Original Article

A Study of Controlled Ovarian Stimulation with Clomiphene Citrate or Letrozole in Combination with Gonadotropins and IUI in Unexplained Infertility

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Abstract

Aim: To compare the effect of clomiphene citrate (CC) + human menopausal gonadotropin (hMG) with letrozole + hMG on size, number of follicles, endometrial thickness, serum levels of oestradiol and progesterone and pregnancy rate. **Settings and Design:** Non-randomised interventional study. **Patients and Methods:** A total number of 60 patients in the age group of 20–35 years with unexplained infertility were divided into two groups, 30 in each. Group A received CC + hMG and group B received letrozole + hMG. In both the groups, ovulation was triggered by hCG followed by intrauterine insemination. **Results:** The number of follicles on day 8 were significantly higher in the CC + hMG group than that in the letrozole + hMG group. Serum oestradiol level was significantly higher in the CC + hMG group on day 10 and on the day of hCG administration. Pregnancy rate in the CC + hMG group was 23.3% and 13.3% in the letrozole + hMG group. **Conclusion:** The sequential protocol was cost-effective. CC + hMG could be a preferred ovarian stimulation protocol in couples with unexplained infertility with the added advantage of having no significant complications in properly monitored cycles.

Keywords: *Clomiphene citrate, IUI, letrozole*

Introduction

Infertility is a common problem in gynaecological practice affecting about 21% of couples in the reproductive age group.[1] Unexplained infertility comprises 10–20% among all infertility patients. The treatment of unexplained infertility includes super-ovulation and intrauterine insemination (IUI). The initial management of unexplained infertility is taken care of by oral drugs. If a controlled ovarian stimulation using oral drugs is unsuccessful, then gonadotropin therapy in conjunction with IUI is recommended. The cost of treatment is a limiting factor while using gonadotropin. A sequential regimen with oral drugs and gonadotropin has shown a fecundity rate almost equal to gonadotropin alone.[2] A combination of oral drugs with gonadotropin significantly reduces the cost of treatment without compromising on the outcome.[3] This study was conducted to see the effect of sequential therapy of clomiphene citrate (CC) and letrozole with gonadotropin on the outcome measures in the resource-limited settings.

Patients and Methods

The study was conducted in the department of obstetrics and gynaecology of Lady Hardinge Medical College and Hospital, a tertiary care centre hospital over a period of one year and five months. It was a non-randomised interventional study. Ethical clearance was obtained from the institutional ethical committee of the medical college. Patients visiting the outpatient department (OPD) with infertility in the age group of 20–35 years were subjected to routine tests for infertility. These included husband semen analysis (HSA), endometrial biopsy to check for secretory

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phase endometrium, which indicated ovulation, and hysterosalpingography (HSG). The normal parameters for HSA pertained to World Health Organization criteria 2010. The respective serum levels of day 2 Follicular stimulating hormone (FSH), Luteinising hormone (LH), Thyroid stimulating hormone (TSH) and prolactin were noted. All the above hormone levels were done by two-site sandwich immunoassay using a direct chemiluminescent technique. The couples, in whom all the above hormonal profiles were normal, and those with normal HSA, secretory endometrium in endometrial biopsy and patent fallopian tubes of female partner, were labelled as having unexplained infertility. Written consent was obtained from all the participants of the study after explaining the risks and benefits of the planned treatment. Demographic details namely age, duration of marriage, years of infertility and baseline serum levels of TSH, FSH, LH and prolactin were noted. They were divided into two groups. Groups A and B were allocated alternatively to the subsequent women coming to the OPD. Group A received oral tablet CC 50 mg from day 3 to day 7 of the menstrual cycle. Group B received oral tablet letrozole 5 mg for 5 days from day 3 to day 7 of the cycle. Transvaginal ultrasonography (TVS) was performed in both the groups on day 8. Intramuscular injection of human menopausal gonadotropin (hMG) was given in a dose of 75 IU daily starting from day 8 till the size of the dominant follicle became 18 mm. Intramuscular hMG was given for a maximum of five days. Alternate day TVS was performed to measure the total number of developing follicles, the size of follicles on both the sides and endometrial thickness (ET). The only method for ultrasonography used was transvaginal, and it was performed by a single observer throughout the study. The power of transvaginal probe used was 7.5 MHz. The day when the size of at least one dominant follicle reached 18 mm, ovulation was triggered by an intramuscular injection of 10,000 units of human chorionic gonadotropin (hCG). Ovulation was expected after 36 h of injection hCG, and it was confirmed by TVS. Crenation of the follicle and appearance of fluid in the pouch of Douglas were considered to be the signs of rupture of follicle. IUI was performed after 36 h of trigger either by swim up technique or single density method depending on the sperm count and motility. Serum oestradiol levels were noted on day 10 and on the day of hCG administration. Serum progesterone level was done on day 21 to check ovulation and adequacy of luteal phase support. Both serum oestradiol and progesterone levels were recorded by competitive immunoassay using the direct chemiluminescent technology. For all the hormone levels, the kit used was ADIANA Siemens. Cycles with large follicles more than six in number were deferred from trigger to prevent Ovarian hyperstimulation syndrome (OHSS). There were a total of 80 cycles in the CC + hMG group and 84 cycles in the letrozole + hMG group. The primary outcomes were number and size of follicles, ET as well as S. oestradiol and S. progesterone levels. Secondary outcome was pregnancy rate.

Statistical evaluation

Statistical analysis was performed using the Statistical Package for the Social Sciences version 19.0 software (SPSS Inc., Chicago, IL, United States). The results were expressed as mean ± standard deviation. Unpaired *t* test and Fisher's exact test were used at an appropriate place to calculate the *P* value. The *P* value was considered significant when it was <0.05.

Results

The demography and baseline investigations of both the groups were as given in Tables 1 and 2, respectively. The respective *P* values for all the parameters were more than 0.05 indicating that no significant differences between the groups and both the groups were comparable. The numbers of follicles, size of follicle and ET were as tabulated in Table 3. The serum levels of

Table 1: Demographic parameters of Group A and Group B

Parameter	CC + hMG group ($n = 30$)	Letrozole + hMG group ($n = 30$)	*P* value
Age (years), mean ± SD	26.87 ± 3.55	28.27 ± 2.89	0.09
Duration of infertility (years), mean ± SD	6.8 ± 2.44	7.87 ± 2.61	0.12

CC = clomiphene citrate, hMG = human menopausal gonadotropin and SD = standard deviation. *P* value ≤0.05 significant.

Table 2: Baseline investigations of Group A and Group B

Parameters	CC + hMG group ($n = 30$)	Letrozole + hMG group ($n = 30$)	*P* value
Sperm count (million/ml), mean ± SD	81.83 ± 20.39	86.2 ± 24.29	0.22
S. TSH (μIU/ml), mean ± SD	3.00 ± 1.40	2.72 ± 1.29	0.21
S. FSH (IU/L), mean ± SD	8.99 ± 5.04	7.41 ± 3.85	0.08
S. LH (IU/L), mean ± SD	5.02 ± 2.57	4.07 ± 0.06	0.06
S. prolactin (ng/ml), mean ± SD	15.52 ± 4.79	13.39 ± 6.54	0.07

SD = standard deviation, hMG = human menopausal gonadotropin and ng = nanogram. *P* value ≤0.05 significant.

oestrogen and progesterone and dose of hMG required are shown in Table 4.

In total, three patients were deferred from trigger. It was noted a development of a total of eight follicles in one of the cycles of the CC + hMG group, with the largest follicle being 20 mm. The day 10-serum oestrogen level was 650 pg/ml in the same cycle. Therefore, the cycle was cancelled anticipating OHSS. She conceived with natural intercourse in the same cycle. It was a twin pregnancy. One cycle in the CC + hMG group and one cycle in the letrozole + hMG group were cancelled due to the development of follicular cyst. When the size of follicle was more than 30 mm, it was considered as a follicular cyst. They were advised natural timed intercourse. Day 21 progesterone level indicated ovulation in all cycles.

The number and percentage of normal pregnancy, twin pregnancies, abortions, ectopic pregnancy and live births were as given in Table 5. There were no congenital anomalies in the foetuses in any of the groups.

The results of the present study were compared with two closely similar studies in Table 6.

Discussion

CC is known to exert an action through the depletion of central oestrogen receptors, which in turn reduces the negative feedback mechanism of oestrogen on hypothalamus and pituitary. On one hand, the effect is an increase in gonadotropin secretion leading to multiple follicular developments. On the other hand, letrozole causes a decrease in peripheral oestrogen production, which is responsible for the increased gonadotropin secretion in the early part of the cycle; however, due to its short half-life, its effect wears off in the late follicular phase resulting in monofollicular development in the late follicular phase of the cycle.[4] In this study, the average number of follicles, which started developing on day 8, were higher in the clomiphene + hMG group than in the letrozole + hMG group (1.78 vs. 1.51), which is possibly due to the increased gonadotropin secretion by CC leading to the development of multiple follicles. The increase in mean number of follicles persisted on day 10 (1.58 vs. 1.39) and on day 12 (1.59 vs. 1.33), although on these two days, the difference did not attain statistical significance. On the day of hCG administration, however, the mean numbers of follicles were within 1.2 in both the groups.

Table 3: Comparison of Follicular development and endometrial thickness in Group A and Group B

Parameters	CC + hMG (n = 80)	Letrozole + hMG (n = 84)	P value
No. of follicles on			
Day 8	1.78 ± 0.63	1.51 ± 0.58	0.04
Day10	1.58 ± 0.55	1.39 ± 0.39	0.06
Day 12	1.59 ± 0.88	1.30 ± 0.33	0.05
No. of follicles ≥18 mm on the day of hCG administration	1.21 ± 0.35	1.21 ± 0.30	0.44
Size of follicle in mm			
Day 8	13.22 ± 2.06	13.27 ± 1.40	0.45
Day 10	16.04 ± 2.27	16.37 ± 1.29	0.24
Day 12	18.67 ± 1.76	18.83 ± 1.38	0.36
Mean size of follicle ≥18 mm on the day of hCG administration	19.79 ± 1.06	19.92 ± 0.93	0.20
ET in mm on			
Day 8	6.69 ± 1.26 mm	6.41 ± 1.34 mm	0.20
Day 10	7.58 ± 1.54 mm	7.21 ± 1.28 mm	0.15
Day 12	8.21 ± 1.31 mm	7.92 ± 1.30 mm	0.20
Mean ET in mm on day of high administration	8.27 ± 0.66	7.73 ± 0.12	0.06

ET = endometrial thickness, hMG = human menopausal gonadotropin, hCG = human chorionic gonadotropin and mm = millimeter. P value ≤0.05 significant.

Table 4: S. oestradiol level during induction, amount of gonadotropin required and day 21 progesterone level

Parameters	CC + hMG (n = 80)	Letrozole + hMG (n = 84)	P value
S. oestradiol (pg/ml) on day 10	245.43 ± 92.89	200.67 ± 24.67	0.006
S. oestradiol (pg/ml) on the day of hCG administration	264.09 ± 99.09	218.98 ± 24.44	0.009
Amount of hMG (IU) required to reach follicle size ≥18 mm	264.17 ± 66.70	263.33 ± 55.12	0.47
S. progesterone (ng/ml) on day 21	23.48 ± 11.14	24.24 ± 10.23	0.39

CC = clomiphene citrate, hCG = human chorionic gonadotropins, hMG = human menopausal gonadotropin, IU = international unit, pg = picogram and ng = nanogram. P value ≤0.05 significant.

This can be explained by the fact that the dose of CC, letrozole and hMG used in this study is low resulting in development of a single dominant follicle. In a study by Akbary-Asbagh *et al.*,[5] the mean number of dominant follicles were 1.8 in the CC + hMG group as compared to 1.4 in the letrozole + hMG group. In another study by Jee *et al.*,[6] the mean number of dominant follicles in the letrozole + hMG group were 3.2 ± 1.7, and in the CC + hMG group, it were 5.6 ± 2.4, which was statistically significant with a *P* value of <0.0001.[6]

The antioestrogenic effect of CC is responsible for thinner endometrium in the CC-treated groups. Though statistically not significant in the present study, the ET in the CC + hMG group was higher than that in the letrozole + hMG group. The endometrial response as seen in both the groups shows a progressive increase in ET from 6.69 mm on day 8 to 8.27 mm on the day of hCG administration in the CC + hMG group and from 6.41 to 7.73 mm in the letrozole + hMG group. The increased ET co-relates with the higher oestradiol levels seen in the CC + hMG group. The adverse effect of CC on endometrial growth has been seen at higher doses or with longer duration and may be offset by the higher oestradiol levels seen with CC.[7] Although the supraphysiological levels of oestradiol in clomiphene-induced cycles may have deleterious effect on endometrium, in the present study, the mean oestradiol level was 264.09 ± 99.09 pg/ml, which is not exceptionally high. In addition, sequential therapy with hMG in the present study might be a factor for good endometrial response. Higher oestradiol level in the CC + hMG group correlated with an improved ET (8.27 vs. 7.73 mm), and this could have been the reason for a better pregnancy rate in the CC + hMG group. The ET of <6 mm on the day of hCG administration is associated with poor results, and in the present study, none of the women who became pregnant had ET <6 mm. The present study is supported by a study by Al-Fozan *et al.*,[8] in which ET in the CC group (8.2 ± 0.6 mm) was higher than that in the letrozole group (7.1 ± 0.2 mm). In a study conducted by Bayar *et al.*,[9] the mean ET in both the groups were similar, that is 8 mm. In the present study, the dose of CC was 50 mg, which was lower compared to other studies with the result that there was less antioestrogenic effect and better ET than letrozole group.

In the present study, the respective E2 levels on day 10 and on the day of hCG administration were significantly higher in the CC + hMG group. The higher oestradiol level correlated with improved ET in the CC + hMG group. Other studies have similarly shown lower peak E2 level in the letrozole + hMG group. In a study conducted by

Table 5: Pregnancy outcome in Group A and Group B

Parameters	CC + hMG	Letrozole + hMG	*P* value
Total pregnancy	7 (23.33%)	4 (13.33%)	1.158
Twin pregnancy	1 (14.28%)	0	
Abortion	1 (14.28%)	2 (50%)	0.227
Ectopic pregnancy	1 (14.28%)	0	
Live births	6 (85.71%)	2 (50%)	0.49

CC = clomiphene citrate and hMG = human menopausal gonadotropin. *P* value ≤0.05 significant.

Table 6: Comparison of different studies on parameters of ovulation induction and pregnancy outcome

Studies	Present study		Akbary-Asbagh *et al.*[5]		Jee *et al.*[6]	
Parameters	CC + hMG	Letrozole + hMG	CC + hMG	Letrozole + hMG	CC + hMG	Letrozole + hMG
No. of DF	1.2 ± 0.35	1.2 ± 0.30	1.8	1.4	5.6 ± 2.4	3.2 ± 1.7
Mean size of DF (mm)	19.97 ± 1.06	19.92 ± 0.93	–	–	–	–
ET on the day of hCG (mm)	8.27 ± 0.66	7.73 ± 0.12	6.4 ± 0.8	7 ± 1.1	9.1 ± 1.7	9.3 ± 1.7
E2 on the day of hCG administration (pg/ml)	264.09	218.98	619 ± 451.3	209 ± 195.1	1371.7 ± 750.5	231.0 ± 179.8
Day 21 progesterone (pg/ml)	23.48 ± 11.14	24.24 ± 10.23	22.8	20.7	–	–
Pregnancy rate (%)	23.3%	13.3%	23%	28%	25.9%	18.2%
Twin pregnancy	1	0	–	–	1	1
Ectopic pregnancy	1	0	–	–	–	1
Miscarriage	1	2	–	–	1	0

DF = dominant follicle, ET = endometrial thickness, pg = picogram, hMG = human menopausal gonadotropins and hCG = human chorionic gonadotropin.

Akbary-Asbagh *et al.*,[5] E2 level on the day of hCG administration was 209.7 ± 195.1 pg/ml in the letrozole + hMG group, and it was 619 ± 451.3 pg/ml in the CC + hMG group, which was statistically significant.

Healy *et al.*[10] found that the dose of gonadotropin required to reach dominant follicle size in gonadotropin-only group was 940.6 ± 464.2 IU as against 600.6 ± 405.7 IU in the letrozole + gonadotropin group. If we compare the results of the above study with that in the present study, much lesser amount of gonadotropin was required in both the groups in the present study. In a study conducted by Jee *et al.*,[6] the total ampoules (150 IU) of hMG required were 8.2 ± 2.8 in the letrozole + hMG group and 8.4 ± 2.3 in the CC + hMG group, which was comparable for both the groups. In the present study, the total dose of hMG required was comparatively less than other studies making it cost-effective.

In contrary to other studies,[2,5] in a study by Jee *et al.*,[6] the pregnancy rate in the CC + hMG group was higher than in the letrozole + hMG group, which was similar to our study. The present study had higher ET in the CC + hMG group as compared to other studies, and this may be the reason behind higher pregnancy rate than that in the letrozole + hMG group.

Jee *et al.*[6] had one twin pregnancy in both the CC + hMG group and the letrozole + hMG group. In the present study, there was one twin pregnancy, and it was in the clomiphene group.

All the abortions in both the groups were early abortions, which may be due to poor endometrial receptivity or some genetic factor. Probably, the thicker endometrium in the CC + hMG group may be the reason behind the lesser rate of abortion in the CC + hMG group.

In the present study, there was one ectopic pregnancy in the CC + hMG group. The woman with ectopic pregnancy had a history of ectopic pregnancy in the past, which may be a risk for recurrence of ectopic pregnancy. There was only one ectopic pregnancy in the letrozole + hMG group, and no ectopic pregnancy in the CC + hMG group in a study conducted by Jee *et al.*[6]

Conclusion

The sequential protocol was cost-effective. CC + hMG could be a preferred ovarian stimulation protocol in couples with unexplained infertility with the added advantage of having no significant complications in properly monitored cycles.

Strength and weakness

The study could have been better if the sample size were larger, which could not be accomplished due to limitation of time, because it was a post-graduate level dissertation with a fixed duration of study.

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Conflicts of interest

There are no conflicts of interest.

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