

Original Article

The effects of letrozole+GnRH antagonist versus mere GNRH antagonist during IVF in patients with poor ovarian response

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Abstract: Background: poor ovarian response (POR) is inadequate response of ovaries to ovarian stimulation. Different types of therapies have been used in women with POR. Antagonists of gonadotropin releasing hormone (GnRH) have been indicated beneficial results. Letrozole as an aromatase inhibitor reduces the conversion of androstenedione and testosterone to estrone and estradiol. Here we aimed to evaluate and compare therapeutic effects of letrozole+ GnRH antagonist during in vitro fertilization (IVF) in women with POR. Methods: the current study was a randomized clinical trial performed in 2018 in Beheshti hospital Isfahan, Iran. Patients diagnosed with POR who were candidates for IVF were entered and divided into 2 groups: Intervention received 2.5 mg letrozole daily for 5 days from the third day of menstruation cycle and Placebo received placebo. GnRH antagonists were also administered. Data regarding to number of extracted ovocytes, frequency of pregnancy and IVF failure, levels of gonadotropin, duration of gonadotropin usage, initial endometrial thickness, serum levels of Estradiol and number of transferred embryos were collected and analyzed. Results: The two groups of the study were similar in terms of demographic and clinical data prior to the interventions (P -value >0.05). The comparison of the two groups in terms of response to the treatment manifestations, including total gonadotropin ($P=0.49$), luteinizing hormone ($P=0.21$), follicular stimulating hormone ($P=0.16$), anti-mullerian hormone ($P=0.94$), estradiol ($P=0.85$), Right ovary size ($P=0.48$), left ovary size ($P=0.84$), endometrial thickness ($P=0.17$), Oocytes number ($P=0.80$), and successful pregnancy ($P=0.74$) revealed insignificant differences. Conclusion: Adding letrozole to GnRH antagonists was not associated with better pregnancy results in women with POR who were candidates for IVF. We suggest more studies on larger populations should be performed.

Keywords: Poor ovarian response, in vitro fertilization, letrozole, GnRH antagonist

Introduction

In recent decades, new technology and therapeutic methods have been evolved to improve in vitro fertilization (IVF) but still, one of the most important prognostic factors is the number of mature ovocytes following hormone stimulations [1]. Poor ovarian response (POR) is defined as insufficient response of ovaries to ovarian stimulation [2]. As a result, evaluating ovarian reserve before stimulations seems to be essential. As a sign of ovarian function potential, ovarian reserve is used by physicians [3].

POR is diagnosed based on a very low estradiol level, reduced mature follicles and failure in IVF despite adequate gonadotropin stimulation [4].

Usage of GnRH antagonists have been suggested in some previous studies [5]. They reported similar results for fertility or failure in usage of both GnRH agonists and antagonists [6]. As a result, it seems that new therapeutic strategies should be administered in order to treat POR. Administration of aromatase inhibitors along with GnRH antagonists have been suggested by some previous studies but their results were not completely in line with each other.

Letrozole is an aromatase inhibitor which acts selectively and is non-steroidal [7]. The mechanism of action of letrozole is by reducing conversion of androstenedione and testosterone to estrone and estradiol [8]. The main usage of letrozole is to stimulate ovulation in women

with poly cystic ovarian disease (PCOD) or women undergoing IVF [9]. Some studies have suggested that clomiphene is the first line therapy in such women but on the other hand, the use of letrozole has indicated promising results. On the other hand, peripheral anti-estrogen effects of clomiphene were not observed in treatments with letrozole [10]. As a result, letrozole could be known as an effective treatment for women undergoing IVF. Previous studies have also evaluated effects of letrozole therapy in women with POR [11, 12]. As well as combination of letrozole and GnRH antagonist in poor ovarian response have different results in previous studies [13]. Here in the current study, we aimed to investigate and evaluate therapeutic effects of letrozole therapy along with GnRH antagonist in women undergoing IVF.

Methods and material

Study design

This study was a randomized clinical trial performed in Beheshti hospital Isfahan, Iran in 2018. The current study was approved by research committee of Isfahan University of Medical Sciences and the ethical committee of the university has confirmed it (IR.MUI.MED.REC.1398.076). Inclusion criteria were included patients with POR based on Bologna criteria [14] who were candidates for IVF in Beheshti hospital, Isfahan. The POR based on Bologna criteria was having at least two of these 3 criteria: 1) history of at least one failed IVF despite long term usage of GnRH agonists and presence of less than 4 mature ovocytes 2) reduced ovarian reserve to: antral follicle count <5-7 or Anti-Müllerian hormone (AMH) <1.1 ng/ml 3) age of more than 40 years for the father. Exclusion criteria were: having metabolic or endocrine diseases including: hyperprolactinemia, hypo-hyperthyroidism, endometriosis, history of previous ovarian surgery, body mass index (BMI) more than 30 kg/m² and the father's azoospermia. The patients have informed consent for participation to study.

Study assessment and management

Study populations were selected from patients who referred to Beheshti hospital based on inclusion and exclusion criteria. Basic information of the patients including: age, duration of infertility, BMI and previous failed IVF were collected. All of the patients underwent transvaginal

ultrasound study in the second day of menstruation cycle in order to assess endometrial thickness and counting antral follicles. Serum levels of Luteinizing Hormone (LH), Follicle-stimulating hormone [15], Estradiol (E2), progesterone and AMH were also evaluated. Patients were then randomly divided into 2 groups using RANDOM ALLOCATION software including intervention and placebo. Intervention group received 2.5 mg letrozole daily for 5 days from the third day of menstruation cycle and second group received placebo instead letrozole. Ovarian stimulation was performed in both groups using recombinant human FSH (rh FSH) with dosage of 225 units. RhFSH was administered in the third day of menstruation cycle subcutaneously. Ultrasound studies and serial measurements of serum levels of E2 were performed in order to evaluate follicular maturity. Dosage of rhFSH were then adjusted based on personal responses of each patient. GnRH antagonist, letrozole, was administered when follicular diameter was 14 millimeter or more with dosage of 250 mg daily until ovulation. After ovulation and until 4-8 cell embryo, cells were placed with special catheters inside the uterus. One day after extraction of matured ovocyte 400 mg vaginal suppository of progesterone 2 times daily were administered until 2 weeks and was continued until 8 weeks, if pregnancy occurred. Serum levels of B-HCG were measured 2 weeks after IVF in order to evaluate pregnancy.

Statistical analysis

Data regarding the number of extracted ovocytes, frequency of pregnancy and IVF failure, levels of gonadotropin, duration of gonadotropin usage, initial endometrial thickness, serum levels of E2 and number of transferred embryos were collected and analyzed using SPSS software version 25. Data were showed as mean and SD or frequency and percentage. Independent T test was used to compare quantitative variables between groups and Chi Square and Fisher's Exact test were used to compare qualitative variables between groups. *P*-value <0.05 was also assessed as significance threshold.

Results

Initial variables

In the present study, 86 patients were included and divided into 2 groups of GnRH

Gonadotropin releasing hormone

Table 1. Comparison of demographic data between two groups

Variable		Intervention (n=43)	Placebo (n=43)	P-value
Age (years) (mean ± SD)		35.11±4.63	33.88±4.22	0.20 ^a
BMI (kg/m ²) (mean ± SD)		23.87±1.46	23.46±1.71	0.23 ^a
Age of the spouse (years) (mean ± SD)		39.86±4.64	39.06±9.31	0.62 ^a
Infertility duration (years) (mean ± SD)		4.18±2.59	4.59±2.98	0.50 ^a
Successful pregnancies n (%)	No	37 (43%)	38 (44.2%)	0.74 ^b
	Yes	6 (7%)	5 (8.5%)	
Failed IVF n(%)	0	15 (17.4%)	12 (14%)	0.60 ^c
	1	26 (30.2%)	27 (31.4%)	
	2	2 (2.3%)	4 (4.7%)	

a: Independent T test, b: Chi square test, c: Fisher's Exact Test, IVF: in vitro fertilization, BMI: Body mass index.

Table 2. Evaluation and comparison of parameters between two groups

Variable		Intervention (n=43)	Placebo (n=43)	P-value
Total gonadotropin (mean ± SD)		10.22±1.2	9.96±0.7	0.49 ^a
Total days (mean ± SD)		10.20±1.18	9.93±0.77	0.21 ^a
LH (mean ± SD)		5.55±4.38	4.41±2.97	0.16 ^a
FSH (mean ± SD)		7.07±4.21	6.37±3.57	0.41 ^a
AMH (mean ± SD)		0.74±0.35	0.75±0.35	0.94 ^a
TSH (mean ± SD)		2.06±0.65	2.01±0.61	0.74 ^a
Estradiol (mean ± SD)		969.83±518.56	989.67±514.84	0.85 ^a
Right Ovary size (mean ± SD)		2.94±1.07	2.79±0.93	0.48 ^a
Left Ovary size (mean ± SD)		2.67±1.15	2.62±1.26	0.84 ^a
Endometrial thickness (mean ± SD)		8.79±1.08	9.07±0.77	0.17 ^a
Oocytes number (mean ± SD)		4.34±2.30	4.46±2.08	0.80 ^a
Pregnancy test n (%)	-	37 (43%)	38 (44%)	0.74 ^b
	+	5 (5.8%)	6 (7%)	

a: Independent T test, b: Chi-square, LH: Luteinizing Hormone, FSH: Follicle-stimulating hormone, AMH: Anti-Müllerian hormone, TSH: thyroid stimulating hormone.

antagonist+letrozole (n=43) and GnRH antagonist+placebo (n=43). Our primary analysis indicated that there were no significant differences between two groups regarding the age (P=0.20), BMI (P=0.23), age of the spouse (P=0.62), duration of infertility (P=0.50), frequencies of successful pregnancies (P=0.74) and numbers of failed IVF (P=0.60). These data are summarized in **Table 1**.

Outcomes

Evaluation of the other parameters related to the ovarian function and response to the therapeutic approach revealed insignificant differences between the two groups (P>0.05) (**Table 2**). Besides, the comparison of the two groups in terms of successful pregnancy revealed no difference (P-value =0.74) (**Figure 1**).

Discussion

In the present study, 86 women with POR who were candidates for IVF were evaluated. We compared the therapeutic effects of GnRH antagonist plus letrozole therapy versus GnRH antagonist plus placebo and showed insignificant efficacy in terms of successful pregnancy.

Schoolcraft and colleagues performed a study on 534 women with POR who were also candidates for IVF. They compared effects of letrozole therapy+GnRH antagonists versus GnRH agonists. Their results showed no significant differences between groups of patients regarding to numbers of oocytes, fertilization rate and number of transferred embryos. They also showed that the maximum levels of E2 were significantly lower in patients receiving letro-

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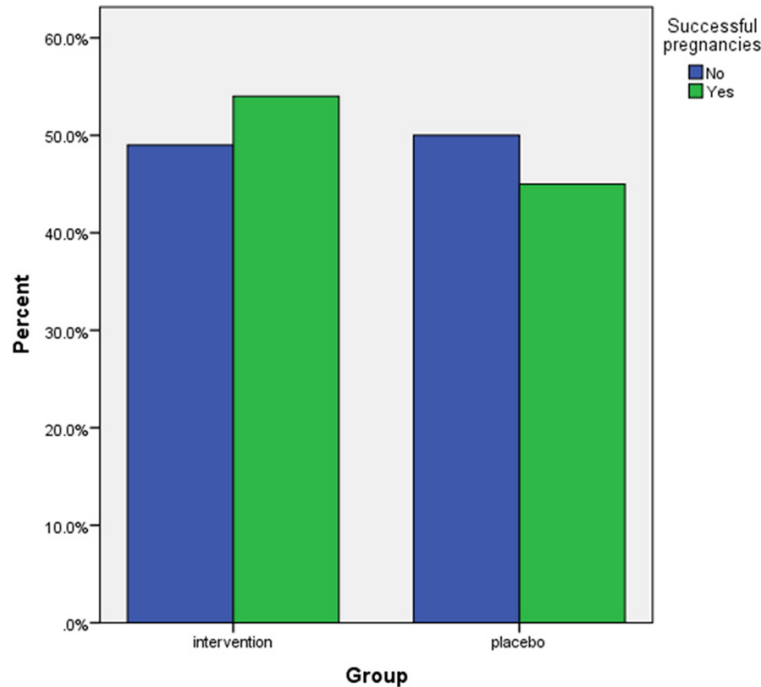


Figure 1. Percentage of successful pregnancy between intervention and placebo.

zole and GnRH antagonists. Furthermore, they declared that the number of pregnancies lasting more than 12 weeks were significantly higher in patients treated with GnRH agonists. They concluded that treatments with GnRH agonists have more beneficial results than letrozole therapy+GnRH antagonists [16]. These findings are somehow in line with our results. We also showed that letrozole therapies might not be associated with higher chances of pregnancy in women with POR. Another study was performed by Goswami and colleagues in 2004 in India on women with POR. In this randomized controlled trial, 38 women were entered and effects of letrozole therapy along with recombinant FSH were assessed. They reported that adding letrozole was not associated with increased rates of pregnancy [17]. Ebrahimi and colleagues also evaluated 70 women diagnosed with POR in 2016 and reported that no significant differences could be observed between letrozole+GnRH antagonists and placebo+GnRH antagonists groups regarding to pregnancy rates [13]. These studies are in line with the results of the current study. We believe that letrozole therapy has no significant effects on increasing the chances of pregnancy in women diagnosed with POR.

On the other hand, some paradoxical results have also been reported by previous studies. In a review study by Revelli and colleagues in 2017, they evaluated therapeutic effects of letrozole and GnRH antagonists in patients with POR and reported that the total pregnancy rate might not be different between this method and the previous treatments but using letrozole along with GnRH antagonists could be beneficial and associated with reduced dosage and duration for treatments with gonadotropins [18]. In another study by Garcia and others in 2005, 71 patients with POR who were candidates for IVF were evaluated. They assessed therapeutic effects of high doses of FSH/ Generic human menopausal gonadotropin (hMG) antago-

nist therapy with or without letrozole and showed that adding 2.5 mg letrozole to higher dosages of FSH/hMG antagonists could lead to more regular menstruation cycles and also higher chances of pregnancy [19]. These results are not in line with our findings. We believe that the most important reason is differences in study populations.

Yang and colleagues also performed a study in 2016 on 220 women with POR who were also candidates for IVF. They evaluated therapeutic effects of letrozole associated with GnRH antagonists and showed beneficial results for this method [20]. They reported significantly higher LH levels and Human chorionic gonadotropin (hCG) in patients receiving letrozole for 5 days. They also concluded that administrating letrozole for 5 days overlapping GnRH antagonists is an effective method which can have promising prognosis for women with POR. Bastu and others evaluated different dosages of GnRH in 95 women with POR with or without letrozole in 2016. They indicated that the first therapeutic option for such patients should be increasing the dosage of GnRH. They also mentioned that this treatment method will not increase the pregnancy chances in all of the patients, but adding letrozole

could affect the prognosis [21]. These findings are somehow in line with our study but here we indicated that letrozole therapies are not associated with higher pregnancy rates.

In contrast to our findings, Moini et al. conducted a study with similar pattern of design to ours on 160 females with POR. The experiment group received 5 mg letrozole within the first five days of ovarian stimulation in combination with 150 IU of rFSH and 150 IU of HMG, while the controls received regiment containing GnRH antagonist plus placebo who eventually, underwent either in vitro fertilization or intracytoplasmic sperm injection and fresh embryo transfer. By the end of this study, all of the manifestations defined as successful outcomes, including the number of retrieved oocytes, the metaphase II oocytes, and the clinical pregnancy rate were remarkably higher among those treated with letrozole plus GnRH antagonist whereas the dosage of hMG used, the duration of ovarian stimulation and antagonist administration were notably less. Therefore, they eventually recommended adding letrozole to the GnRH antagonist [11]. The latter study in agreement with the use of letrozole in combination with GnRH antagonist was conducted by Ozmen et al. that prescribed 5 mg of letrozole daily to a fixed dosage (450 IU/day) of r-hFSH and represented improved cycle outcomes by the administration of letrozole that led to dramatically fewer costs and higher rate of successful IVFs [22]. Consistent with mentioned studies, Sekhon and colleagues evaluated the response rate of 90 POR females to GnRH antagonist alone or in combination with letrozole and noted to considerable decline in gonatropin requirement as well as increased improvement in the rate of implantation and ongoing pregnancies [23].

Despite the diverse recommendations for the doses of letrozole from dialy dose of 2.5 mg to 7.5 mg added to GnRH antagonists for the improvement in the pregnancy outcomes among POR females, our results in agreement with several studies showed inefficacy of this therapeutic approach, while some of the other investigations have strongly favored the use of letrozole, even Garcia et al. administered 2.5 mg of daily letrozole for their patients and achieved the desired outcomes [19]. We want to assume that the discrepancies in the out-

comes are attributed to the definitions used as POR in different studies, such as the exact definition for POR, E2 levels on the day of HCG injection, the ovarian reserve test cut-off values, and the number of retrieved oocytes. The other factors such as ethnicity and environment should not be underestimated, as well.

Conclusion

Adding letrozole to GnRH antagonists was not associated with better pregnancy results in women with POR who were candidates for IVF. Previous studies have also reported variable results and this issue could be due to population differences [24]. Therefore, we suggest that more studies on larger populations should be performed in order to evaluate exact effects of letrozole in patients with POR.

Acknowledgements

The current study was approved in the Isfahan University of Medical Sciences with ethical code (IR.MUI.MED.REC.1398.076).

Disclosure of conflict of interest

None.

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