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Prospective randomized trial comparing efficacy of letrozole step-up protocol with letrozole plus gonadotropins for controlled ovarian stimulation and intrauterine insemination in patients with unexplained infertility

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Abstract

Purpose Empirical treatment options for unexplained infertility treatment include controlled ovarian stimulation (COS) with clomiphene citrate, letrozole or gonadotropins followed by intrauterine insemination (IUI). Achieving consistent multifollicular development with letrozole generally requires addition of gonadotropins. However, the cost and discomfort of injections remains a drawback of this regimen. Therefore, there is a need for evolving newer cost-effective regimens for COS/IUI using orally administered drugs like letrozole.

Methods Sixty couples with unexplained infertility (on standard infertility investigations) visiting the infertility clinic at a tertiary centre in North India were randomized into two groups. Group A COS was done by step-up protocol of letrozole from day 2 or 3 of menstrual cycle, starting with 2.5 mg and increased by 2.5 mg per day for next 3 days (2.5 mg, 5 mg, 7.5 mg, 10 mg). Group B COS was done with combination of letrozole and hMG (human menopausal gonadotropin). Starting from day 2 or 3 of menses, tablet letrozole 2.5 mg twice a day was given for 5 days. Intramuscular injection of hMG 150 IU was given every alternate day starting from day 7 and titrated according to the response. HCG was given when leading follicle was 17 mm and IUI done 36 h after HCG.

Results Twenty-eight couples in letrozole step-up group (group A) and 30 couples in letrozole plus hMG group (group B), completed follow up for 44 and 55 cycles, respectively. Mean numbers of follicle $\geq$ 16 mm in both groups were comparable: 1.74 ($\pm$ 0.83) in group A and 1.94 ($\pm$0.68) in group B ($p=0.178$). Ovulation rate of 90.9% (40/44) was achieved in group A, whereas it was 100% (55/55) in group B ($p=0.022$), although there was no significant difference in clinical pregnancy rate per patient, which was 3/28 (10.7%) in group A versus 5/30 (16.67%) in group B ($p=0.707$). The mean (SD) cost of medicines was significantly lower in group A Rs. 345.00 (00) compared to group B Rs. 2148.64 (515.67) [$p<0.0001$]. There was one case each of hyperstimulation and multiple pregnancies in group B, while none in group A.

Conclusion It is possible to achieve multifollicular development with use of novel letrozole step-up protocol, even without addition of gonadotropins, at significantly lower cost.

Keywords Unexplained infertility · Controlled ovarian stimulation · Letrozole · Superovulation · Insemination

Introduction

In the absence of an identifiable anomaly, treatment of unexplained infertility has been largely empirical. Treatment regimens include expectant management, controlled ovarian stimulation (COS), intrauterine insemination (IUI), COS with IUI, and assisted reproduction [1]. Patients with unexplained infertility are expected to have normal ovulation, i.e. release of oocyte from one dominant follicle per cycle. Therefore, increasing the number of oocytes

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(superovulation) for fertilization is expected to increase chances of conception in these patients and forms the basis of most of the treatment strategies for unexplained infertility [2]. Deciding the optimal strategy involves consideration of age, treatment efficacy, side effects like multiple pregnancy and cost effectiveness. Apart from in vitro fertilization (IVF), highest pregnancy rate per cycle (up to 27%) has been reported with combination of gonadotropin therapy and IUI [3]. However, the discomfort and expense of injectable therapy remain a deterrent to this treatment arm and efforts to find alternatives with comparable efficacy are still ongoing.

Letrozole is one such candidate oral drugs. Although the use in infertility treatment is as yet off label, it has certain advantages over clomiphene, like no negative effects on endometrium and cervical mucus [4]. Conventional regimens of letrozole generally lead to monofollicular development and therefore find limited use in superovulation [4]. For the purpose of superovulation, i.e. multifollicular growth, gonadotropins need to be added to letrozole [5]. Modification of regimes of oral letrozole like extended letrozole and letrozole step-up protocol is being explored to increase the chances of superovulation, without requirement of gonadotropins [6, 7].

We undertook this randomized study, to compare the efficacy of letrozole plus gonadotropin regimen with letrozole step-up regimen for COS and IUI in patients with unexplained infertility, with an aim to find less expensive and painless alternative to gonadotropins.

Materials and methods

This randomized controlled trial was conducted in the infertility clinic of a tertiary centre in northern India, over a period of 3 months. Ethical clearance for the study was obtained from Institute ethical committee (IEC No. INT/IEC/2018/000168). Standard infertility investigations included endometrial biopsy for histopathology and acid fast bacilli smear and culture, to rule out genital tuberculosis, baseline ultrasound to rule out structural pathologies of uterus and ovaries like endometriomas or polycystic ovaries and confirmation of tubal patency by HSG (hysterosalpingography) or laparoscopy. If no cause was identified after complete standard fertility investigations, a diagnosis of unexplained infertility was made. Over a period of 6 months, 780 newly registered cases of infertility were screened. Out of these, 65 patients with unexplained infertility were evaluated for inclusion in the study. Two patients refused to give consent (opted for pure gonadotropin stimulation); one patient opted for in vitro fertilization, one was excluded due to cyst on baseline ultrasound, while one patient's husband refused for intrauterine insemination.

Detailed history was taken again, including coital history, menstrual history (to rule out ovulatory dysfunction), obstetric history, and past medical history for chronic illness or malignancy. General physical, systemic, abdominal, and local examination of the female partner, including weight, height, and BMI were noted. Husband's semen analysis was interpreted according to the World Health Organization 2010 criteria [8]. Baseline transvaginal ultrasound was done on day 2 or 3 for antral follicle count in both ovaries (antral follicles were identified as 2–10 mm size range of well-defined anechoic cysts with smooth margins and absence of internal septations or nodularity). Baseline serum anti-mullerian hormone and day 3 serum follicle-stimulating hormone (FSH) were measured as additional indicators of ovarian reserve.

Totally 60 patients meeting the eligibility criteria were randomized by block randomization with block size of four, 30 in each group. Two patients from group A were excluded after randomization, as they did not come for follow up. Patients were explained about the rationale and mechanism of action of the drugs and especially about the off label use of letrozole for the purpose of ovulation induction. Study group A: controlled ovarian stimulation was done by letrozole step-up regimen. Starting from day 2 or 3 of menses, with a dose of 2.5 mg, daily increments of 2.5 mg were made for total of 4 days. For example, if COS was started on day 2, the day 2 dose was 2.5 mg, day 3 was 5 mg, day 4 was 7.5 mg, and day 5 dose was 10 mg (Total cumulative dose 25 mg).

Study group B: controlled ovarian stimulation was done with combination of letrozole and human menopausal gonadotropin (hMG). Starting from day 2 or 3 of menses, tablet letrozole 2.5 mg twice a day was given for 5 days. Intramuscular injection of hMG 150 IU was given every alternate day starting from day 7.

Patients in both groups were monitored by transvaginal ultrasonography, alternate day starting from day 11, till the leading follicle reached a size of > 17 mm (average of diameter measured in three dimensions). Intramuscular injection of highly purified human chorionic gonadotropin (5000 IU) was administered to trigger ovulation. IUI was done 36 h later.

Statistical analysis

Statistical analyses were performed using IBM SPSS Statistics ver. 23.0 software (IBM Co., Armonk, NY, USA) and Microsoft Excel 2007 (Microsoft, Redmond, WA, USA). Discrete categorical data were represented in the form of either a number or a percentage. The normality of quantitative data was checked by QQ plot and Shaprio–Wilk test of normality. Continuous data, normally distributed, were reported in the form of its mean and standard deviation and when it was skewed it was written in the form of its median

and inter-quartile range, as per the requirement. Student's *t*-test was used for comparing means for normally distributed data. Proportions were compared using Chi-squared or Fisher's exact test, depending on their applicability for the two groups. All the statistical tests were two-sided and were performed at a significance level of $p < 0.05$.

Results

A total of 44 COS and IUI cycles were carried out in 28 couples of group A, whereas 55 cycles were given to 30 couples of group B. Baseline characteristics like age, body mass index (BMI), fertility history, and semen parameters were comparable between the two groups and are detailed in Table 1.

Median serum AMH level in group A was 2.14 (1.41–2.90), whereas it was 3.45 (2.15–4.25) in group B and showed significant difference between the two groups ($p = 0.005$). Other than serum AMH levels, other markers of ovarian reserve like antral follicle count and baseline serum FSH were not significantly different between the two groups ($p > 0.05$), as shown in Table 2.

Ultrasonographic findings, including number and size of dominant follicles and endometrial thickness, on the day of hCG trigger are summarized in Table 3. Ovulation rate was 90.9% (40/44) in group A and 100% (55/55) in group B ($p = 0.022$). However, 14.5% (8/55) cycles in group B required more than two HMG injections for achieving adequate size of dominant follicle. In group A, development of more than one dominant follicle (≥ 16 mm) was seen in 68.1% (30/44) cycles, whereas in group B, it was seen in 72.7% (40/55) cycles and the difference was not statistically significant ($p = 0.211$). Comparison of pregnancy rates between the two groups is shown in Table 4.

The mean cost of medicines was significantly less at Rs. 345.00 (00) in group A, compared to Rs. 2148.64 (515.67) in group B ($p < 0.0001$).

Table 1 Baseline characteristics of the study population

Variable	Group A ($n = 28$)	Group B ($n = 30$)	p value
Age (years)	29.21 (3.14)	30.8 (3.19)	0.062
BMI (kg/m^2)	23.86 (3.3)	22.7 (3.1)	0.196
Fertility history			
Duration of infertility (years)	5.50 (3.02)	6.33 (3.14)	0.308
Type of infertility[#] Primary secondary	19/28 (67.8%) 9/28 (32.2%)	21/30 (70%) 9/30 (30%)	0.860
Semen analysis [mean (SD)]			
Sperm concentration (million/ml)	62.00 (18.64)	72.30 (24.00)	0.075
%Motility (total)	60.11 (10.30)	61.30 (10.89)	0.675

Data presented as proportions: Chi Square Test

Mean (SD) for rest of the variables : t-Test

Table 2 Baseline hormone analysis and parameters of ovarian reserve

Variable	Group A ($n = 28$)	Group B ($n = 30$)	p value
Hormone analysis—mean (SD)			
Serum TSH (uIU/ml)	2.43 (0.82)	2.76 (1.11)	0.199
Serum prolactin (ng/ml)	14.19 (6.65)	13.72 (5.67)	0.773
Serum FSH (day 3) (mIU/ml)	5.63 (1.84)	5.26 (1.33)	0.383
Serum AMH* [median (IQR)]	2.14 (1.41–2.90)	3.45 (2.15–4.25)	0.005
Baseline ultrasonographic findings—mean (SD)			
Antral follicle count	10.25 (2.52)	11.43 (2.18)	0.062

* Median (IQR): Mann- Whitney U test

Table 3 Comparison of ultrasonography findings on the day of hCG trigger [mean (SD)]

Variable	Group A ($n = 28$)	Group B (n-30)	p value
Mean number of dominant follicles (≥ 16 mm)	1.74 (0.83)	1.94 (0.68)	0.178
Mean size of the dominant follicle (mm)	18.65 (2.16)	18.60 (2.24)	0.859
Mean mid-cycle endometrial thickness (mm)	8.18 (1.80)	8.07 (1.26)	0.721

Table 4 Comparison between pregnancy rates in the two groups

	Group A ($n=28$) (%)	Group B ($n=30$) (%)	p value
Clinical pregnancy rate per cycle	3/44 (6.8)	5/55 (9.1)	0.730
Clinical pregnancy rate per patient	3/28 (10.7)	5/30 (16.67)	0.707

There was one case of hyperstimulation (more than 3 follicles ≥ 16 mm) in letrozole plus hMG group, whereas none in group A. In this case, hCG trigger was withheld and patient did not develop signs or symptoms of ovarian hyperstimulation syndrome. Two patients in group B complained about significant pain at injection site (requiring analgesics). None of the patients in either group reported hypoestrogenic symptoms. Cyst formation was noted in one patient of group A, whereas it was seen in two patients in group B. Only one multiple pregnancy (twin conception) was seen in group B, whereas all conceptions were singletons in group A.

Discussion

Gonadotropin use in COS/IUI is riddled with problems of expense, cold storage, painful injections, risk of ovarian hyperstimulation syndrome, and multiple pregnancies [9, 10]. Therefore, much research has been concentrated on finding alternative regimens, with oral dosing, potentially reducing cost and side effects, with comparable pregnancy outcomes, especially in low resource countries.

Traditionally letrozole has been associated with monofollicular development. Various modifications of letrozole regimen have been tried to ensure continued FSH availability, even after the selection of dominant follicle, thus resulting in multifollicular development. This can be achieved by sequential addition of gonadotropins or extended letrozole or letrozole step-up protocol [5, 6, 11, 12]. This is the first study reporting direct comparison between letrozole step-up regimen against letrozole plus sequential gonadotropin regimen.

Multifollicular development

In the letrozole step-up group, multifollicular development was achieved in more than 2/3 cycles, i.e. 68.1% with a mean number of follicles on day of trigger, being 1.74 (±0.83). Previously Mitwally et al. have reported mean of 2.2 (±1.5) follicles, whereas Galal et al. found a mean number of 1.5 (±0.7) follicles in their randomized study [7, 13]. Our study results were comparable to previous studies using letrozole step-up protocol.

In the other group, using letrozole and gonadotropin combination, we found multifollicular development in 72.7% cycles with a mean number of follicles calculated to be 1.94 (±0.68). Our results were nearly similar to that of a large prospective randomized trial by Pourali et al. in which they found that mean number of follicles ≥ 16 mm, per cycle was 2.28 (± 0.1) in the group of patients receiving combination of letrozole and FSH [11].

The above results reiterate that it is possible to achieve multifollicular growth by modifying dosing of letrozole, even without addition of gonadotropins.

Endometrial thickness

The mean mid-cycle endometrial thickness was 8.18 mm (±1.8) in group A and 8.07 (±1.26) in group B ($p=0.721$). Although it is slightly lower than the mean endometrial thickness reported by Mitwally i.e. 9.8 (±2.1) mm, when using letrozole step-up regimen, it is still higher than the conventional cutoff of 8 mm [7]. Pourali et al. had reported a mean endometrial thickness of 8.99 (±0.65) mm with combined protocol.

Ovulation and pregnancy rates

Ovulation rate was 90.9% with letrozole step-up protocol, with only four non-ovulatory cycles. Previous studies by Mitwally and Galal have not reported the non-response rates. Our study found a clinical pregnancy rate (per patient) of 10.7% in letrozole step-up group and 16.67% in the combination group, whereas if calculated per cycle, these rates were 6.8% and 9.1%, respectively. Overall, these were lower compared to those reported in earlier studies. Mitwally et al. have reported 27.3% per cycle, while Galal et al. have reported 16% with letrozole step-up [7, 13]. Pourali et al. have reported a clinical pregnancy rate of 26.51% with letrozole and hMG combination [11]. In a large randomized trial, Diamond et al. have reported a clinical pregnancy in 22.4% of cycles with conventional letrozole dosing, without any modification or adjuvant gonadotropins [9]. Possible explanations could be lesser number of patients in our study and relatively younger age group of patients in other studies. For example, in Galal's study [13], mean age was 26.3 years compared to 29.21 years in our study. Overall duration of infertility in our study was also more than 5 years, which in itself is a poor prognostic factor [14].

Adverse events

Ovarian hyperstimulation syndrome was not seen in either group, although one patient in letrozole plus gonadotropins

group had four dominant follicles. No patients complained about hypoestrogenic symptoms like hot flashes, vaginal dryness in any group. Previous studies have not reported adverse effects with letrozole step-up protocol per se, although studies reporting conventional 5 mg /day dosing have reported hot flashes in 16.8% patients [9]. Significant pain at injection site was reported by two patients in group B (6.67%). A large study by Diamond et al. has reported up to 10.8% incidence of injection site reactions, when using gonadotropin COS in 297 patients [9].

Cost of medication (for COS)

The cost of medication is significantly different between the two groups. As there is out of pocket cost (especially with poor or no insurance coverage) which is recurring for these couples, the letrozole step-up regimen scores over combination of gonadotropin and letrozole.

Strengths and limitations

Our study is the first randomized trial which compares letrozole step-up regimen with letrozole plus gonadotropins for superovulation in women with unexplained infertility planned for COS/IUI. Small sample size was a limitation of our study. Two observers did the ultrasound instead of one (due to logistic reasons) and the lack of blinding could have introduced bias.

Future direction

Prospective RCT with larger number of patients and adequate blinding can be planned for establishing a definite benefit of this orally administered regimen (Letrozole step-up protocol), entailing lesser cost and discomfort to the patient.

Conclusion

In patients with unexplained infertility, undergoing controlled ovarian stimulation and intrauterine insemination, it is possible to achieve multifollicular development with use of letrozole step-up protocol, without addition of gonadotropins. Multifollicular development rates, mean endometrial thickness, clinical pregnancy rates are comparable with lesser side effects and cost, thus eliminating the need for injectable medication. Further randomized controlled studies with larger sample size can be executed to validate the above findings.

Author contributions JK: protocol development, data collection, data analysis, manuscript writing. VS: protocol development, data collection, manuscript proofreading. SG: review of literature, Manuscript proofreading, and editing. AA: review of literature, manuscript proofreading, and editing.

Compliance with ethical standards

Conflict of interest All authors declare that they have no conflict of interest.

References

1. Practice Committee of the American Society for Reproductive Medicine (2006) Effectiveness and treatment for unexplained infertility. Fertil Steril 86:111–114
2. Gunn DD, Bates GW (2016) Evidence-based approach to unexplained infertility: a systematic review. Fertil Steril 105:1566–1574
3. Crosignani PG, Walters DE, Soliani A (1991) The ESHRE multicentre trial on the treatment of unexplained infertility: a preliminary report. European Society of Human Reproduction and Embryology. Hum Reprod 6:953–958
4. Casper RF, Mitwally MF (2012) A historical perspective of aromatase inhibitors for ovulation induction. Fertil Steril 98:1352–1355
5. Healey S, Tan SL, Tulandi T, Biljan MM (2003) Effects of letrozole on superovulation with gonadotropins in women undergoing intrauterine insemination. Fertil Steril 80:1325–1329
6. Fouda UM, Sayed AM (2011) Extended letrozole regimen versus clomiphene citrate for superovulation in patients with unexplained infertility undergoing intrauterine insemination: a randomized controlled trial. Reprod Biol Endocrinol 9:84
7. Mitwally MF, Said T, Galal A, Chan S, Cohen M, Casper RF, Magarelli PC (2008) Letrozole step-up protocol: a successful superovulation. https://doi.org/10.1016/j.fertnstert.2008.02.071
8. World Health Organization (2010) WHO laboratory manual for the examination and processing of human semen, 5th edn. World Health Organization, Geneva, p 271
9. Ager J, Huang H, Hansen KR, Baker V (2015) Letrozole, gonadotropin, or clomiphene for unexplained infertility. N Engl J Med 373:1230–1240
10. Bedaiwy MA, Forman R, Mousa NA (2006) Cost- effectiveness of aromatase inhibitor co-treatment for controlled ovarian stimulation. Hum Reprod 21:2838–2844
11. Pourali L, Ayati S, Tavakolizadeh S, Soleimani H, Sani FT (2017) Clomiphene citrate versus letrozole with gonadotropins in intrauterine insemination cycles: a randomized trial. Int J Reprod BioMed 15:49
12. Shahrokh Tehrani Nejad E, Abediasl Z, Rashidi BH, Azimi Nekoo E, Shariat M, Amirchaghmaghi E (2008) Comparison of the efficacy of the aromatase inhibitor letrozole and clomiphene citrate gonadotropins in controlled ovarian hyperstimulation: a prospective, simply randomized, clinical trial. J Assist Reprod Genet 25:187–190
13. Galal AF (2015) Letrozole step-up protocol: the effect of a novel superovulation induction protocol to enhance pregnancy rate in a couple with unexplained infertility undergoing intrauterine insemination. Fertil Steril 104:e328 **(poseidon)**
14. Muhamad W (2016) The relationship between duration of infertility and intrauterine insemination: a multi-centers study. Dev Biol 1:3