ORIGINAL RESEARCH

Study to compare the ovulation induction rate and conception rate by letrozole and clomiphene citrate in infertile women at a tertiary care centre

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ABSTRACT
Background: To compare the ovulation induction rate of letrozole and clomiphene citrate in infertile women and the conception rate in both groups. **Materials & Methods:** One hundred fifty women in reproductive age (20-35yrs) were recruited. The male partner was also evaluated and semen analysis (based on WHO 2010 criteria) was done. Infertile women were randomized using a computer generated table in 2 groups-**Group 1:**-Women who received letrozole 2.5 mg to 7.5 mg for max 6 cycles and **Group 2:**-Women who received clomiphene citrate 50mg-150mg for max 6 cycles. The Chi-square test was used to compare the categorical variables. All the analysis was carried out on SPSS 16.0 version (Chicago, Inc., USA). **Results:** The correlation of ET on the day of trigger in clomiphene and letrozole group was found to be statistically significant ($p<0.001$), on application of t-test. The correlation of number of follicles on Day 14 in clomiphene and letrozole group was found to be statistically significant ($p <0.001$), on application of t-test, which shows that clomiphene does multi follicular development while letrozole does mono follicular development mainly. **Conclusion:** Higher pregnancy rate was found in letrozole treated cycle as compared to clomiphene it is statisticallysignificant ($p<0.05$)
Key words: Conception rate, letrozole, clomiphene

INTRODUCTION

Infertility is a major social stigma which can cause significant mental trauma to a female. Infertility is defined as one year of unprotected intercourse without pregnancy. This condition may be further classified as primary infertility, in which no previous pregnancy has occurred and secondary infertility, in which a prior pregnancy, although not necessarily a live birth has occurred. About 17% of couples in industrialized countries seek help for infertility, which may be caused by ovulatory failure, tubal damage or endometriosis, or a low sperm count. In developed countries, 80% to 90% of couples attempting to conceive are successful after 1 year and 95% after 2 years. Although there is no evidence of a major change in the prevalence of female infertility, many more couples are seeking help than previously [1].

The World Health Organization (WHO) estimates that 60 to 80 million couples worldwide currently suffer from infertility. Infertility varies across regions of the world and is estimated to affect 8 to 12 per cent of couples worldwide [2]. The WHO estimates the overall prevalence of primary infertility in India to be between 3.9 and 16.8 per cent. Estimates of infertility vary widely among Indian states from 3.7 per cent in Uttar Pradesh, Himachal Pradesh and Maharashtra to 5 per cent in Andhra Pradesh and 15 per cent in Kashmir [2]. Underlying these numbers exists a core group of couples, estimated to be 3 to 5 per cent, who are infertile due to unknown or unpreventable conditions. A prevalence of infertility above this level suggests preventable or treatable causes. Fecund ability is the probability of achieving pregnancy within a menstrual cycle and fecundity is the probability of achieving a live birth within a single menstrual cycle. The fecund ability of a normal couple has been estimated to be 20 to 25% [3]. On the basis of this estimate, about 90% of couples should conceive within 12 months of unprotected intercourse. In couples with unexplained infertility, IUI significantly improves fertility outcome when performed in stimulated cycles. Clomiphene citrate

(CC) was used for inducing ovulation in infertile women for last 40 yrs. It is administered orally, relatively safe and inexpensive. In contrast, alternative treatments usually involved gonadotrophins that were significantly more complicated and uncomfortable to administer, expensive and associated with more frequent and serious complications. CC was also found to have adverse effects, especially in the form of common anti-estrogenic, endometrial and cervical mucus changes that could prevent pregnancy in the face of successfully induced ovulation [4].

In addition, there is significant risk of multiple pregnancies with CC [4]. Due to disappointing results of clomiphene citrate treatment, aromatase inhibitors (AIs) has been proposed as new ovulating agents. AIs are orally administered, easy to use and relatively inexpensive with minor side effects. The most widely used aromatase inhibitor is letrozole. Compared to CC, letrozole is associated with thick endometrium, higher pregnancy rates and lower multiple gestation rates. In clinical use, third generation, non-steroidal aromatase inhibitor are generally well tolerated. The side effects are hot flushes, headaches and leg cramps [5-7]. Letrozole, an aromatase inhibitor, has been demonstrated to be effective as an ovulation induction and controlled ovarian hyper stimulation agent. In some studies which compared the effect of CC and letrozole in infertile women undergoing superovulation, they found that there was no difference in pregnancy rate or endometrial thickness. Though the miscarriage rate was higher in CC [8]. Endocrinological environment of cycles, stimulated with CC and letrozole, are compared and found that letrozole associated with significantly lower estradiol concentration compared with CC [9]. About the safety of drugs, there was no difference in overall rates of major and minor congenital malformations who conceived after letrozole or CC treatment [10, 11]. A combined analysis of literature on unexplained infertility yielded estimated pregnancy rate of 4% per cycles for control cycles and IUI cycles, 8% per cycle for superovulation cycles and 18% per cycle of superovulation and IUI. Hence, this study was conducted to compare the ovulation induction rate of letrozole and clomifene citrate in infertile women and the conception rate in both groups.

AIMS & OBJECTIVES

1. To compare the ovulation induction rate of letrozole and clomiphene citrate in infertile women.
2. To study the conception rate after administration of letrozole and clomiphene citrate in both groups.

MATERIALS & METHODS

A prospective randomized controlled trial conducted in the Department of Obstetrics and Gynaecology. The patients were recruited from the gynecology OPD of Batra hospital and Medical research centre,New Delhi.One hundred fifty women in reproductive age (20-35yrs) were recruited. The study was conducted for a period of 18 months.The male partner was also evaluated and semen analysis (based on WHO 2010 criteria) was done. Infertile women were randomized using a computer generated table in 2 groups-Group 1:-Women who received letrozole 2.5 mg to 7.5 mg for max 6 cycles and Group 2:-women who received clomiphene citrate 50mg-150mg for max 6 cycles. To determine the diameter of the follicle, the mean of measurements in two perpendicular directions was taken. The numbers of follicles in both ovaries were added for the total antral follicle count (AFC).The follicles visualized and counted by TVS in the early follicular phase were 2-10 mm in size. Patients were subdivided into 3 groups on the total antral follicle counts: Group 1 - <5, Group 2 - 5-15 and Group 3 - >15. The results are presented as frequencies, percentages and mean± SD. The Chi-square test was used to compare the categorical variables. The unpaired t- test was used to compare the continuous variables between the groups. The p-value <0.05 was considered significant. All the analysis was carried out on SPSS 16.0 version (Chicago, Inc., USA).

RESULTS

On analyzing the data, the correlation of ET on day 2 in clomiphene and letrozole group was found to be statistically insignificant ($p>0.05$) on application of t-test. The correlation of ET on the day of trigger in clomiphene and letrozole group was found to be statistically significant ($p<0.001$), on application of t-test. The correlation of number of follicles on Day 14 in clomifene and letrozole group was found to be statistically significant ($p <0.001$), on application of t-test, which shows that clomiphene does multi follicular development while letrozole does mono follicular development mainly. The correlation of maximum size of follicle on day 14 in both groups was found to be statistically significant ($p<0.001$), on application of t-test. Mean of maximum size follicle in clomiphene group was found to be 18.06±1.52 while in letrozole group it was found to be 19.86±2.05. The correlation of total sperm count in clomiphene and letrozole group was found to be statistically insignificant ($p>0.05$). On application of t-test for equality.

Table 1: Distribution of women in relation to various parameters

	Groups		P Value
	Drug C	Drug L	
	Mean ± SD	Mean ± SD	
ET on Day 2 in Mm	2.97 ± 0.92	3.19 ± 0.79	0.151
No of Follicles on Day 2	13.75 ± 4.71	13.66 ± 4.69	0.911
Max Size of Follicle on Day 2	5.83 ± 1.21	5.76 ± 1.31	0.744
ET on Day 14 in Mm	8.01 ± 1.00	8.88 ± 0.98	<0.001
No of Follicles on Day 14 in Both Ovaries	4.35 ± 1.68	2.17 ± 0.88	<0.001
Max Size of Follicle on Day 14	18.06 ± 1.52	19.86 ± 2.05	<0.001
Total sperm count in million	67.78 ± 22.68	71.11 ± 30.24	0.480

Table 2: Distribution of women in relation to estradiol levels on day 2 and on the day of trigger in both groups

	Groups				P Value
	Drug C		Drug L		
	Mean ± SD	Median (IQR)	Mean ± SD	Median	
Estradiol level on day 2 in pg/ml	37.37 ± 9.51	36 (31.95-45.45)	35.79 ± 10.11	35 (28.70-45.0)	0.389
Estradiol level on the day of trigger in pg/ml	669.92 ± 250.10	704 (469.5-790)	787.58 ± 241.57	760 (631-966)	0.042

The correlation of estradiol level on day 2 in both groups was found to be statistically insignificant (p>0.05). The correlation of estradiol level on the day of trigger was found to be statistically significant (p=0.042), on application of t-test.

Table 3: Distribution of ovulation rate in clomiphene and letrozole group

Follicle after trigger	Groups				P Value
	Drug C		Drug L		
	Frequency	%	Frequency	%	
Rupture	43	66.2%	54	83.1%	0.027
Not Rupture	22	33.8%	11	16.9%	
Total	65	100%	65	100%	

The ovulation rate in clomiphene group was 66.2% (43/65) and in letrozole group was found to be 83.1% (54/65). It was found to be statistically significant (p=0.027).

Table 4: Distribution of pregnancy rate in letrozole and clomiphene group

Pregnancy Test	Groups				P Value
	Drug C		Drug L		
	Frequency	%	Frequency	%	
Negative	58	89.2%	47	72.3%	0.014
Positive	7	10.8%	18	27.7%	
Total	65	100%	65	100%	

Higher pregnancy rate was found in letrozole treated cycle as compared to clomiphene which is statistically significant (p<0.05)

Table 5: Distribution of outcome of pregnancy in clomiphene and letrozole group

Outcome of pregnancy	Groups				P Value
	Drug C		Drug L		
	Frequency	%	Frequency	%	
Ongoing	3	42.9%	13	72.2%	0.054
Delivered	2	28.6%	5	27.8%	
Abortion	2	28.6%	0	0.0%	
Total	7	100%	18	100%	

Miscarriage rate was found higher in clomiphene group 28.6% (2/7) as compared to letrozole group 0% in our study which was statistically insignificant (p>0.05).

Table 6: Distribution of multiple pregnancies in clomiphene and letrozole group

Multiple Pregnancy	Groups				P Value
	Drug C		Drug L		
	Frequency	%	Frequency	%	
No	5	71.4%	17	94.4%	0.180
Yes	2	28.6%	1	5.6%	
Total	7	100%	18	100%	

In this study one multiple pregnancy was found in letrozole group only 5.6% (1/18).It was found to be statistically insignificant, on application of fisher'sexact test ($p>0.05$) and Pearson's chi-square test ($p>0.05$).

Table 7: Distribution of ovarian stimulation in clomiphene in letrozole group

Ovarian Hyper stimulation	Groups				P Value
	Drug C		Drug L		
	Frequency	%	Frequency	%	
Absent	63	96.9%	64	98.5%	1.000
Present	2	3.1%	1	1.5%	
Total	65	100%	65	100%	

Only one case of ovarian hyper stimulation was found in letrozole group 1.5% (1/65),whereas in clomiphene group 3.1% (2/65) cases had ovarian hyper stimulation, which was statistically insignificant ($p>0.05$).

DISCUSSION

Infertility has been a source of misery from times immemorial. The infertile couples not only undergo emotional trauma but also are an object of social contempt. Now with the advent of newer modalities of treatment of infertility, a friendly hand can be extended with confidence even though a lot remains to be solved. About 17% of couples in industrialized countries seek help for infertility, which may be caused by ovulatory failure, tubal damage or endometriosis or a low sperm count. In developed countries, 80% to 90% of couples attempting to conceive are successful after 1 year and 95% after 2 years. [1] Unexplained infertility is defined when all standard investigations (semen analysis, assessment of ovulation, demonstration of tubal patency), yields normal results. Around 10-15% of infertile couple will ultimately reach this clinical diagnosis [12]. Hence, this study was conducted to compare the ovulation induction rate of letrozole and clomiphene citrate in infertile women and the conception rate in both groups.

In the present study, on analyzing the data, the correlation of ET on day 2 in clomiphene and letrozole group was found to be statistically insignificant ($p>0.05$) on application of t-test. The correlation of ET on the day of trigger in clomifene and letrozole group was found to be statistically significant ($p<0.001$), on application of t-test. The correlation of number of follicles on Day 14 in clomiphene and letrozole group was found to be statistically significant ($p <0.001$), on application of t-test, which shows that clomiphene does multi follicular development while letrozole does mono follicular development mainly. The correlation of maximum size of follicle on day 14 in both groups was found to be statistically significant ($p<0.001$), on application of t-test. Mean of maximum size follicle in clomifene group was found to be 18.06±1.52 while in letrozole group it was found to be 19.86±2.05. The correlation of total sperm count in clomiphene and letrozole group was found to be statistically insignificant ($p>0.05$). On application of t-test for equality. The correlation of estradiol level on day 2 in both groups was found to be statistically insignificant ($p>0.05$). The correlation of estradiol level on the day of trigger was found to be statistically significant ($p=0.042$), on application of t-test. Mean FSH level were comparable to other previous studies conducted by Rooiji *et al*., [13] who calculated mean value of 6.6 mIU/ml. Cem Ficiciog [14]*et al*., found the mean value of 7.49+-2.56 mIU/ ml. Hung Yu Ng [15] also found mean FSH levels among fertile Chinese population to be 6.1 mIU/ml. Day 2 LH was measured by chemi-illuminiscense technique, mean value obtained was 6.80±3.18 mIU/ml in letrozole group and 6.92±3.58 mIU/ml in clomiphene group. Day 2 estradiol essay in cases revealed a mean value of 35.79±10.11 pg/ml in letrozole group and 37.37±9.51 pg/ml in clomiphene group. Cem Ficiciog *et al*., found the mean values of Day 2 E2 was found to be 33.97±13.98. Estradiol levels less than 50pg/ml considered as normal. No significant difference was found in letrozole and clomiphene group. No significant correlation was found between day 2 AFC and day 2 E2 level. The number of dominant follicles (size>16mm),in letrozole group (less than 2 dominant follicles) was 88.20% cycles and in clomiphene group (between 2 to 5 dominant follicle) was found in 79.60% cycles. It was found to be statistically significant ($p<0.05$). Fatemi *et al*., (2003) [16], found that significantly more follicles developed in patients in clomiphene group (n=8) as compared with letrozole group. The endometrial thickness on the day of trigger, mean value obtained was 8.88±0.98 mm in letrozole group and 8.01±1.00 mm in clomiphene group, which is

statistically significant. Fariba Seyedoshohadaei, Laleh Tangestani and Naser Rashadmanesh (2016) [17] also found significant difference in endometrial thickness between the two groups (letrozole group 8.17mm and clomiphene group 7.26mm (p=0.021).

In the present study, miscarriage rate was found higher in clomiphene group 28.6% (2/7) as compared to letrozole group 0% in our study which was statistically insignificant (p>0.05). One multiple pregnancy was found in letrozole group only 5.6% (1/18). It was found to be statistically insignificant, on application of fisher's exact test (p>0.05) and Pearson's chi-square test (p>0.05). Only one case of ovarian hyper stimulation was found in letrozole group 1.5% (1/65),whereas in clomiphene group 3.1% (2/65) cases had ovarian hyper stimulation, which was statistically insignificant (p>0.05). Clomiphene is a non-steroidal triphenylethylene derivative with both estrogen agonist and antagonist properties. However, in almost all circumstances clomiphene acts purely as an antagonist or anti estrogen: its weak estrogenic action are clinical apparent only when endogenous levels are very low. Clomiphene is cleared through the liver and excreted in the stool, approximately 85% is eliminated within a week, but traces can remain in the circulation for longer. Clomiphene is a racemic mixture of two different stereoisomers, enclomiphene (originally known as cis-clomiphene) and zeclomiphene (originally known as trans-clomiphene). Enclomiphene is the more potent isomer and the one responsible for ovulation-induction actions. The half-life of enclomiphene is relatively short, so serum concentrations rise and fall quickly during and after treatment. Zuclomiphene is cleared much more slowly; serum levels remain detectable for weeks after a single dose and may even gradually accumulate over a series of cycles, but there is no evidence that residual Zuclomiphene has any important clinical effects or consequences [18, 19]. Currently available clomifene compounds are skewed toward en-clomiphene predominance. Classically CC treatment has been reported to induce ovulation in 60-80% of properly selected candidate. More than 70% of those who ovulate respond at the 50 or 100 mg dosage level. Cumulative conception rates upto 70% were observed after upto three successfully induced ovulatory cycles [20, 21, 22]. In another study, cumulative conception rate of 73% was achieved within nine CC induced ovulatory cycles. Overall, cycle fecundity is approximately 15% in women who ovulate in response to treatment with higher chance of pregnancy in the first cycle. It is important to realize that these figures apply to young women in whom ovulation is the sole reason preventing them from conceiving. In the reality of daily clinical practice, such a group of patients does not frequently exist, particularly in the subspecialty referral infertility practice, in which much lower pregnancy rates are observed with CC induction of ovulation. Age, presence of other infertility factors, treatment history, and duration of infertility in addition to androgen levels are important factors affecting treatment outcomes. Amenorrhea women are more likely to conceive than oligomenorrheic women, probably because those who already ovulate, albeit inconsistenly, are more likely to have other coexisting infertility factors. Generally speaking, failure to conceive within 6 CC induced ovulatory cycles should be regarded as a clear indication to expand the diagnostic evaluation to exclude other factors or change the overall treatment strategy when evaluation is already complete [22].

CONCLUSION

Multifollicular growth occurred in clomiphene cycle as compared to letrozole which is statistically significant (p<0.05). Significant difference was found in ovulation rate in both groups. Higher pregnancy rate was found in letrozole treated cycle as compared to clomiphene which is statistically significant. (p<0.05).

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