



Original Article

Comparative Evaluation of Sequential Regimes of Gonadotropins with Clomiphene Citrate and Letrozole for Ovulation Induction

Kashika Gupta^{1,*}, Banashree Das²¹ Senior resident, Department of Obstetrics & Gynecology, VMMC & Safdarjung Hospital, Delhi-110029, India.² Consultant & Professor, Department of Obstetrics & Gynecology, VMMC & Safdarjung Hospital, Delhi-110029, India.

ARTICLE INFO

A B S T R A C T

Received: 12 Mar 2015
Accepted: 24 Apr 2015

Objective: This study was conducted with the aim to evaluate and compare the efficacy of sequential use of gonadotropins with clomiphene citrate (CC) and letrozole as second line regimes for ovulation. Patients who fail to conceive on CC may benefit from regimes combining use of these oral ovulation induction drugs with gonadotropins, which would decrease the costs and risks involved in gonadotropin use. **Methods:** A prospective clinical study was conducted involving 107 treatment cycles in infertile patients undergoing ovulation induction with two different regimes. Forty eight patients with unexplained or anovulatory infertility who had failed to conceive after three or more cycles of CC and were receiving second line treatment regimes were included. According to the treatment received, patients were designated into two groups. Group 1 (22 patients)– received letrozole followed by hMG (human menopausal gonadotropin). Group 2 (26 patients)– received clomiphene citrate (CC) followed by hMG. Both the groups received Inj. hCG (human chorionic gonadotropin) when the mature follicle reached a size of 18 mm and IUI (intrauterine insemination) was being done 36-40 hours after inj. hCG. Patients were evaluated for different ultrasound parameters, and ovulation and pregnancy rates were compared. Data was analysed with SPSS for Windows 14.0. Results: Forty patients were included in the final analysis. The number of follicles ≥ 10 mm on day 10 was significantly higher in clomiphene group (2.47 ± 0.97) as compared to letrozole group (1.9 ± 0.45). p value 0.023. The mean number of mature follicles (≥ 18 mm) was more in clomiphene group than in letrozole group, but not significantly higher. The endometrial thickness was comparable in the two groups. The hMG dose required was lesser in letrozole group than in the CC group. Of all cycles, 79.44% resulted in ovulation. The ovulation and pregnancy rates were comparable in the two groups. **Conclusions:** Sequential use of gonadotropins with letrozole and clomiphene are effective second line regimes for ovulation induction with comparable outcomes. CC with hMG is associated with multifollicular development more often and the peripheral anti estrogenic effects of CC were not clinically evident in terms of any adverse effects on endometrial thickness or pregnancy rates. Letrozole with hMG leads to a significantly lower gonadotropin requirement in successive treatment cycles as compared to CC. **Key words:** ovulation induction, clomiphene citrate, letrozole, gonadotropins, Intrauterine insemination

Corresponding author *

Dr. Kashika Gupta
17/2 Shakti Nagar, Delhi-110007, India
E mail id: drkashika@gmail.com.
Ph number: 09873438122

1. INTRODUCTION

The therapeutic armamentarium for ovulation induction has been ever expanding. The quest for the ideal regime for every patient continues. Clomiphene citrate(CC) is a well accepted first line agent for ovulation induction. Aromatase inhibitors(AI) have emerged as a promising alternative to CC for ovulation induction^{1,2,3}and have been in use for this indication since 2001.

In patients who fail to respond to oral ovulation induction drugs, gonadotropins remain the preferred option because of their high efficacy. However, the high cost, need for multiple injections and intensive monitoring, and risk of ovarian hyperstimulation are the limiting factors in their use.

Considering the high costs and logistic constraints involved in use of gonadotropins, which is a very significant factor in developing nations, sequential and overlapping regimes of gonadotropins with CC and letrozole have emerged as a very promising alternative. These regimes have shown pregnancy rates comparable to use of gonadotropins alone, with a decreased overall requirement of gonadotropins, thus reducing the cost and risks.^{4,5}

Studies comparing sequential regimes of gonadotropins with CC versus gonadotropins with letrozole have shown conflicting results.

A prospective pilot study⁴ showed that letrozole cycles have a significantly higher pregnancy rate than CC in gonadotropin combined IUI (intrauterine insemination) cycles. However, in this study, letrozole was administered in sequential regime with gonadotropins whereas CC was given in overlapping regime.

Other studies comparing the use of letrozole with hMG(human menopausal gonadotropin) vs CC with hMG in overlapping regimes have shown comparable pregnancy rates with no significant statistical difference.^{5,6}

Although, it has been demonstrated that sequential or overlapping regimes of gonadotropins with clomiphene or letrozole may be as effective as gonadotropins alone with a lower overall cost and risks, it remains to be established which of the two regimes is more effective. There is paucity of studies evaluating the effectiveness of sequential regimes of gonadotropins with clomiphene and letrozole.

Keeping this in mind, this study was conducted with an aim to evaluate and compare the effectiveness of sequential use of gonadotropins with clomiphene and letrozole in terms of ovulation, follicular growth, endometrial thickness, pregnancy rates and total hMG dose required in the Indian population. This study aims to devise an economical and effective second line treatment regime for infertile patients.

2. METHODS

This prospective study was conducted in the Department of Gynaecology, in a tertiary care hospital in Delhi from May 2011 to March 2012.

The study was approved by the Ethical Committee of the Institution. Written informed consent was taken from all the subjects.

Patients undergoing ovulation induction with sequential regimes of gonadotropins with clomiphene citrate or letrozole as second line regime were included in the study.

All patients had either unexplained or anovulatory infertility(WHO group II) with patent fallopian tubes confirmed on HSG(hysterosalpingography)/laparoscopy and normal husband semen analysis. These patients were less than 35 yrs of age and had failed to conceive after previous treatment with clomiphene citrate for three or more cycles.

Patients with premature ovarian failure, elevated FSH levels(>15 mIU/ml) or thyroid dysfunction were excluded from the study.

Forty eight patients with primary or secondary infertility undergoing ovulation induction with two different regimes of drugs were included in the study and observed.

In Group I: Patients who received Letrozole 2.5 mg/day from day three to day seven of the cycle (22 patients) were included

In Group II: Patients who received CC 50 mg/day from day three to day seven of the cycle (26 patients) were included.

Both the groups received Inj hMG from day seven onwards (sequential regime) until at least one mature follicle reached a size of 18 mm. This was followed by Inj. hCG (human chorionic gonadotropin) 10,000 IU when the mature follicle reached a size of 18 mm and IUI was being done 36-40 hours after inj hCG.

Monitoring was being done by transvaginal ultrasound (TVS) in which the number of follicles, size of follicles and endometrial thickness was recorded till ovulation occurred. Patients receiving the above treatment were observed for three consecutive cycles, and the outcomes were recorded. Two patients in group I and six patients in group II were lost to follow up. So, forty patients (20 patients in each group) were included in the final analysis.

The outcome measures included ovulation rate, the number of mature follicles 18 mm, maximum size of dominant follicle, the endometrial thickness on the day of hCG administration, total dose of hMG required in the two groups and pregnancy rates. Clinical pregnancy rate was defined when an intrauterine gestational sac was visible on ultrasonography.

STATISTICAL ANALYSIS: Data was analysed with SPSS for Windows 14.0. Quantitative data (endometrial thickness/ follicular size) and demographic data was analysed using Student's t-test/ Wilcoxon Ranksum test as appropriate. Qualitative variables were compared between the two groups using

chi-square test / Fisher's exact test as appropriate. p value <0.05 was considered statistically significant. Mann Whitney test was used as appropriate.

3. RESULTS

The mean age of the female partner was 29.08 ± 2.68 years (range 25-35) and there was no significant difference between the mean ages of patients in the two groups.

The mean age of patients who conceived was 28.29 ± 3.2 while that in patients who could not become pregnant was 29.24 ± 2.6 , with no significant difference. (p value 0.2)

The overall mean duration of infertility was 6.7 ± 2.48 years.

Age of the female partner, duration of infertility, number of previous treatment cycles, and profile of infertility factors were comparable in the clomiphene and letrozole group (as shown in Table I).

Table I: Baseline characteristics of patients in the two groups

	GROUP I	GROUP II	P
	Letrozole + hMG (n=20)	Clomiphene + hMG (n=20)	value
Female age (y)	28.90 ± 2.614	29.25 ± 2.807	0.686
Duration of infertility (y)	6.41 ± 2.34	6.97 ± 2.64	0.69
No. of previous treatment cycles	3.15 ± 0.489	3.5 ± 0.946	0.15
Infertility factors			0.49
Unexplained (n=28)	15	13	
Anovulatory (n=12)	5	7	

Table 2: Outcome parameters in the two groups

	GROUP I	GROUP II	P
	(Letrozole plus hMG) (n=20)	(CC plus hMG) (n=20)	value
Number of follicles ≥ 10 mm on day 10	1.9 ± 0.45	2.47 ± 0.97	0.023
Number of mature follicles (< 18mm)	1.367 ± 0.47	1.5 ± 0.737	0.5

Maximum size of dominant follicle	19.31 ± 1.4	18.26 ± 2.26	0.085
Endometrial thickness(mm)	8.79 ± 1.52	8.86 ± 1.44	0.874
Number of ampoules of hMG required	1.98 ± 0.66	2.5 ± 1.25	0.112
Day of 18 mm follicle size	11.15 ± 1.53	11.89 ± 1.23	0.124
Clinical pregnancy	3	4	
Twin gestation	1	0	
Ectopic pregnancy	0	1	

The outcome parameters in the two groups have been summarised in table II. The mean number of follicles 10 mm on day 10 of cycle was significantly more in the clomiphene plus hMG group (p value 0.023). The number of follicles 18 mm was higher in the clomiphene group but the difference was not statistically significant. There was no significant difference in the endometrial thickness on the day of hCG administration in the two groups.

In all the patients taken together, the average number of ampoules of 150 IU of hMG required was 2.24 ± 1.02 in each cycle, whereas those required in Group I was 1.98 ± 0.66 ampoules and in group II was 2.5 ± 1.25 ampoules but the difference in the two groups was not found to be statistically significant with a p value of 0.112.

The total dose of hMG required was lesser in letrozole group as compared to the CC group and the difference was statistically significant in the second and third cycles.

One case of OHSS was seen in CC + hMG group.

A total of 107 treatment cycles were given, of which 56 were in letrozole group and 51 in CC group. Eighty five cycles (79.44% of all cycles) resulted in ovulation. The ovulation rate was higher in letrozole plus hMG group as compared to CC plus hMG group (83.92% vs. 74.5%), though not statistically significant.

The clinical pregnancy rate was comparable in the two groups.

The pregnancy rate per patient was 15% (3/20) in letrozole group and 20% (4/20) in CC group. The pregnancy rate per cycle was 5.35% (3/56) in letrozole group and 7.84% (4/51) in CC group. One patient in CC plus hMG group had a tubal ectopic pregnancy which was treated by medical management. Another patient in the CC group had a spontaneous abortion at 7 weeks gestation. One patient in the letrozole group experienced a twin gestation. No congenital anomalies were observed in either group.

4. DISCUSSION

Ovulation induction remains the mainstay in the management of anovulatory (WHO groups I & II) and unexplained infertility. There has always been a quest for an ideal ovulation induction regime in the management of these patients.

This study was conducted with an aim to compare the effectiveness of sequential regimes of letrozole with gonadotropins (Group I) and clomiphene citrate with gonadotropins (Group II) as second line ovulation induction regimes in patients who failed to conceive on oral ovulation induction drugs.

In our study, the number of follicles 10 mm on day 10 were significantly higher in the clomiphene group but there was no significant difference in the number of mature follicles.

In a prospective randomized controlled trial by Barroso *et al.*⁷, comparing letrozole (2.5 mg) and CC (100mg) in sequential regimes with r FSH, no significant differences were found in the number of follicles 10 mm on day 10.

Similar to the findings in the current study, Mitwally MFM and Casper RF⁴ and F. Akbary-Asbagh *et al.*⁵ found no significant difference in the number of mature follicles in the two groups of patients receiving letrozole versus CC combined with gonadotropins.

Barroso G *et al.* (2006)⁷ also found no significant difference in the number of mature follicles in the two

groups receiving letrozole versus CC sequentially with gonadotropins.

In a study by B.C. Jee *et al.*⁶, comparing overlapping regimes of letrozole and CC with hMG observed significantly more number of mature follicles in the CC group as compared to the letrozole group. This may be due to the higher dose of clomiphene citrate (100 mg) used as compared to 50 mg in the present study.

The endometrial responsiveness (assessed by the endometrial thickness on TVS) was comparable in the two groups in our study, similar to the findings of Jee B.C. *et al.*⁶

Contrary to these findings, three studies^{4,5,7} using clomiphene and letrozole in combination with gonadotropins, found a significantly higher endometrial thickness in the letrozole group and attributed this to the peripheral antiestrogenic effect of CC. In these three quoted studies, there was no significant difference in the number of mature follicles. In our study, the number of follicles 10 mm on day 10 were significantly higher in the clomiphene and hMG group. Also the number of mature follicles was also higher in the clomiphene group. This may indicate that the higher amount of estrogen production from the greater number of growing follicles may cause endometrial development in the clomiphene group comparable with the group on treatment with letrozole. This might also indicate release of the hypothalamus and/or pituitary and the peripheral tissues (endometrium and cervical mucous) from the anti-estrogenic effect of CC allowing rising estrogen to cause favourable endometrial development.

It was also observed in the present study that the mean endometrial thickness was more in patients who conceived as compared to those who could not conceive (9.16±2.1 vs. 8.7 ±1.32), though this difference was not statistically significant.

Various authors have reported that addition of oral antiestrogens (CC or AIs) to gonadotropins reduces the requirement of hMG^{4,5,8} decreases the cost and gives similar treatment outcomes. Similarly, in our study we required far less mean hMG dose (2.24 ampoules) due to addition of oral antiestrogens (CC or Letrozole).

In our study, overall requirement of hMG was similar in both the groups with no statistically significant difference. The findings in the present study are concurrent with the observations of various other authors.^{4,5,6,7}

It was also observed in our study that, the dose of gonadotropin required in the second and third cycle was significantly higher in the clomiphene group; thus emphasizing the advantage of lower gonadotropin requirement in successive cycles of letrozole.

This may be explained by the much shorter half-life and absence of any antiestrogenic effects of aromatase inhibitors which are not associated with any deleterious effects on the final stages of follicular development and oocyte maturation. Administration of letrozole in the early follicular phase results in very low or absent drug levels in the body during the peri-ovulatory and luteal phases of the cycle, which may promote better follicular development as compared to clomiphene citrate, thus decreasing the gonadotropin dose needed for COH even more. Thus, successive treatment cycles with letrozole cause a greater reduction in gonadotropin requirement as compared to CC, without compromising ovulation and pregnancy rates.

However, these findings need to be confirmed by larger randomized trials.

In our study there was no significant difference in the day of follicle size 18 mm, which was concurrent with the findings of other similar studies^{4,6,7}; thus indicating that a similar duration of stimulation is needed with both the protocols.

In the present study, the overall ovulation rate was 79.44%. The ovulation rate was higher in the letrozole group (83.93%) as compared to the CC group (74.5%), but the difference was not statistically significant (p value 0.23).

In a similar study by Sipe *et al*⁹ in 50 patients comparing aromatase inhibitor (anastrozole) and clomiphene along with gonadotropins in superovulation and IUI cycles, the ovulation rate was 80% (20/25) in anastrozole group and 88% (22/25) in clomiphene group.

In the present study, there was no significant difference in the pregnancy rates in the two groups. Pregnancy rate per patient in letrozole group was 15% (3/20) as compared to 20% (4/20) in clomiphene group. The overall pregnancy rate per patient was 17.5%.

Similar to the findings in our study, Jee B *Cet al.*⁶ in their study of Letrozole versus CC combined with gonadotropins in IUI cycles reported a comparable pregnancy rate per patient in Letrozole group (18.2%) and in CC group (25.9%).

F. Akbary-Asbagh *et al.*⁵ also demonstrated similar pregnancy rates in patients receiving letrozole and clomiphene (28% vs. 23%) in hMG combined IUI cycles.

However, Mitwally MFM and Casper RF⁴ reported a significantly higher pregnancy rate in letrozole combined FSH cycles when compared with CC+FSH cycles. This was attributed to the peripheral antiestrogenic effects of CC. In this study, letrozole 2.5 mg /day was given from day three to day seven followed by FSH from day seven onwards (sequential regime). On the other hand CC was given later in the cycle from day five to day nine with FSH in an overlapping regime starting from day five. Giving CC later and in an overlapping regime (which was given from day three to day seven with sequential hMG in our study) may be responsible for persistence of

antiestrogenic effects, thus resulting in a thinner endometrium and lower pregnancy rates in comparison to our study and other similar studies.

5. CONCLUSION

This study shows that Letrozole with hMG has similar ovulation and pregnancy rates compared with clomiphene with hMG in superovulation cycles but larger randomized studies are required to conclusively establish which regime is superior as a second line regime. These regimes reduce the costs and risks involved in gonadotropin treatment cycles, thus optimizing outcomes.

CC with hMG is associated with multifollicular development more often and the peripheral antiestrogenic effects of CC were not clinically evident.

Successive cycles of letrozole with hMG lead to a significantly greater reduction of gonadotropin requirement for ovulation induction as compared to CC with hMG. Similar effects on ovarian stimulation and endometrial responsiveness were observed.

Future research should strive to analyse and confirm if letrozole cycles lead to a greater gonadotropin dose reduction in comparison to CC, and the mechanism behind the same.

6. ACKNOWLEDGEMENTS

The authors thank the hospital staff and authorities of VMMC & Safdarjung Hospital for their support throughout.

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Conflict of interest statement: The authors have no actual or potential conflicts of interest relevant to this article.

Funding: none