Original research article

Comparative Study of Letrozole Versus Clomiphene Citrate for Ovulation Induction in Anovulatory Infertility

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Abstract

Introduction: Infertility is a multidimensional health problem with social & economic consequences. Female factor remains the foremost reason (40%-55%) and ovulatory disorders is the most common identifiable female factor. Ovulation induction is one of the most successful treatment of infertility due to anovulation.

Aim: To compare the effects of letrozole and clomiphene citrate (CC) for ovulation induction in women with anovulatory infertility.

Materials And Methods: In this assessor blind randomized controlled trial 80 infertile women with anovulatory infertility were randomized to receive either letrozole or CC for ovulation induction in incremental doses for a maximum of three cycles. Main outcomes studied were endometrial thickness, ovulation rate, pregnancy rate and rate of mono-follicular development. Both the groups were followed by ultrasound until the dominant follicle reached a diameter ≥18 mm, human chorionic gonadotropin (hCG) 10,000 IU was given, and timed intercourse was advised.

Results : The mean age, duration of infertility, body mass index, and endocrine status in both the groups were similar at baseline. Ovulation occurred in 36 subjects (90.0%) in the letrozole group and 29 (72.5%) in the CC group, with a statistically significant difference between the two groups (P=0.045). Mono-follicular development was seen in 60.0% of ovulatory cycles in letrozole group compared to 22.5 % in clomiphene group (p=0.001). Pregnancy was achieved in 37.5% women in letrozole group and 25.0% in clomiphene group (p=0.228). There was no statistically significant difference in endometrial thickness between the two groups at the time of hCG administration (9.71±1.16mm vs. 9.6±1.03 mm with letrozole and clomiphene respectively (p = 0.648).

Conclusion: The effect of letrozole showed a better ovulation rate and monofollicular development as compared with CC. Letrozole may have a role as a first-line treatment for anovulatory patients with anouvulatory infertility.

Keywords: Clomiphene citrate, letrozole, anovulatory infertility , PCOS, pregnancy

Introduction

Infertility is a multidimensional health problem with social & economic consequences. As achieving parenthood is the most basic and desired goal in adulthood, infertility is more than a physical problem. Infertility, defined as failure to achieve pregnancy within 12 months of unprotected intercourse or therapeutic donor insemination in women younger than 35 years or within 6 months in women older than 35 years.[1] The prevalence of infertility is about 10%–15% of reproductive age couples.[2] Estimates suggest that between 48 million couples and 186

million individuals live with infertility globally.[3] Boivin et al reported 9% prevalence of infertility (of 12 months) with 56% of couples seeking medical care. [4] The overall prevalence of primary infertility in India is between 3.9 and 16.8%[5]. Infertility may be primary or secondary. Primary infertility denotes those patients who have never conceived. Secondary infertility indicates history of previous pregnancy but failure to conceive subsequently. [6] Approximately 85–90% of healthy young couples conceive within 1 year, most within 6 months. Poor lifestyle choices, stress, ambitions regarding career, free radical environmental injuries and late age of marriage have increased the incidence of infertility.
Among the causes of infertility, female factor (40%-55%) remains the foremost reason followed by male factor (30%-40%), combined factor (10%), whereas in 10% cases, etiology remains unexplained. [7] The most common identifiable female factors, which accounted for 81% of female infertility, are : ovulatory disorders (25%), endometriosis (15%), pelvic adhesions (11%), tubal blockage (11%) other tubal abnormalities (11%), hyperprolactinemia (7%).[8]

In WHO classification of anovulation , Group 2 i.e. Hypothalamic-pituitary dysfunction is most common which is characterized by irregular or anovulatory menses, normal FSH, estrogen, and prolactin. Most common causes are PCOS and androgen disorders.[9] Polycystic Ovarian syndrome is most common disorder, often complicated by chronic anovulatory infertility.[10] The diagnosis of PCOS is based on the Rotterdam criteria for the presence of any two of the following conditions: (i) chronic anovulation, (ii) clinical/biochemical parameters for hyperandrogenism, and (iii) polycystic ovaries on ultrasonography.[11]

Ovulation induction is one of the most successful treatment of infertility due to anovulation.[12] Ovulation induction is a therapeutic restoration of the release of one egg per cycle in the woman who either has been not ovulating regularly or has not been ovulating at all. Clomiphene citrate has been the most widely used treatment for fertility enhancement for the past more than 40 years . The primary site of clomiphene action is the hypothalamus, where it appears to bind to and deplete hypothalamic ERs, thereby blocking the negative feedback effect of circulating endogenous estradiol. This results in an increase in hypothalamic gonadotropin-releasing hormone (GnRH) pulse frequency and increased serum concentrations of follicle-stimulating hormone (FSH) and luteinizing hormone (LH). Clomiphene acts primarily as an antiestrogen in the uterus, cervix, and vagina. CC has a negative effect on the cervical mucus and endometrium, leading to the discrepancy between ovulation rate 60-80% but a conception rate of only 20%.[13] CC has side effects like multi-follicular development, hyperstimulation syndrome and cyst formation.

Letrozole, is the most effective aromatase inhibitor, for ovulation induction in women with PCOS (anovulatory infertility).It results in decreased estradiol secretion via ovarian suppression, a rise in follicle-stimulating hormone (FSH) (due to release from the negative feedback effect of estradiol), follicular development, and estradiol production (as the effect of the aromatase inhibitor diminishes).[14] As the dominant follicle grows and estrogen levels rise, normal negative feedback occurs centrally because aromatase inhibitors do not deplete estrogen receptors in the brain. FSH is then suppressed, and the smaller-growing follicles become atretic, resulting in monofollicular ovulation in most cases.[9] The potential for mono-ovulation represents a theoretical advantage over clomiphene citrate, which is associated with an increased risk of multiple gestations.

MATERIALS AND METHODS

A hospital-based double blinded prospective cohort study was conducted at Obstetrics and Gynaecology Department of a tertiary care hospital, Sri Guru Ram Das Institute of

Medical Sciences and Research ,Amritsar for a time period April 2021 to July 2022. During the study period, a total of 126 patients visiting infertility clinic were screened for recruitment. Out of which, 42 patients did not meet inclusion criteria and a total of 84 patients met the criteria and were recruited into the study. Out of those 84 patients, 4 patients lost to follow up. Thus a total of 100 study participant's data was subjected to the final analysis. It has been approved by the Ethics Committee of SGRDIMSAR, Amritsar.

INCLUSION CRITERIA

The study included the following women:

1) All patients coming with anovulatory infertility with no other obvious cause of infertility associated
2) Patient with bilateral or unilateral fallopian tubes patent observed at laproscopic chromopertubation or hysterosalpingography.
3) Their spouse should have male factor fertility confirmed by adequate seminal parameters according to latest WHO guidelines.
4) No history of heart, liver, kidney disease.

EXCLUSION CRITERIA-

1) Women with uncontrolled hypo/hyperthyroidism /pituitary causes of infertility.
2) Male factor infertility.
3) Women with bilateral tubal block identified at HSG or laproscopic chromoperteubation.
4) Patients with unexplained infertility.

A complete medical and gynaecological examination including complete blood counts, liver, thyroid and renal function tests, prolactin, FSH/LH on day 2 of menstruation , etc. was done. A baseline Trans vaginal sonography was done on first visit for AFC (Antral follicle count). Patients were randomly assigned in two different groups A and B after counselling. Starting on any day between 3 to 5 of menses, lowest dose of Clomiphene citrate 50 mg or Letrozole 2.5 mg was prescribed. Follicular monitoring was done by TVS on day 9 of the cycle till a mature follicle of size 18 to 20 mm or more was detected. A single injection of hCG 10,000IU IM was given if atleast one follicle would be of 18-20 mm and the endometrial thickness atleast more than 8 mm. A second TVS was done after 24 to 48 hours of hCG to observe the release of egg. If the follicle was found unruptured, a third TVS was done after 72 hours of the hCG injection to observe a luteinized unruptured follicle. Ovulation was ascertained by observing rupture of the follicle by TVS- collapsed follicle ,fluid in pouch of Douglas .Endometrial thickness was measured, a trilaminal diameter of >/=8mm was considered a satisfactory response.Timed intercourse was advised 24 to 48 hours after hCG on two consecutive days and the luteal phase was supplemented with micronized progesterone.

Women in group A were prescribed lowest dose of Clomiphine citrate i.e. 50 mg for first cycle followed by 100 mg if no ovulation induction achieved with 50 mg CC , followed by 125 mg CC for one cycle and another cycle with 125mg in case of failure, i.e. total of four cycles. Similar protocal was followed for group B Letrozole with 2.5 mg for one cycle followed by 5 mg for next cycle in case of failure, 7.5 mg for next cycle and another cycle with 7.5mg in case of failure, i.e. total of four cycles with each drug . If no follicle developed , the cycle was cancelled, and another cycle was started with increased dose of the same drug. Once ovulation was documented for a particular dose of the drug, patients were advised the same dose for 3 cycles in total.

OUTCOME:

Primary outcome: • Number and maximum size of mature follicles.
• Ovulation rate
• The endometrial thickness on the day of HCG administration,

Secondary outcome: The occurrence of pregnancy (biochemical / ultrasonography) by measuring beta-HCG levels in urine/blood.

Women in both the groups with no evidence of ovulation and with negative pregnancy tests were asked to follow up on the respective schedule of treatment in subsequent cycles for a maximum of 3 cycles for each woman.

Statistical Analysis:

Statistical analyses were performed using a Statistical Package for Social Sciences software (SPSS) version 22. Student t-test (independent t-test) was applied to compare the effects of letrozole and clomiphene citrate (CC) for ovulation induction in women with anovulatory infertility. The Chi-square test was applied to see the association between outcome and demographic characteristics. The Receiver Operating Curve (ROC) was constructed . For all statistical analyses, $p < 0.05$ was considered significant, and $p < 0.001$ was considered highly significant.

RESULTS

During the study period, a total of 126 patients were analyzed for recruitment. 42 patients did not meet inclusion criteria and 4 patients were lost to follow-up in between the study, and therefore, 80 patients entered and completed the study. The comparison of baseline parameters of participants is shown in Table 1. There were no statistically significant differences between the two groups in age, duration of infertility, BMI, and endocrine status at baseline level.

Table 1: Baseline characteristic of the patients with primary infertility in both groups

PARAMETERS	Clomiphene Citrate (n-40)	Letrozole (n-40)	p value
Age (years)	29.75±4.47	28.48±3.37	0.154
Duration of mean infertility period (years)	3.86±2.51	3.71±2.23	0.778
Body Mass Index (kg/m^2)	24.59±3.14	23.9±2.29	0.265
FSH (IU/mL) on day 2 of cycle	6.24±1.82	6.47±1.61	0.554
LH(IU/mL) on day 2 of cycle	8.27±2.08	8.91±2.39	0.203
TSH (IU/mL)	2.41±1.02	2.52±1.06	0.650
Prolactin (ng/mL)	19.19±8.52	17.21±4.7	0.201

The number of follicles ≥18 mm was statistically significantly higher in the letrozole group (90%) compared with the CC group (72.5%) ,p value -0.045 (Table-2).

Table 2: Percentage of successful ovulation

	Clomiphene		Letrozole			
OVULATION	N	%age	N	%age	Total	P value
N	11	27.5%	4	10.0%	15	0.045
Y	29	72.5%	36	90.0%	65	
Total	40	100.0%	40	100.0%	80	

Mono follicular development was highly significantly higher in Letrozole Group (CC 22.5%, Let 60%) ,

p value-0.001 (Table 3).

Table 3: Follicular development among group Clomiphene citrate and letrozole

	RESPONSE	Clomiphene citrate		Letrozole		Total	pvalue
No. of follicles		n	%age	n	n%age		
	No dominant follicle	3	7.5%	4	10.0%	8	
	Mono-follicular	9	22.5%	24	60.0%	30	0.001
	Multi-follicular	28	70.0%	12	30%	33	
	Total	40	100.00%	40	100.00%	80	

There was no statistically significant difference in pretreatment endometrial thickness between the two groups. Mean endometrial thickness was 9.71±1.16mm in letrozole group and 9.6±1.03 in those receiving CC, however the difference was not statistically significant (p value- 0.648). (Table 4).

Table 4: Endometrial thickness (mm) among group CC and Letrozole

	Clomiphene citrate		Letrozole		
	Mean	SD	Mean	SD	p value
Endometrial thickness (mm)	9.71	1.16mm	9.6	1.03	0.648

The pregnancy rate was higher in letrozole group as compared to clomiphene (L- 37.5% versus CC-25%) (Table 5).

Table 5: Percentage of pregnancy rate or conception rate

	Clomiphene		Letrozole			
PREG	N	% age	N	%age	Total	P value
N	30	75.0%	25	62.5%	55	0.228
Y	10	25.0%	15	37.5%	25	
Total	40	100.0%	40	100.0%	80	

DISCUSSION

The first line of ovulation inducing agent CC is not equally effective in all situations for induction of ovulation or super ovulation. Clomiphene resistance occurs in 15–20% of the patients. The use of CC may be associated with poor cervical mucous and endometrial thinning in 15–50% of the patients due to prolonged estrogen receptor depletion in the endometrium and possibly in the cervix. For many years, the first treatment of choice for ovulation induction in PCOS was CC, but up to 58% of such patients are resistant to it and do not ovulate. The PR per cycle remains relatively low. Letrozole, which is an aromatase inhibitor, has been explored as a good alternative by many researchers but the evidence about its efficacy as compared to clomiphene is conflicting.

In this study the number of follicles ≥18 mm was statistically significantly higher in the letrozole group (90%) compared with the CC group (72.5%),p value -0.045. It is supported by previous studies carried by Soni et al in 2020 in which ovulation rate was statistically significantly greater in letrozole group (Let-82% ,CC-62% ,p value-0.045).[15] Hu S et al in 2018 did a meta analysis which revealed that letrozole increased the ovulation rate (RR = 1.18; 95% CI 1.03-1.36, P = 0.01).[16] Study by Legro et al in 2014, resulted in significantly higher ovulation rates in cycles of letrozole as compared to cycles with clomiphene citrate (61.7% with letrozole and 48.3% with CC, p<0.05).[17] In study of Elsemary et al in 2015 ovulation occurred in 68.2% of letrozole cycles and 74.3% of C C cycles without a statistically significant difference. [18]

In this study, the mono-follicular response was 60% in group Letrozole and 22.5% in group CC respectively. It is supported by previous studies carried by Soni et al in 2020 monofollicular development was statistically significant greater in letrozole group (CC 18%, Let 66% ,p value-0.0001)[15]. In study by Shavina et al in 2020 monofollicular development was seen in 68.4% of ovulatory cycles in the letrozole group compared with 44.8% in the CC group (*P*=0.000).[19] In study done by Wafa Y. S. et al in 2017, multi-follicular development was statistically significantly higher in group CC as compared to letrozole (CC 48.0% and Letrozole 26.0% p value- 0.023).[14] In contrast study by Kumar Roy et al in 2005-2010, the mean number of dominant follicles was comparable in both letrozole and CC group (p=0.126) 1.86±0.26 and 1.92±0.17, respectively.[20]

In this study mean endometrial thickness was 9.71±1.16mm in letrozole group and 9.6±1.03 in those receiving CC, however the difference was not statistically significant (p value-0.648). It is supported by study of Elsemary et al in 2015 which stated that endometrial thickness by transvaginal ultrasound at the time of human chorionic gonadotropin administration was not statistically significantly different in the two groups (7.8 ± 2.2 and 8.1 ± 1.2 mm in letrozole and CC groups, respectively).[18] In study by Shavina et al in 2020 , mean endometrial thicknesses were 9.86 ± 2.32 mm and 9.39 ± 2.06 mm with letrozole and CC, respectively , not statistically significant (*P*=0.751).[19] However in study by Chakravorty R et al. in 2016 the endometrial thickness at the time of hCG administration was statistically significantly greater in the letrozole group (9.82 ± 0.7 vs. 8.13 ± 0.56; p<0.001).[21]

In our study, the pregnancy rate with group Letrozole was 37.5% and with the group, CC was 25% ,the difference was not statistically significant (p value= 0.228). Pregnancy rates were similar to the use of letrozole and clomiphene citrate. There were 2 twin pregnancies in CC group and 1 twin & 1 triplet pregnancy in letrozole group. Yland et al in 2022 stated probability of pregnancy was 43% for letrozole v/s 37% for clomiphene.[22] In study of Shavina et al in 2020 pregnancy was achieved in 42.2% of women in the letrozole group and 20.0% of women in the CC group (*P*=0.04).[19] According to Wafa Y.S. et al, pregnancy rates were higher with the use of letrozole as compared to clomiphene citrate in ovulation induction in PCOS women (48.0% with letrozole and 28.0% with CC.[14]

CONCLUSION

The results of this double blinded prospective cohort study suggest that letrozole is a good alternative in term of ovulation and conception rates with significantly better mono follicular development. There was no difference between endometrial thickness between the two groups. Therefore, letrozole is a safe and better alternative to CC in ovulation induction

protocol and it may be recommended as the first-line drug for ovulation induction in cases with anovulatory infertility.

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Conflict of Interest The authors declare no conflict of interest.

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