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Gonadotropin Versus Gonadotropin/Letrozole Protocol in Previously Failed Antagonist Cycles in Patients with Low Prognosis

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Abstract: Letrozole is an aromatase inhibitor which has been used for ovulation induction. Our aim is to evaluate the effectiveness of adding letrozole to gonadotropins in early follicular phase of gonadotrophin-releasing hormone antagonist (GnRH-a) protocol poor ovarian-responders who had failed in the preceding GnRH-a cycle. Ninety-eight patients with poor ovarian response who had previously failed GnRH-a cycle were included. Patients (n; 58) who were treated with letrozole plus gonadotropins (LzGA) were compared with patients (n; 40) who only received gonadotropins (GA). The number of total oocytes retrieved, the number of MII oocytes and fertilized oocytes, fertilization and implantation ratios, the rate of cycle cancellation as well as clinical pregnancy and live birth rates**.** LzGA group had a significantly shorter duration of GnRH-a stimulation and higher progesterone level at trigger day (p=0.005, p=0.049 respectively). Letrozole administration demonstrated lower estradiol levels at trigger day and the total dose of gonadotropins used were lower in LzGA group with no significant difference (p=0.13, p=0.13 respectively). Adding letrozole to gonadotropins in GnRH-a protocol in patients with poor prognosis did not improve pregnancy outcomes. But it seems to decrease IVF costs by reducing the GnRH-antagonist and gonadotropin dosage.

Keywords: Letrozole; Gonadotropin; Poor Ovarian Reserve; IVF.

1. Introduction

A third-generation aromatase inhibitor, letrozole, is now considered as a potent ovulation induction agent [1, 2]. The addition of aromatase inhibitors to gonadotropins has been shown to increase pre-ovulatory follicles without any adverse effect on pregnancy outcomes [2]. Recent studies hypothesized that the addition of letrozole to controlled ovarian stimulation protocols will reduce the total gonadotropin dose and decrease the costs of the IVF cycle. Previous studies have investigated the results of using gonadotropins and aromatase inhibitor together or sequentially in patients with low ovarian reserve, but the results are inconsistent [3-10].

We aimed to evaluate whether it is beneficial to add letrozole in the succeeding cycle of previously failed GnRH-a cycles of patients in the specific cohort of poor

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ovarian response including POSEIDON group III and group IV.

2. Material and Methods

2.1. Participant cohort

In our article, a retrospective study was presented, utilizing data collected between June 2018 and August 2021 at the İzmir Tepecik Research Hospital IVF Centre, affiliated with the University of Health Sciences.

Inclusion criteria encompassed patients designated within POSEIDON groups III or IV, and those who had encountered only one previous unsuccessful cycle involving GnRH-a. The patient cohort was divided into two distinct categories: the study group, composed of 58 individuals subjected to LzGA cycles, and the control group (n: 40), who solely underwent gonadotropin administration. Exclusion criteria entailed patients diagnosed with other infertility factors such as polycystic ovarian syndrome (PCOS), tubal factor and male factor infertility. Additionally, we excluded individuals who had more than 6 months interval between the treatment cycles.

The study was approved by the Ethics Committe of the University of Health Sciences Turkey (Protocol Number: 2022/07-14, date: 18.07.2022).

2.2. Ovarian stimulation

During the gonadotropin cycle, recombinant FSH (Gonal-F; Merck-Serono, Istanbul, Turkey) and highly purified hMG (Merional; IBSA, Istanbul, Turkey) were administered at doses varying from 225 to 300 IU daily. The dosage of FSH and hMG were modified in alignment with the ovarian response that was determined by transvaginal sonography and blood E2 measurements. A non-rigid GnRH antagonist protocol (Cetrotide, 0.25 mg per day, Merc-Serono, Istanbul, Turkey) was introduced when leading follicle achieved an average diameter of 14 mm and/or the serum E2 concentration increased above 350 pg/mL. This regimen was sustained until day of human chorionic gonadotropin (hCG) administration. To induce follicle maturation, recombinant hCG in a dose of 250 μg (Ovitrelle; Merck-Serono, Istanbul, Turkey) was administered, once at least three follicles had attained diameter of 17 mm in average.

Within LzGA cycles, hormonal and sonographic assessments were conducted on the second day of the menstrual cycle. Commencing on either day 2 or 3, Letrozole (Femara; Novartis, Istanbul, Turkey) was administered with a daily dose of 5 mg (2.5 mg twice a day) over 5 days. Ovarian stimulation, GnRH-a protocol, and triggering follicle maturation were applied as similar to previous cycles.

Oocytes were retrieved 36 hours subsequent to the administration of the hCG trigger. The retrieved oocyte cumulus complexes underwent a washing process using GMOPS Plus medium, followed by the detachment of cumulus cells bound to the eggs through the application of 80 IU hyaluronidase (Vitrolife). The standard intracytoplasmic sperm injection (ICSI) procedure was performed after removingcumulus cells attached to the oocytes. The ICSI process was carried out by an inverted Olympus microscope (IX 71) equipped with a Narishige micromanipulator (Narishige, Tokyo, Japan) and a heated stage. Sperm selection was conducted under magnification ranging from ×200 to ×400, with focus on selecting sperms displaying nearly normal morphology for injection into the oocyte. Fertilization was evaluated 16-20 hours following insemination, as evidenced by emergence of two distinct pronuclei and two polar bodies. Resultant zygotes were placed into G1 Plus medium (Vitrolife) within an Esco Miri incubator at 37°C (6% CO2, 5% O2, and 89% N2) for subsequent days. Embryo transfer was performed either on third or fifth day of development.

2.3. Outcome measurements

Primary outcomes were categorized based on chemical pregnancy rates, clinical pregnancy rates, and live birth rates. To verify pregnancy, a serum ß-hCG level was assessed on day 12 following transferring embryo. ß-hCG level exceeding 5 IU/L was deemed positive. Confirmation of clinical pregnancy was established when a fetal cardiac heartbeat was detected via transvaginal ultrasound at the 6th week of gestation. The implantation rate was calculated by dividing count of gestational sacs detected on ultrasound by the number of embryos initially transferred. Live birth was defined as delivery of an infant beyond the 24th week of gestational duration.

The secondary outcomes cover a range of measures, including cumulative gonadotropin dose on average, duration of gonadotropin stimulation on average, duration of GnRH antagonist stimulation on average, average serum estradiol concentration on hCG administration day, mean number of retrieved oocytes, mean number of mature oocytes (metaphase II oocytes), mean number of fertilized oocytes (average count of 2 pronuclear zygotes), fertilization rates, and average number of transferred embryos.

2.3. Statistical analysis

Statistical analyses were performed via SPSS for Windows 23.0 (SPSS, Chicago, USA). Results are given as Mean±standard deviation. Continuous variables were analyzed with Student's t-test, while categorical variables were assessed using the chi-square test. A p value <0.05 was considered as statistically significant.

3. Results

Overall, 98 patients identified to have poor prognosis according to Poseidon Classification, were eligible for the study. Of the 41 patients in LzGA group 25 were in Poseidon 3 and 16 were in Poseidon 4 group. Of the 57 patients in GA group 33 were in Poseidon 3 and 24 were in Poseidon 4 group. There was no significant difference according to the Poseidon groups between LzGA and GA. Table 1 summarizes the baseline patient

characteristics. Two groups were comparable with respect to women's age, body mass index, menstrual cycle length, antral follicle count, the duration of infertility as well as AMH levels. Basal FSH, E2 levels and TMSC were similar.

Table 1: Patient characteristics and basal hormone levels between groups.

Parameters	LzGA Group (N=58)	GA Group (N=40)	*P*
Age (years)	34.34±3.96	35.00±3.60	0.40
Body mass index (kg/m^2)	25.56±4.76	24.73±4.54	0.38
Menstrual cycle (days)	26.93±2.82	26.85±2.72	0.89
Paternal age (years)	37.05±6.03	37.63±6.01	0.64
Duration of infertility (years)	6.33±4.75	6.12±4.02	0.84
Total motile sperm count (million)	56.67±55.67	42.28±51.46	0.19
Antral follicle count	4.60±2.56	4.90±2.77	0.59
Serum FSH on day 3 (mIU/ml)	12.68±4.94	11.28±4.42	0.15
Serum LH on day 3(mIU/ml)	4.73±2.26	4.17±2.14	0.21
Sserum E2 on day 3 (pg/ml)	46.09±24.09	43.85±17.22	0.15
Day-3 serum P (ng/ml)	0.80±0.62	0.78±0.42	0.92
AMH (ng/mL)	0.69±0.35	0.64±0.30	0.49
TSH (IU/mL)	1.82±0.93	2.17±2.30	0.30
PRL (ng/mL)	15.47±6.19	14.58±6.31	0.48
Ratio of POSEIDON Group 3	0.60(25/41)	0.57(33/57)	0.83
Ratio of POSEIDON Group 4	0.40(16/41)	0.43(24/57)	0.83

Mean±SD (range) or percentage (number/total); LzGA=letrozole plus gonadotropins; GA= gonadotropins only. Statistical significance: $p<0.05$. FSH: Follicle-stimulating hormone, LH: Luteinizing hormone, E2: Estradiol, P: Progesterone, AMH: Anti-mullerian hormone, TSH: Thyroid-stimulating hormone, PRL: Prolactin

Ovarian stimulation parameters were compared in Table 2. According to the Table 2, the LzGA group had a significantly shorter duration of GnRH-a stimulation period and higher progesterone levels at trigger day when compared with GA group. Although letrozole administration depicts lower E2 levels at trigger day and lower dose of total gonadotropin use, these results were not statistically significant. Duration of ovarian stimulation, GnRH antagonist starting day and β-hCG application day were comparable.

Table 3 depicts the comparison of cycle outcomes. Mean follicle (>14mm) count, total count of follicles on trigger day, mean number of retrieved oocytes, mean number of metaphase II oocytes, mean number of 2PN zygotes and the top-quality embryos were similar in the two groups. In addition, fertilization and implantation rates, as well as the number of transferred embryo, the day of embryo transfers, and cycle cancelation rates were similar. On the other hand, MII oocyte ratio was higher in LzGA group without statistical significance. Fertilization failure and developmental failure accounted for the majority of the causes of cycle cancellation.

Table 4 shows that fertilization failure and developmental failure accounted for the majority of the causes of cycle cancellation. Degenerated oocytes were the least encountered cause for cycle cancellation.

Pregnancy results is shown in table 5. No significant difference was found in chemical pregnancy, clinical pregnancy, and live birth rates between the groups (*p*-value 0.83, 0.56, and 0.47 respectively).

Table 2: Comparison of ovarian stimulation outcomes between groups.

Variables	LzGA Group (N=58)	GA Group (N=40)	p-Value
Duration of ovarian stimulation (days)	8.12±2.16	8.18±2.22	0.90
The total dosage of Gonadotropins (IU)	2293.33±731.00	2534.38±827.27	0.13
Antagonist starting day	7±2.74	6.15±2.08	0.10
Duration of Antagonist Usage (days)	3.91±1.41	5.03±2.39	0.005
hCG trigger day	10.21±2.60	10.18±2.24	0.95
E2 level at hCG day (pg/ml)	711.37±866.87	955.90±714.27	0.13
Progesterone level at hCG day (ng/ml)	1.23±1.37	0.79±0.35	0.049
LH level at hCG day (mIU/ml)	3.18±2.72	3.02±2.12	0.36

Mean±SD (range) or percentage (number/total); LzGA=letrozole plus gonadotropins; GA=gonadotropins only. Statistical significance: $p<0.05$.

4. Discussion

Managing and treating poor ovarian responders are still matter of debate in the field of IVF 1, 2. Although there have been many strategies suggested in the management of poor responder patients, there is no consensus on most beneficial approach. In our study, we aimed to demonstrate whether the use of letrozole with gonadotropins has any additional beneficial effects on IVF outcomes when compared to single use of gonadotropins among patients who were classified as POSEIDON groups 3 and 4.

Our study has some limitations including retrospective design and small sample size. Meanwhile, the inclusion of a relatively homogenous group of only POSEIDON group 3 and 4 patients and patients with only one failed cycle was its strengths. The low implantation and pregnancy rates can be explained by selecting patients who were classified as POSEİDON group 3 and 4 and already who had failed IVF cycles due to several reasons.

Management of patients with low ovarian reserve is still controversial. It has been recommended that adding letrozole to gonadotropins in GnRH-a cycle in patients with low ovarian reserve may have beneficial effects 5-10. Goswami et al. published the first article in literature which showed the effectiveness of letrozole in IVF treatment in patients with low ovarian reserve and concluded that adding 2.5 mg letrozole to r-FSH significantly decreased the cost of IVF cycle due to use of lower gonadotropin doses 7.

To the extent of our understanding, the only study similar to our findings was published by Ozmen et al. Similar to our investigation, in the LzGA group, the mean total dose of rFSH and serum estradiol concentrations on the day of human chorionic gonadotrophin administration were notably lower. Their findings indicated that the supplemental utilization of letrozole appeared to enhance IVF cycles by reduction cycle cancellation rate and to decrease the cost by lowering the overall gonadotropin dose required [8].

We found cancellation rates to be similar in both groups in contrast with this study. Similar with recent studies, we found that patients with poor prognosis had less need for gonadotropins and GnRH antagonists in the letrozole-added group in agreement with the studies investigating effectiveness of letrozole in patients with low ovarian reserve [7, 8, 17]. Therefore, adding aromatase inhibitors to treatment protocols of low responders seems cost-effective.

Table 3: Cycle outcomes between groups.

Variables	LzGA Group (N=58)	GA Group (N=40)	*p*-Value
Follicles >14mm on hCG day	3.34±2.53	3.13±1.89	0.64
Follicles >17mm on hCG day	1.97±1.46	1.95±1.33	0.95
Total follicles	5.57±3.22	5.73±3.11	0.81
Top-quality embryos	0.78±0.87	1.15±1.33	0.97
Day of embryo transfer	2.10±1.68	2.50±1.82	0.27
M2 oocytes	2.71±1.80	2.58±1.72	0.71
Retrieved oocytes	3.17±2.12	3.20±2.27	0.95
M2 oosit ratio	89.76±19.32	81.78±22.76	0.06
2PN zygotes	1.90±1.63	1.90±1.72	0.99
Fertilisation ratio	65.99 ± 37.25	66.58 ± 37.02	0.93
Implantation ratio	22.09 ± 38.27	22.41 ± 39.15	0.97
No of transferred embryos	0.98 ± 0.76	1.13 ± 0.82	0.38
Embryo transferred cycles rate	70.67 (41/58)	72.50 (29/40)	0.84
Cycle cancelation rate	29.33 (17/58)	27.50 (11/40)	0.84
Day 2 transfer	10	5	0.86
Day 3 transfer	24	15	0.52
Day 5 transfer	7	9	0.17

Mean ±SD (range) or percentage (number/total). LzGA=letrozole plus gonadotropins. GA=gonadotropins only. Statistical significance: $p<0.05$.

Table 4: The causes of cycle cancelation for each cycle.

	Fertilisation Failure	Development Failure	No Oocytes	Morphologic Problem	Abnormal fertilisation	Degenerated oocytes	Total
Previous cycle of the trial group Gonadotropins only	10	5	7	6	1	1	30
Gonadotropins+letrozol Antagonist cycle	5	7	2	1	1	1	17
Previous cycle of the control group Gonadotropins only	3	6	1	2	2	0	14
Gonadotropins only Antagonist cycle	5	5	0	1	0	0	11
Total	23	23	10	10	4	2	72

Table 5: Pregnancy results.

Cycles	Chemical pregnancy positive %	Clinical pregnancy positive %	Live Birth positive %
Previous cycle of the trial group (Gonadotropins only)	5 (3/58)	3(2/58)	0(0/58)
LzGA Group	20.7 (12/58)	15.5(9/58)	10.3(6/58)
Previous cycle of the control group (Gonadotropins only)	15(6/40)	10(4/40)	0(0/40)
GA Group	22.5(9/40)	20(8/40)	10(4/40)

Percentage (number/total). Statistical significance: $p<0.04$ (Chi-square test). There were no statistically significant differences between the two groups. (*p*for chemical pregnancy: 0.83, clinical pregnancy: 0.56, and live birth:0.47).

On the other hand, Garcia-Velasco et al. revealed the efficacy of letrozole co-administration. In contrast to our study, 2.5 mg letrozole given in the first 5 days of stimulation in the high-dose FSH/hMG, GnRH-a protocol resulted in a higher number of oocytes retrieved and increased implantation rate[6]. Unfortunately, such promising results have not been obtained in subsequent randomized controlled trials. In an another study with Moini et al., the total oocyte counts and MII oocyte number were statistically significantly higher in the letrozole group. However, there was no difference in implantation, fertilization, and pregnancy rates[11]. Shapiro et al. concluded that, MII oocytes, 2PN embryos, and good quality embryos were significantly higher in gonadotropin-letrozole cycles [5]. Similarly, in our study, the ratio of MII oocyte was found to be higher in the letrozole group.

Yan Lee et al. found that the letrozole group used a significantly lower dose of HMG than the controls and the HMG duration was significantly shorter. Although less were oocytes collected in the letrozole group, the number of transferred oocytes was similar. In contrast to our study, they reported that the duration of the GnRH-antagonist treatment was similar in both groups. Similar to our study; cancellation rate, implantation rate, ongoing pregnancy rate, live birth rate, and cumulative live birth rate were comparable for both groups [20]. However, other studies did not find any positive effect of letrozole and the results of the available studies are conflicting [4, 13, 16, 17]. In our study, adding letrozole to the treatment did not change the results statistically.

5. Conclusions

As a result, we found that addition letrozole to gonadotropins during early follicular phase to GA protocol did not have a contribution to pregnancy outcomes. However, a significant reduction in the GnRH antagonist dose and a significantly higher progesterone level at hCG day was detected. There was no difference between the groups in terms of chemical, clinical pregnancy and live birth rates.

Limitations of the Study

The main limitation in present study is the relatively smaller sample size.

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Conflict of Interests

Authors declare no conflict of interest. All authors read and approved final manuscript.

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Author Contributions

CK; Design: CK, AD, EŞG; Supervision: AD, EŞG; Data Collection and/or Processing: KK; Analysis and/or Interpretation: CK, KK; Literature Review: CK, KK; Writing: CK; Critical Review: AD, EŞG**.**

Ethical Approval

The study was approved by the University of Health Sciences Research Ethics Committee (Protocol Number: 2022/07-14, Date: 18.07.2022).

Data sharing statement

The data that support the findings of this study are available on request from the corresponding author

Consent to participate

Consent was obtained from the all patients participating in the study.

Informed Statement

The all patients who agreed to participate in the study signed the informed consent form.

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