs. 4.3) in POR patients. [11] However, Ebrahimi and colleagues determined that adding letrozole to GnRH antagonist protocol as an adjuvant therapy has no significant effect on oocyte count (2.8 ± 1.09 in letrozole+ antagonis group vs. 2.6 ± 1.5 in placebo+ antagonist group). Furthermore, they achieved the mean oocyte II number of 2.03 ± 0.12 by using letrozole. [10] Another study reported the mean number of 1.6 ± 0.8 oocyte retrieved from poor ovarian responders underwent let+rFSH protocol, which was not significantly different in comparison with GnRH agonist +FSH protocol. [12] In our investigation of letrozole effects in infertile women with DOR, higher number of retrieved oocytes were achieved (3.95 ± 3.3), while all of them had previously represented low response (lower than 3 retrieved oocytes) following gonadotropin-only stimulation protocol.

Poor responders are used to represent more cancelled cycles of which not proceeding to oocyte retrieval leading to low pregnancy rate. In our study, chemical and clinical pregnancy rates were 19.69% and 16.66% respectively. Ebrahimi reported 25.7% chemical and 14.3% clinical pregnancy among 35 patients after treating by letrozole+GnRH antagonist and it was higher than GnRH protocol; however it has been revealed in their study that letrozole effect on pregnancy rate was not significant. [10] Whereas our study revealed that letrozole presented a successful role in treatment of patients who were poor responder to gonadotropin-only protocol.

In our study the cancelation rate was very low (4.34%) despite the higher sample size compare to similar works have been done by other investigations. [10,12] Bastu et al. reported the cancellation rate of 24% per 31 patients received letrozole combined with low dose gonadotropin, though it was not different to whom not receiving letrozole in their therapeutic regimen. [14] Other work in relatively similar protocols confirmed this result and reported the cancellation rate of 15.5% due to low response in letrozole+FSH/hMG-ant protocol. [11]

While, our patients represented mean endometrial thickness 5.74 ± 1.6 mm, other studies reported more endometrial thickening. Goswami et al. indicated the mean endometrial thickening of 8.5 ± 0.4 mm in FSH+letrozole group on day of hCG administration. [12] Also, Mitwally et al. reported similar endometrial thickening of 8.8 ± 0.09 mm following administration of letrozole+FSH, while it was not significantly different to FSH-only group. [9] However, in comparison to clomiphene citrate, letrozole seems to have a notably effect on endometrial thickness which has been stated by recent studies. [15]

One of the factors that are probable to be associated with ovarian response to stimulation is the serum level of anti-mullerian hormone (AMH) that its prognostic value has been studied recently. [16-18] Knez et al. investigated AMH value of prediction of response to ovarian stimulation in 623 patient undergoing stimulation protocol by agonist or antagonist of GnRH and determined that AMH level is significantly associated with number of retrieved oocytes (r=0.667, P<0.001) and could be an accurate predictor of excessive and poor responses to stimulation protocols as well. [17] The correlation of AMH level and retrieved oocytes count has been proved in literature. [19-21] Our data analysis represented a notably positive correlation between AMH level and number of retrieved oocytes (r=+0.27, P=0.028). We also found no correlation between patient's BMI and retrieved oocytes (P>0.2). Furthermore, we investigated whether the number of retrieved oocyte is associated with patient's age and consequently found that there was a negative actuarial association between age and the number of retrieved oocytes (r=-0.256, P=0.040). This result was in concordance with the available literature. [13,16] However, Saliva et al. found age as an independent marker for ovarian response to stimulation and has no correlation with retrieved oocytes count. [17]

Conclusion

Administration of letrozole in combination with gonadotropin regimen in patient with DOR who represented inadequate response to gonadotropin-only stimulation protocol, could improve ovarian response and lead to successful clinical pregnancy (rate of 16.66%). Consequently, combination use of letrozole is a suggestive choice in the case of unsuccessful gonadotropin stimulation. Increase number of retrieved oocytes is statistically associated with younger ages and higher level of AMH as well. Hence, it is predictable that infertility treatment in patients with DOR with lesser age is relatively more successful and considering to AMH level we could evaluate outcome prognosis in these patients. However, further investigations are needed to more accurately predict this prognosis.

Competing Interest

The authors declare that they have no competing interests.

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