Antagonist/letrozole protocol versus microdose flare-up protocol in poor responders: a randomized study

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ABSTRACT

Objective: To compare the efficacy of gonadotropin-releasing hormone (GnRH) antagonist/letrozole (AL) and micro-dose flare-up (MF) protocols on cycle parameters and clinical outcomes in poor responders.

Patients & methods: A randomized prospective study comprised of 150 infertile women undergoing controlled ovarian stimulation (COS) and intracytoplasmic sperm injection (ICSI) and classified as past or potential poor responders based on specific criteria. Participants were prospectively randomized to receive AL protocol (group I, n=75) or MF protocol (group II, n=75). Clinical pregnancy was the primary outcome. Cycle cancellation rate, dose of gonadotropin used, serum E2 levels, number of retrieved oocytes, fertilization rate, and embryo quality were secondary outcomes

Result(s): Patient characteristics were similar between the two protocol groups. There were no significant differences in mean age, number of oocytes, fertilization rates, number of embryos transferred, or embryo score. Peak E2 levels were lower in the AL group, although this difference did not reach statistical significance. Clinical pregnancy per started cycle (33.3% versus 29.3%, P=0.59) and per embryo transfer (36.8% versus 34.4%, p=0.7) were comparable between AL and MF protocols. Trends toward lower cancellation rates were noted among AL group, but these did not reach statistical significance.

Conclusion(s): The treatment outcomes of gonadotropin-releasing hormone antagonist/letrozole protocol and the microdose flare-up protocol seem to be similar in poor ovarian responders undergoing ICSI.

Key words: GnRH antagonist, letrozole, microdose flare up, poor responder, ovaria stimulation.

INTRODUCTION

The success of in-vitro fertilisation (IVF) and intracytoplasmic sperm injection (ICSI) treatment depends on adequate follicle recruitment. Controlled ovarian stimulation (COS) is gonadotrophin (Gn) induced stimulation of the ovaries for purposes of in vitro fertilisation (IVF) treatment, which specifically aims at inducing ongoing multiple follicle development rather than a single dominant follicle in ovulatory women(1). Protocols for COS are based on this principle. Hence, ovarian response to COS may differ leading to an optimal response or a sub-optimal response, which may be 'poor response' or 'ovarian hyperstimulation syndrome.

Although there is lack of uniform definitions, poor response to COS can be generally defined as unsatisfactory ovarian response in terms of low number of follicles developed, low serum estradiol (E2) levels, and low number of oocytes retrieved despite adequate ovarian stimulation. However, the cutoff points for these parameters that define poor response vary between studies (2, 3).

The ideal controlled ovarian stimulation protocol for poor responders has not been clearly defined. A variety of regimens have been employed including the use of increased gonadotropin doses, decreased GnRH agonist (GnRH-a) doses, flare regimes, adjunctive growth hormone, GnRH antagonists, and microdose flare regimes (3). Several studies have supported the use of a microdose GnRH-a flare protocol in this patient group, which demonstrated improved ovarian responses and clinical outcomes (4-6). This approach takes advantage of the initial release of endogenous Gn that is induced by low-dose GnRH-a administration in the early follicular phase in an effort to enhance response to the subsequent administration of exogenous gonadotropins. Although high doses of gonadotropins are typically added, but the effective stimulation largely has been attributed to endogenous gonadotropin release (7).

GnRH antagonists have been administered to poor responders during gonadotropin stimulation with mixed results (8-12). The use of antagonists allows initiation of gonadotropin stimulation in the absence of prior pituitary gonadotropin down-regulation given that these agents are not typically added to the COS protocol until follicular maturation has already been initiated. The aromatase inhibitor letrozole has been employed as a novel approach

to improving gonadotropin response. This agent acts by blocking E2 synthesis with a resulting decrease in negative feedback at the level of the pituitary. The resulting increase in endogenous gonadotropin secretion may enhance the ovarian response to exogenous gonadotropins (13, 14). Moreover, the increased intraovarian androgens, in addition to serving as precursors for ovarian estrogen synthesis, also have been shown to have a fundamental trophic role in primate ovarian follicular development by augmenting FSH receptor expression on granulosa cells (15). The preliminary studies in poor ovarian responders have noted decreased gonadotropin consumption (16) and increased number of oocytes retrieved (17) with the use of letrozole. Therefore, the combination of a GnRHantagonist and letrozole in conjunction with gonadotropin may offer a new alternative to the microdose GnRHa flare protocol for poor responders preparing for IVF.

Several investigators have compared flare and GnRH antagonists in poor responders with conflicting results. Fasoulitis et al. described a trend, though not statistically significant, toward higher implantation and clinical pregnancy rates when using antagonists (11). On the other hand, Demirol and Gurgan 2009, in a randomized study, reported that the microdose flare-up protocol seemed to have a better outcome in poor-responder patients, with a significantly higher mean number of mature oocytes retrieved and higher implantation rate (18).

Actually, these two protocols are popular in terms of treatment of poor responders and efforts to define their efficiency and safety will contribute to the improvement of therapeutic management of poor responder patients. Therefore, the aim of this prospective randomized study is to compare the efficacy of gonadotropin-releasing hormone (GnRH) antagonist/letrozole (AL) and micro-dose flare-up (MF) protocols on cycle parameters and clinical outcomes in poor responders.

PATIENTS & METHODS

The current study was conducted, from September 2007 to September 2010, in private and university IVF units following approval of the institutional review board (IRB) at Zagazig University School of Medicine. The study included 150 patients who were candidates for ICSI and classified as poor responders as described below. All women were ≤ 37 years and underwent pre-cycle ovarian reserve testing, which included an assessment of cycle day 3serum FSH and measurement of antral follicle count (AFC) measuring 2–10 mm during the early follicular phase. Criteria for classification as a poor responder included at least one of the following: day 3 serum FSH level>10mIU/mL, <6 total antral follicles, prior cycle cancellation and prior poor response to COS (peak E2 <500pg/mL and/or <4 oocytes retrieved). An informed consent was obtained from all couples.

Treatment Protocols

A total of 75 patients were assigned to the GnRH AL protocol (group I). On day 3 of the cycle, gonadotropin stimulation was initiated with recombinant FSH(Gonal-F, Serono, Rockland, MA) 300 IU and hMG (menogon, Ferring) 150 IU daily. Letrozole (Femara, Novartis, East Hanover, NJ) 2.5 mg daily was also initiated on day 3 and continued for 5 days. A GnRH antagonist, cetrorelix (Cetrotide, Serono) 0.25 mg SC daily was initiated once the lead follicle reached 14 mm in mean diameter. Serial ultrasound examinations and evaluation of serum E2, LH, and P levels were used to assess follicular maturation. Gonadotropin doses were adjusted after 5 days of stimulation

A total of 75 patients were assigned to the MF protocol (group II). Each patient underwent treatment with 20 ug SC administration of GnRH agonist triptorelin (Decapeptyl;Ferring,), twice daily, from second day of the cycle until the day of hCG administration. The exogenous gonadotropin stimulation started on day 3 of the cycle and consisted of recombinant FSH and hMG (in the doses described above).

Human chorionic gonadotropin (hCG) 10,000 IU IM was administered when at least two follicles achieved a mean diameter of 17 mm and serum E2 levels were ≥ 500 pg/mL. Oocyte aspiration was performed 35hours after hCG administration. ICSI was performed in a standard way. Oocytes were examined 16–18 hours after ICSI for pronuclei (PN). Normal fertilization was defined as existence of two pronuclei (2PN). The embryos obtained were categorized on day 2 or 3 into four categories depending on their morphologic appearance, cytoplasmic fragmentation, and blastomere size (grade I [high quality]: embryos with equal blastomeres and no observed cytoplasmic fragmentation; grade II [good quality]: embryos with equal blastomeres and <20% fragmentation of the cytoplasm; grade III [fair quality]: embryos with unequal blastomers and 20%–50% fragmentation of the cytoplasm; grade IV [poor quality]: embryos with unequal blastomers and >50% fragmentation of the cytoplasm)(19). Depending on patient's age, embryo quality, and the number of embryos available, one to four embryos were transferred 2–3 days after oocyte collection. Cycle cancellation was recommended when fewer than three developing follicles of an appropriate growth pattern were noted.

Luteal phase support

In both groups, daily intramuscular injection of progesterone (Prontogest; Ibsa, Switherland) 100 mg, started from the day of embryo transfer and continued until a negative pregnancy test or a positive fetal heart beat was documented by transvaginal ultrasound. In all groups, serum HCG tests were performed on days 18 and 20 after the administration of HCG. An ultrasound scan was done 3 weeks after a positive pregnancy test to confirm a clinical pregnancy. Spontaneous abortion was defined as the spontaneous loss of a clinical pregnancy before 20 completed weeks of gestational age (i.e. 18 weeks after fertilization) (20). Ongoing pregnancy was defined as pregnancy developing beyond 20 weeks gestation

Hormone measurements

Serum concentrations of FSH, LH, oestradiol, progesterone and bHCG were determined using Elecsys 2010 (Roche, Germany). For FSH, the analytical sensitivity was <0.1 IU/l with total precision of 2.9%. For LH, the analytical sensitivity was 0.1 IU/l with total precision of 1.6%. For oestradiol, the analytical sensitivity was 5 pg/ml with total precision of 2.3%. For progesterone, the analytical sensitivity was 0.03 ng/ml (conversion factor = 3.18 nmol/l) with total precision of 2.4%. For quantitative bHCG assay, the analytical sensitivity was 0.5 IU/l with total precision of 2.1%.

Outcome measures

Clinical pregnancy was the primary outcome. Cycle cancellation rate, dose of gonadotropin used, serum E2 levels, number of retrieved oocytes, fertilization rate, and embryo quality were secondary outcomes

Randomization

On day 1 of the cycle, included women were randomized into two groups (I and II) using block randomization. Allocation concealment was performed using 150 identical dark-sealed envelopes, prepared by the statistician and kept in the unit's pharmacy. When the woman was eligible and agreed to participate, she was instructed to open the next available envelope to determine the group to which she was assigned. The randomization key was kept with the pharmacy director and not opened until after statistical analysis was performed.

Statistical Analysis

Prior data comparing the CPR between AL and MF was shown to be 37% and 52%, respectively (21). Therefore, 61 women would be required to be able to reject the null hypothesis that the success rates are equal with a probability (power) of 0.8 and Type I error probability of 0.05 using the Chi-square (χ^2) statistic. Since the rate of cancelation during COS was reported to be up to 24% (22), a total of 75 women were included in each arm. Data were statistically described in terms of mean ± standard deviation (SD) and percentages, where appropriate. Comparison of quantitative variables was done using Student t- test for independent samples. For comparing categorical data, Chi square (χ^2) test was performed, except when the expected frequency of events was less than five, in which case the Fisher's exact test was used. Relative risk and 95% confidence intervals (CI) and/ or probability value (p-value) are presented. A *p*-value <0.05 was considered to be statistically significant. All statistical calculations were done using computer programs Excel version 7(Microsoft Corporation, NY, USA) and SPSS version 15 (SPSS, Chicago, IL, USA).

RESULTS

Seventy-five cycles were performed with the AL protocol, and 75 were performed with the MF protocol. The mean age of the patients, mean duration of infertility, basal FSH level and AFC were similar in both groups (table I). Number of previous IVF cycles was comparable (P = 0.66). A trend toward higher cycle cancellation rates that did not reach statistical significance was experienced among patients assigned to MF as opposed to AL (14.6 % vs. 9.3%, P = 0.31). In AL group, a total of seven cycles were cancelled (three cycles owing to impaired response, one to absent oocytes on retrieval, one to absent mature oocytes, one to fertilization failure and one to arrested embryo development) while in MF group a total of eleven cycles were cancelled (four cycles owing to impaired response, cyst was formed in three cases, two cycles duo to absent oocytes on retrieval and two to fertilization failure). These cases were included in the intention-to-treat analyses

The results of COS are displayed in table II. There were no differences in duration or doses of gonadotropins required, numbers of retrieved or mature oocytes. As would be expected, lower peak E2 levels were noted with AL, although this difference did not reach statistical significance. Endometrial thickness and progesterone level on day of hCG were comparable. Fertilization rates, high quality embryos (grade 1 & 2) and number of transferred embryos were also similar between the two groups. There were no statistically significant differences in clinical pregnancy rates (per started cycle and per embryo transfer) between the two groups. Similarly, abortion and ongoing pregnancy rates were also comparable between both groups (table III).

Table I: Baseline characteristics of the AL and MF groups

Group	AL(n = 75)	MF(n = 75)	P value
Age (years)	31.17 ± 3.9	31.85 ± 3.5	0.26
Infertility duration(years)	5.5 ± 1.5	5.14 ± 1.6	0.14
BMI(Kg/m2)	23.13 ± 1.6	23.17 ±1.6	0.88
Basal FSH(IU/L)	7.92 ± 1.4	7.69 ±1.4	0.33
AFC	5.05 ± 1.1	4.88 ±1.1	0.34

Data presented as mean ± SD. P > 0.05 non-significant

Table II: Controlled ovarian stimulation characters of the AL and MF groups.

Group	AL(n = 75)	MF(n = 75)	P value
Stimulation duration	11 ± 1.47	11.2 ± 1.45	0.44
Ampoules number	67.78 ± 10.3	64.9 ± 8.3	0.07
E2 on hCG day	1713.39 ± 308.2	1816.81 ± 359.7	0.07
P on hCG day	1.11 ± 0.33	1.10 ± 0.32	0.9
Endometrial thickness	8.45 ± 1.04	8.41 ± 1.03	0.18
Retrieved oocytes	5.19 ± 1.6	4.86 ± 1.5	0.22
Mature oocytes	4.54 ± 1.3	4.41 ± 1.2	0.55
Fertilization rate(%)	68.33 ± 15.1	66.98 ± 18.1	0.63
Grade I & II embryos	3.86 ± 1.5	3.44 ± 1.1	0.06
Transferred embryos	3.56 ± 1.14	3.32 ± 0.82	0.18

Data presented as mean ± SD unless otherwise specified. P > 0.05 non-significant

Table III: Pregnancy outcome of AL and MF groups

Group	AL(n = 75)	MF(n = 75)	P value	RR(95% CI)
Clinical pregnancy/ started cycle, n (%)	25/75(33.3%)	22/75(29.3%)	0.59	1.2(0.57-2.55)
Clinical pregnancy/ ET, n (%)	25/68(36.8%)	22/64(34.4%)	0.77	1.11(0.51-2.41)
Abortion n (%)	2/25(8%)	2/22(9.1%)	1	0.87(0.08-9.78)
Ongoing pregnancy/ ET, n (%)	23/68(33.8%)	20/64(31.3%)	0.75	1.12(0.51-2.49)

Chi-square test, or Fisher exact test when appropriate. P > 0.05 non-significant

DISCUSSION

Despite improvements in the success of IVF and ICSI in all age groups, the treatment of poor responders remains controversial. One of the difficulties in critically evaluating various COS protocols is the lack of a single universally accepted definition of the poor responder (2, 3). A variety of criteria have been used alone or in combination as inclusion criteria for proposed protocols. The current investigation has not relied on a single criterion but rather on the evidence of prior poor response or presumed poor response based on standard evaluations of ovarian reserve (23). Older women were excluded to avoid conflicting results of mixing women with physiologic age related ovarian insufficiency and non physiologic impaired ovarian response, so as to evaluate a more homogenous population in a truly randomized trial. There is no question that it would be advantageous to predict a challenging group of young women who will respond poorly and tailor an appropriate protocol in advance rather than allowing them to fail to respond to more standard regimens before introducing a more appropriate stimulation regime during a second cycle.

In the current study, a stimulation protocol involving the use of an aromatase inhibitor initiated in the early follicular phase along with gonadotropin stimulation and subsequent GnRH antagonist suppression was compared with a more standard microdose flare GnRH-a regimen. Stimulation parameters, including duration of stimulation and gonadotrophin ampoules were comparable between the two groups. Lower mean peak E2 levels were appreciated in the group receiving the GnRH antagonist, which was presumed to be due to aromatase inhibition induced by letrozole (14). Even so, quantitative results of stimulation were similar between the two protocols as judged by the number of retrieved, mature and fertilized oocytes obtained as well as the number of high quality and transferred embryos (Table 2). There were no statistically significant differences in clinical pregnancy (per started cycle and embryo transfer) and ongoing pregnancy rates, between the two protocols.

Prior randomized and nonrandomized studies have offered varied results. Traditional flare regimes in which higher-dose GnRH-a is administered with minimal delay before initiation of gonadotropin COS are associated with significant increases in follicular-phase serum P and androgen levels, which may exert deleterious effects on follicular development and oocyte quality (24). In an effort to minimize this effect, while maintaining the benefit of stimulating endogenous gonadotropin release, the administration of lower doses of GnRH-a was proposed. Three separate trials employing lower daily doses of GnRH-a, in appropriately selected poor responders, have demonstrated significant benefit over more traditional regimes with regards to improved ongoing pregnancy rates and decreased cancellation rates (4-5,25). Surrey et al. reviewed different regimens used in the poor responders and concluded that a microdose GnRH agonist flare protocol was more uniformly beneficial to the cycle outcome than other regimens (5).

The introduction of GnRH antagonists brought new hope to the treatment of poor responders. Nikolettos et al. showed that patients in the GnRH antagonist group required fewer ampoules of gonadotropins and needed a statistically significantly shorter treatment duration compared with the long GnRHa protocol to achieve the same number of follicles (26). Craft et al. reported a significant reduction in cycle cancellation rates and more oocyte production in a mixed group of patients with poor response or failed cycles during prior standard GnRH-a down-regulation cycles(8). Nevertheless, only an 11.8% live birth rate per completed cycle was achieved. In Cochrane Database Systematic Review 2010, higher number of retrieved oocytes and lower Gn doses were reported upon using antagonist versus long agonist protocol. However, there were no differences in CPR, cancellation rates or miscarriage rates between the two protocols (27).

Although the use of an antagonist eliminates the effect of excessive pituitary gonadotropin suppression induced by a GnRH-a, poor responders may benefit from additional stimulation of endogenous gonadotropin release in the early follicular phase. D'Amato et al. employed a protocol including clomiphene citrate, high dose gonadotropins, and delayed antagonist administration to a group of poor responders and compared results with patients undergoing a long protocol in a prospective nonrandomized trial (28). Although cancellation rates were significantly decreased, no significant differences in pregnancy rates were noted. Once again, low implantation rates (13.5%) were noted. Some possible causes for this phenomenon may be the antiestrogenic effects of clomiphene on the endometrium and potential deleterious effects on oocytes (29). The aromatase inhibitor letrozole acts to increase endogenous gonadotropin release but does not deplete estrogen receptors at the level of the endometrium and could theoretically serve as an ideal alternative to clomiphene citrate in this model (30). Controversy has surrounded the subject of the overall safety of letrozole therapy. A published abstract has suggested a possible increased risk in birth defect rates with letrozole use, which is a source of additional concern (31). However, a larger retrospective trial evaluating 911 newborns who were conceived after administration of this agent to mothers as infertility therapy found no greater overall rates of major or minor malformations than in those who were conceived after administration of clomiphene citrate (32).

Two studies have demonstrated that the addition of letrozole to gonadotropins in poor-responder patients undergoing COS improved follicular response and lessened gonadotropin requirements (14, 33). Garcia-Velasco and colleagues reported the results of an observational pilot trial designed to assess the effects of the addition of letrozole to an antagonist-gonadotropin regimen in a group of poor responders (17). No enhancement of pregnancy rates was noted, but implantation rates were improved. In the current investigation, gonadotropin requirements, oocyte number, and embryo quality as well as pregnancy outcomes were similar between the two treatment groups assigned to either AL or ML protocols. Demirol and Gurgan, in a randomized controlled study upon 90 poor responders, compared the efficacy of the microdose flare-up and multiple-dose antagonist protocols. The two protocols had comparable CPR. However, the MF protocol had significantly higher mean number of mature oocytes retrieved and higher implantation rate (18). Of note, the study included different patient population (older women and those having basal FSH > 15 IU/L) and letrozole was not added to the antagonist group. On the other hand, two recent studies had concluded that microdose flare-up protocol and multiple dose GnRH antagonist protocol seem to have similar efficacy in improving treatment outcomes of poor responder patients (34, 35). Interestingly, in a recent retrospective study upon 1383 poor responders (predicted to have or with a history of poor ovarian response), the MF protocol was used in 1026 cycles and the AL protocol was used in the remaining 357 cycles. The clinical pregnancy and implantation rates were comparable between the two groups (36). So, based on current study findings and others (21, 36), it could be assumed that AL and MF are equally effective protocols for management of poor responders.

In the current study, a trend toward higher cycle cancellation rates that did not reach statistical significance was experienced among patients assigned to MF as opposed to AL (14.6 % vs. 9.3%, P = 0.31). Cancellation due to impaired response, absent oocytes on collection or fertilization failure was comparable between both groups. However, three cases developed cyst formation during COS using the MF protocol while none developed cyst among the AL group. Interestingly, these three cases had AFC ≤ 3 and two of them had been cancelled previously due to impaired response with development of only one follicle during COS. In such group of whom we can call "extreme poor responders" the initial release of endogenous gonadotropins induced by GnRH-a administration in combination with exogenous Gn might have caused these cysts. Actually, none of the published studies reported on cyst formation development and cycle cancelation is generally mentioned broadly without going into much details. So, the current study findings definitely warrant further study in a larger group of these patients.

In conclusion, in the present study, the treatment outcomes of gonadotropin-releasing hormone antagonist/letrozole protocol and the microdose GnRH-a flare-up protocol seem to be similar. Further prospective randomized trials with larger numbers of patients and large meta-analyses with strict inclusion criteria are needed to assess the efficacy of the two protocols in the poor responders.

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