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Original Research Article

Effect of Letrozole and Clomiphene Citrate versus Letrozole Alone for Ovulation Induction in Women with Polycystic Ovary Syndrome

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Abstract

Background: Polycystic ovary syndrome (PCOS) is a common endocrinopathy characterized by oligo-ovulation or anovulation signs of androgen excess and multiple small ovarian cysts. It is thought to be one of the leading causes of female sub-fertility. It has been estimated that PCOS affects 5-10% of females in reproductive age. In this study, we used combination of letrozole and clomiphene vs letrozole alone in ovulation induction in infertile PCOS women. ***Objective:*** The objective of the study was to compare the effectiveness of the combination of letrozole and clomiphene citrate and letrozole alone in ovulation induction in infertile women with PCOS. ***Methods:*** Randomized controlled trial conducted in the Department of Reproductive Endocrinology and Infertility, Bangabandhu Sheikh Mujib Medical University, Dhaka, from July 2020 to June 2021. A total of 50 women 18-40 years of age with a diagnosis of infertility and PCOS as defined by the Rotterdam criteria and no other known cause of infertility were included in this study. Participants were randomly assigned into two groups by using a computer-generated random table to either 5 mg letrozole alone or the combination of 2.5 mg letrozole and 50 mg CC daily on cycle days 3-7 for two treatment cycle. Statistical analysis was performed by the SPSS program for Windows, version 22.0. Main outcome measured by number & size of mature follicles, endometrial thickness, day 21 serum progesterone & ovulation rate in both groups. The secondary outcome includes pregnancy rate and complication of both treatment arms. ***Results:*** Regarding demographic criteria, there was no significant difference between two groups. In the current study, clinical characteristics, laboratory parameters and cycle characteristics were also comparable to both groups and showed no significant difference. Dominant follicles were found 24(52.2%) in group A (CC+Letrozole) and 29(63.0%) in group B (Letrozole Alone) without any significant difference. Mono follicular development was found 18(75.0%) in group A and 24(82.8%) in group B which was not statistically significant. Endometrial thickness and serum progesterone level at 1st and 2nd cycle were not statistically significant between two groups ($p>0.05$). Ovulation rate was higher in group B than group A (76.0% vs 72.0%) with absolute difference 4.0 and relative ratio 0.95. Pregnancy rate was also higher in group B than group A (24.0% vs 16.0%) with absolute difference 8.0 and relative ratio 0.67. Ovulation and pregnancy rate were not statistically significant between two groups. Regarding side effects, no significant difference was found between two groups. ***Conclusion:*** Although it was not statistically significant, ovulation and pregnancy rate was a little higher in alone letrozole group than combined group. The results of this preliminary study suggested that letrozole may have a better role as a first-line treatment for anovulatory patients with PCOS.

Keywords*:* Polycystic Ovary Syndrome (PCOS), Endocrinopathy, Oligo-Ovulation, Letrozole, Clomiphene, Effect.

INTRODUCTION

Polycystic ovary syndrome (PCOS) is undisputedly the most common gynecological endocrinopathy [1]. Although PCOS is under-diagnosed [2], its prevalence ranges between 2.2% and 26% in different countries [3], and 6.8-18% in women of reproductive age [4], using different diagnostic criteria and recruitment method of the study population. The first signs of PCOS are diagnosable in the prepubertal period, and given its heterogeneous nature, the beginning of symptoms in the patient can be accompanied by psychologic disorders such as depression and anxiety, along with irregular menstrual periods in adolescence

and then infertility [5]. The primary clinical indicator of PCOS is irregular or lack of menstrual cycles and infertility. There is a clear link between PCOS and infertility, as PCOS is responsible for 55% to 70% of infertility cases resulting from chronic anovulation, thus, it is among the most common causes of infertility due to ovulation dysfunction [4]. In PCOS patients, excessive androgen secretion results in increased estrogen precursors in granulosa cells. In these patients, luteinizing hormone receptors, in the presence of hyperinsulinemia, appear earlier in granulosa cells, causing activation of aromatase in these cells. This phenomenon results in increased estrogen production, with positive feedback on luteinizing hormone and negative feedback on FSH, and ultimately disruption of folliculogenesis [6]. Hyperandrogenism and insulin resistance cause chronic anovulation and therefore infertility. Even if pregnancy does occur, it is associated with repeated spontaneous miscarriage in the first trimester and with gestational diabetes [7]. In general, clinical signs of PCOS consist of clinical or laboratory evidence of hyperandrogenism, oligoovulation, presence of PCOS, after ruling out other causes such as adrenal hyperplasia and hyperprolactinemia which consist of; 1) clinical or laboratory evidence of hyperandrogenism, 2) oligoovulation, 3) presence of polycystic ovaries in the sonography. Anovulation or oligoovulation are important characteristics of PCOS. Oligoovulation manifests as irregular menstrual bleeding and is seen in 70% of patients [8]. In PCOS patients with a complaint of infertility, the treatment of choice is the induction of ovulation. Different treatment regimens have been used in PCOS patients, but none has had a significant outcome. The reason behind this diversity in treatment options is the multifactorial pathology of PCOS and its different manifestations. Therefore, because of its diverse clinical and endocrine characteristics and unknown pathophysiology, as well as the role of genetics in its pathogenesis, it is difficult to use only one treatment option in PCOS [5]. Multiple treatments have been recommended for infertility in patients with PCOS, including weight reduction, clomiphene citrate, metformin, gonadotropins, pulsed gonadotropin-releasing hormone, gonadotropin-releasing hormone agonists, ovary cauterization, ovarian wedge resection, letrozole, and assisted reproductive technology, such as in vitro fertilization [9, 10]. Clomiphene is still considered first-line therapy for ovarian stimulation in PCOS [11, 12]. Due to its structural similarities to estrogen, clomiphene competitively attaches to nuclear estrogen receptors. By lowering the negative feedback of estrogen, it activates mechanisms that change the secretion pattern of gonadotropin-releasing hormone, which in turn result in increased pituitary gonadotropin hormones. This process ultimately causes ovarian follicles to grow [11]. With clomiphene, ovulation occurs in 80% of cases and pregnancy by 40%. It can be used for 6-12 months, but longer periods of administration could potentially increase the risk of malignant and borderline ovarian tumors [11]. Clomiphene resistance is defined as three cycles of failure to ovulate or six cycles of ovulation without pregnancy [13]. Aromatase is a microsomal enzyme that mediates the conversion of androstenedione to estrogen, and testosterone to estradiol. It is present in several tissues, including the ovary, brain, placenta, adipose tissue, muscle, liver, and breast. Aromatase is a good target to control estrogen secretion because estrogen is the final step in the biosynthetic pathway. Several studies have demonstrated the effectiveness of aromatase inhibitors in the induction of ovulation [14 -17]. Monofollicular ovulation is another example of the advantages of the aromatase inhibitors, especially when PCOS patients have an exaggerated response to gonadotropins. Recently, aromatase inhibitors have become an alternative to clomiphene citrate as first-line therapy for stimulation of ovulation in ovulating and nonovulating infertile women [18]. Letrozole, as opposed to clomiphene, is rapidly excreted [19], and causes ovulation in 60%-80% of patients [20]; in clomiphene- resistant patients, it caused ovulation in 62% of cases, and pregnancy occurred in 14.7% of patients. Letrozole does not have any adverse effects on the fetus and is safe. Letrozole decreases the secretion of estrogen both in the brain and in the periphery and causes an increase in gonadotropins, which in turn causes maturation of the ovarian follicles. The Pregnancy and Polycystic ovary syndrome (PPCOS) II trial, a randomized controlled trial comparing letrozole and CC, demonstrated that letrozole was associated with a higher live birth rate (27.5% vs. 19.1%; P=.007; rate ratio 1.44, 95% confidence interval [CI] 1.10-1.87) and cumulative ovulation rate (61.7% vs. 48.3%; P<.001) among women with PCOS [21].There is one study that prospectively reviewed treatment outcomes using a combination of letrozole and clomiphene who had previously failed CC for 6 cycles and letrozole for 4 cycles. Their study enrolled 100 patients and showed an ovulatory rate (defined by the development of dominant follicle) of 82.9%of cycles (213/257) with the combination treatment, However, their study was not randomized and used a limited population of women with PCOS that was resistant to both clomiphene and letrozole alone [4]. There is another randomized controlled trial of using both letrozole and CC versus letrozole alone. Seventy patients were randomized: 35 to letrozole alone and 35 to letrozole and CC. Results were analyzed according to the intention-to-treat principle. Women who received the combination of letrozole and CC had a statistically higher ovulation rate compared with those who received letrozole alone (27 of 35 women [77%] vs. 15 of 35 women [43%]). There were no serious adverse events or multiple-gestation pregnancies in either group. Letrozole or CC for ovulation induction, there are few treatment options available to PCOS patients except proceeding to gonadotropin injections or in vitro fertilization, both of which are associated with increased cost and risk. Letrozole decreases the secretion of estrogen both in the brain and in the periphery and causes an increase in gonadotropins, which in turn causes maturation of the

ovarian follicles. So in a prospective randomized fashion, I designed a study to evaluate the ovulatory rate and effectiveness of this combination treatment compared with letrozole alone. If the combination results in a higher ovulatory rate, larger studies evaluating the pregnancy and delivery rates with the combined therapy would be indicated. Therefore, I aim to test the hypothesis that combined therapy of letrozole and CC is effective and superior to the use of letrozole alone to achieve ovulation in women with PCOS. Secondary objectives included pregnancy rate and characterizing the side-effects profile on this treatment regimen

OBJECTIVES

General Objective

To evaluate the effect of combined letrozole and clomiphene citrate or letrozole alone for ovulation induction in infertile PCOS patient.

Specific objectives:

- To measure the size and number of mature follicles, endometrial thickness all determined by mid cycle ultrasound in both groups and compare the values between two groups.
- To measure the Day 21 serum Progesterone in both groups and to compare the values between two groups.
- To determine the ovulation rate of both combined and only letrozole group & compare between two groups.
- To find out the clinical pregnancy rate and compare between two groups.
- Monitoring complications and side effects of both treatment arms.

MATERIALS AND METHODS

It was a randomized controlled trial study. The patients were selected by purposive sampling method. A total of 50 sample was collected, 25 in each group. The study was conducted July 2020 to June 2021 at the department of Reproductive Endocrinology & Infertility, Bangabandhu Sheikh Mujib Medical University (BSMMU), Dhaka. Bangladesh.

Inclusion Criteria:

- Age group between 18-40 years.
- BMI 18 to <30 kg/m^2.
- Diagnosed case of infertility
- Diagnosed case of polycystic ovary syndrome based on Revised Rotterdam criteria.
- Normal sperm parameter according to the World Health Organization cutoff points.

Exclusion Criteria:

- Current use of hormonal contraception.
- Other known causes of infertility- endometriosis, tubal factor, uterine abnormalities.
- Uncorrected thyroid disease.
- Untreated hyperprolactinemia.
- Medical conditions in which avoiding pregnancy is recommended until under improved poorly controlled Type 1 or Type 2 diabetes, poorly controlled hypertension
- Contraindications to clomiphene citrate: hypersensitivity to CC or any of its components, history of liver disease or known liver disease, unknown cause of abnormal uterine bleeding.
- Contraindications to letrozole: hypersensitivity to letrozole or any of its components.
- Use of medications known to affect reproductive function or metabolism.
- If patients are suspected based on clinical findings for other etiologies that mimic PCOS, workup must be completed to exclude other etiologies before enrollment (i.e congenital adrenal hyperplasia, Cushing's syndrome, androgen-secreting tumor).

Study Procedure

Participants were enrolled after all eligibility criteria confirmed and informed consent was completed. Randomization occurred during the first 3 days of spontaneous menses or while taking medroxyprogesterone (10 mg/d) to induce withdrawal bleed. Women was randomly assigned to receive 5 mg letrozole daily or a combination of 2.5 mg letrozole and 50 mg CC daily on cycle days 3-7 for two treatment cycles. Participants received the oral ovulation medication, one mid-cycle ultrasound, Day 21 progesterone level and urine pregnancy tests or serum beta HCG were done. Participants were instructed to keep track of bleeding, intercourse, side-effects, and use of other medications. Couples were instructed to have regular intercourse, 2-3 times per week. A mid-cycle ultrasound was performed on cycle day 12-14 using same transvaginal ultrasonography machine. All images were reviewed by the principal investigator. Serum progesterone level was obtained on cycle day 21 or 22.

Data Analysis Procedures

Statistical analyses were carried out by using the Statistical Package for Social Sciences version 22.0 for Windows (SPSS Inc., Chicago, Illinois, USA). The mean values were calculated for outcome variables and percentages for ovulation. Chi square test was used for categorical variables and unpaired t-test was used for continuous variables. P values <0.05 was considered as statistically significant.

Ethical Implications

Written approval was taken from the concerned authority and the department with due procedure. Informed consent was taken from the patient before collecting data. Privacy, anonymity, and confidentiality were maintained during the procedure. Ethical clearance was taken from the Ethical Committee of BSMMU.

RESULTS

Table 1: Demographic characteristics of study population. (N=50)

Demographic characteristics	Group A (n=25)		Group B (n=25)		P value
	n	%	n	%	
Age (years)					
20-30 yrs.	18	72.0	20	80.0	0.905
31-40 yrs.	7	28.0	5	20.0	
Mean ±SD	26.4±5.1		26.6±4.3		
Range (min-max)	20.0-36.0		20.0-35.0		
BMI (kg/m^2)					
<25.0	14	56.0	15	60.0	0.970
25.0-29.9	11	44.0	10	40.0	
Mean ±SD	23.9 ±3.3		23.8±2.5		
Range (min-max)	19.0-29.4		20.5-29.5		
Duration of infertility					
≤3	17	68.0	19	76.0	0.697
4-6	8	32.0	6	24.0	
Mean ±SD	2.8±1.5		2.6±1.4		
Range (min-max)	1.5-6.0		1.5-6.0		
Fertility history					
Primary	19	76.0	17	68.0	0.529
Secondary	6	24.0	8	32.0	

Table 1 showed that majority patients belonged to age group 20-30 years in both groups. The mean age was found 26.4±5.1 years in group A and 26.6±4.3 years in group B. Mean BMI was 23.9±3.3 kg/m^2 in group A and 23.8±2.5 kg/m^2 in group B. Mean duration of infertility was 2.8±1.5 years in group A and 2.6±1.4 years in group B. Primary infertility was 19(76.0%) and 17(68.0%) in group A and group B respectively. Age, BMI, duration of infertility, fertility history was not statistically significant between two groups (p>0.05).

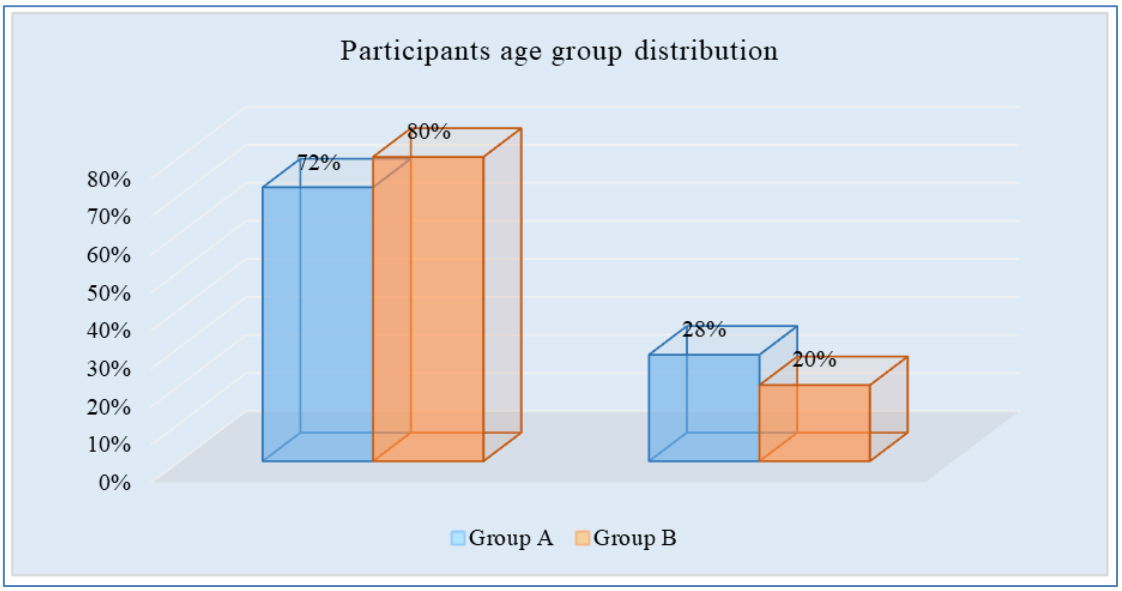

Figure I: Pie chart showed group wise participants age distribution. (N=50)

Table 2: Distribution of the patients by Clinical characteristics of study populations in two groups. (N=50)

Clinical characteristics	Group A (n=25)		Group B (n=25)		P value
	n	%	n	%	
Menstruation variable					
Oligomenorrhea	19	76.0	20	80.0	
Irregular	1	4.0	2	8.0	0.651
Regular	5	20.0	3	12.0	
Clinical variable					
Hirsutism	15	60.0	17	68.0	0.556
Acne	9	36.0	7	28.0	0.544
Polycystic ovaries	20	80.0	19	76.0	0.732

Table 2 showed that oligomenorrhea was found 19(76.0%) in group A and 20(80.0%) in group B. Hirstusim was found 15(60.0%) and 17(68.0%) in group A and group B respectively. Acne was 9(36.0%) and 7(28.0%) in group A and group B respectively. Polycystic ovaries were 20(80.0%) in group A and 19(76.0%) in group B. The difference was not statistically significant (p>0.05) between two groups.

Table 3: Distribution of the patients by Laboratory parameters of study populations in two groups. (N=50)

Laboratory parameters	Group A (n=25)	Group B (n=25)	P value
	Mean ±SD	Mean ±SD	
Serum LH (mIU/mL)	8.2±1.5	8.3±2.7	0.841
Serum FSH (mIU/mL)	5.3±1.6	5.2±1.6	0.794
TSH (mIU/mL)	1.8±0.9	1.8±0.8	0.921
Serum prolactin (ng/dl)	15.1±6.3	15.3±5.4	0.898

Table 3 showed that mean LH, FSH, TSH and serum prolactin were not statistically significant (p>0.05) between two groups.

Table 4: Cycle characteristics of the participants (N=50)

Cycle characteristics	Group A (n=25)		Group B (n=25)		P value
	n	%	n	%	
Progesterone withdrawal					
1st cycle	15/25	60.0	17/25	68.0	0.556
2nd cycle	14/21	66.7	15/21	71.4	0.739
Withdrawal per cycle	29/46	63.0	32/46	69.6	0.508
Ultrasonund cycle (days)	13.0±0.9		12.7±0.8		0.194
Day of serum progesterone level obtian	22.1±0.5		21.9±0.7		0.187

Table 4 showed that progesterone withdrawal cycle, ultrasound cycle and day of serum progestone level were not statistically significant (p>0.05) between two groups.

Table 5: Comparison of Number of study population with different size of follicle. (N=50)

Size of follicle (mm)	Group A		Group B		P value
	n	%	n	%	
1st cycle	(n=25)		(n=25)		
<14	7	28.0	3	12.0	
14-17.9	5	20.0	7	28.0	0.354
≥18	13	52.0	15	60.0	
2nd cycle	(n=21)		(n=21)		
<14	6	28.6	3	14.3	
14-17.9	4	19.0	4	19.0	0.507
≥18	11	52.4	14	66.7	

Table 5 showed that in 1st cycle majority patients had follicle size ≥18 mm in both groups, that was 13(52.0%) in group A and 15(60.0%) in group B. In 2nd cycle most of the patients had follicle size ≥18 cm in both groups, 11(52.4%) in group A and 14(66.7%) in group B. The difference was not statistically significant (p>0.05) between two groups.

Table 6: Comparison of Formation of mature follicles of study population. (N=50)

Formation of dominant follicles	Group A		Group B		P value
	n	%	n	%	
1st cycle	(n=25)		(n=25)		
Yes	13	52.0	15	60.0	
No	12	48.0	10	40.0	0.569
2nd cycle	(n=21)		(n=21)		
Yes	11	52.4	14	66.7	
No	10	47.6	7	33.3	0.346

Table 6 showed that in 1st cycle dominant follicles were found 13(52.0%) in group A and 15(60.0%) in group B. In 2nd cycle dominant follicles were 11(52.4%) in group A and 14(66.7%) in group B. The difference was not statistically significant (p>0.05) between two groups.

Table 7: Comparison of Number of dominant follicles of study population (N=50)

Number of dominant follicles	Group A		Group B		P value
	n	%	n	%	
1st cycle	(n=13)		(n=15)		
Mono follicular	10	76.9	12	80.0	0.600
Multi follicular	3	23.1	3	20.0	
2nd cycle	(n=11)		(n=14)		
Mono follicular	8	72.7	12	85.7	0.378
Multi follicular	3	27.3	2	14.3	

Table 7 showed that in 1st cycle, mono-follicular was found 10(76.9%) in group A and 12(80.0%) in group B. In 2ndcycle, mono-follicular was found 8(72.7%) in group A and 12(85.7%) in group B. The difference was not statistically significant between two groups.

Table 8: Comparison of Endometrial thickness of study population in two groups. (N=50)

Endometrial thickness (mm)	Group A		Group B		P value
	n	%	n	%	
1st cycle	(n=25)		(n=25)		
≥7	15	60.0	17	68.0	
<7	10	40.0	8	32.0	0.577
Mean ±SD	7.8±2.1		8.1±1.6		
2nd cycle	(n=21)		(n=21)		
≥7	13	61.9	14	66.7	
<7	8	38.1	7	33.3	0.464
Mean ±SD	7.5±1.7		7.9±1.4		

Table 8 showed that in 1st cycle mean endometrial thickness was 7.8±2.1 mm in group A and 8.1±1.6 mm in group B. In 2nd cycle mean endometrial thickness was 7.5±1.7 mm and 7.9±1.4 mm in group A and group B respectively. The difference was not statistically significant (p>0.05) between two groups.

Table 9: Comparison of Serum progesterone level of study population in two groups. (N=50)

Serum progesterone (ng/ml)	Group A		Group B		P value
	n	%	n	%	
1st cycle	(n=25)		(n=25)		
>3.0	15	60.0	17	68.0	
≤3.0	10	40.0	8	32.0	0.788
Mean ±SD	9.0±7.2		9.5±6.5		
2nd cycle	(n=21)		(n=21)		
>3.0	13	61.9	14	66.7	
≤3.0	8	38.1	7	33.3	0.859
Mean ±SD	8.8±7.2		9.2±6.6		

Table 9 showed that in 1st cycle mean serum progesterone level was 9.0±7.2 ng/ml in group A and 9.5±6.5 ng/ml in group B. In 2nd cycle mean serum progesterone level was 8.8±7.2 ng/ml and 9.2±6.6 ng/ml in group A and group B respectively. The difference was not statistically significant (p>0.05) between two groups.

Table 10: Comparison of outcomes for cc+letrozole vs letrozole alone. (N=50)

	Group A		Group B		Absolute	Relative ratio	P value
	n	%	n	%	difference (95% I)	(95% CI)	
Formation of	24/46	52.2	29/46	63.0			**0.291**
dominant follicles							
Mono follicular	18/24	75.0	24/29	82.8			0.361
development							
Endometrial thickness (mm)							
1st cycle	7.8±2.1		8.1±1.6				0.577
2nd cycle	7.5±1.7		7.9±1.4				0.464
Serum progesterone (ng/ml)							
1st cycle	9.0±7.2		9.5±6.5				0.788
2nd cycle	8.8±7.2		9.2±6.6				0.859
Ovulation rate	18/25	72.0	19/25	76.0	4.0 (-20.3 to 28.3)	0.95 (0.68 to 1.32)	0.747
Pregnancy rate	4/25	16.0	6/25	24.0	8.0 (-14.1 to 30.1)	0.67 (0.21 to 2.08)	0.480

Tale 10 showed that dominant follicles was found 24(52.2%) in group A and 29(63.0%) in group B without any statistically significance. Mono follicular development was found 18(75.0%) in group A and 24(82.8%)in group B which were not statistically significant. Endometrial thickness and serum progesterone level at 1st and 2nd cycle were not statistically significant between two groups(p>0.05). Ovulation rate was (72%) in group A and 76% in group B with absolute difference 4.0 and relative ratio 0. 95. Pregnancy rate was 4(16.0%) and 6(24.0%) in group A and group B respectively with absolute difference 8.0 and relative ratio 0.67.

Table 11: Side effects of study population. (N=50)

Side effects	Group A (n=25)		Group B (n=25)		P value
	n	%	n	%	
Headache	5	20.0	4	16.0	0.268
Mood change	3	12.0	4	16.0	0.291
Insomnia	2	8.0	2	8.0	0.390
Fatigue	4	16.0	3	12.0	0.291
Hot flash	4	16.0	2	8.0	0.238
Nausea	7	28.0	5	20.0	0.210
Night sweat	3	12.0	2	8.0	0.325

Table 11 showed that, regarding side effects majority 7(28.0%) patients had nausea in group A and 5(20.0%) in group B. Headache was found 5(20.0%) and 4(20.0%) in group A and group B respectively. Fatigue was found 4(16.0%) in group A and 3(12.0%) in group B. Mood change was 3(12.0%) and 4(16.0%) in group A and group B respectively. Headache, moon change, insomnia, fatigue, hot flash, nausea and nigh sweat were not statistically significant (p>0.05) between two groups.

DISCUSSION

Polycystic ovary syndrome (PCOS) is the most common endocrine disorder responsible for subfertility among the young adult [22]. The prevalence of PCOS is increasing and as high as 15-20% [23, 24]. Safe and effective ovulation induction is important for women with WHO group II anovulation 23. Clomiphene citrate has been used for ovulation induction since 1960s [25]. Clomiphene resistance occurs in 15% to 20% of patients [26]. The use of Clomiphene may be associated with poor cervical mucous and endometrial thinning in 15% to 50% of patients due to prolonged estrogen- receptor depletion. This is responsible for discrepancy between ovulation and pregnancy rate [27]. 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 still conflicting [28]. Letrozole is a third generation aromatase inhibitor that acts by preventing negative feedback inhibition of the hypothalamopituitary axis by estrogen, thus increasing FSH level and also increasing the follicular sensitivity to FSH [28]. It is postulated that aromatase inhibitor may have superior ovulation induction properties in terms of mono-follicular growth and endometrium development, which is important for embryo implantation [29]. In this present study it was observed that majority patients belonged to age group 20- 30 years in group. The mean age was found 26.4±5.1

years in group A (clomiphene citrate +Letrozole) and 26.6±4.3 years in group B (Alone Letrozone). The difference was not statistically significant (p>0.05) between two groups. Harira (2018) [30] reported that mean age was 25.9±2.95 years in CC+letrozole group and 26.5±3.5 years in Letrozole+hMG group. The difference was not statistically significant (p=0.14). In our country a study done by Zeba *et al.,* (2018) [31] which showed mean age was 29.3±2.9 years in clomiphene citrate group and 29.1±3.2 years in letrozone group, that was not significant (p=0.612). Their studies findings were almost similar with my study. In this study it was observed that mean BMI was 23.9±3.3 kg/m2 in group A and 23.8±2.5 kg/m2 in group B. The difference was not statistically significant (p>0.05) between two groups. Similarly, a study conducted by Harira (2018) [30] showed that mean BMI was 22.8±2.6 kg/m2 in CC+letrozole group and 23.2±2.15 kg/m2 in Letrozole+hMG group. The difference was not statistically significant (p=0.23). Sahu and Rout (2020) [32] had observed that the mean body mass index in clomiphene is 26.09 with SD of 2.13 and in group letrozole is 26.20 with SD of 2.06. Both the groups are comparable with respect to body mass index with p value 0.809 (>0.05) not significant. In this present study it was observed that mean duration of infertility was 2.8±1.5 years in group A and 2.6±1.4 years in group B. The difference was not statistically significant (p>0.05) between two groups. In our country a study done by Zeba *et al.,* (2018) [31], showed that mean duration of infertility was 3.1±0.7 years in clomiphene citrate group and 3.0±0.8 years in letrozone group, that was not significant (p=0.304). Sahu and Rout (2020) [32], reported that the mean duration of infertility in group clomiphene is 2.93 years and in group letrozole is 2.89 years with p value is 0.819 (>0.05), which is not significant. Both the groups are comparable with respect to mean duration of infertility. In this current study it was observed that primary infertility was 19(76.0%) and 17(68.0%) in group A and group B respectively. Secondary infertility was 6(24.0%) and 8(32.0%) in group A and group B respectively. The difference was not statistically significant (p>0.05) between two groups. Hussain *et al.,* (2013) [33] reported that primary infertility was 59(78.7%) in CC group and 57(76.0%) in letrozole group. Secondary infertility was 16(21.3%) and 18(24.0%) in CC and letrozole group respectively. The difference was not statistically significant (p>0.05) between two groups. In this study we found that oligomenorrhea was found 19(76.0%) in group A and 20(80.0%) in group B. Hirstusim was found 15(60.0%) and 17(68.0%) in group A and group B respectively. Acne was 9(36.0%) and 7(28.0%) in group A and group B respectively. Polycystic ovaries were 20(80.0%) in group A and 19(76.0%) in group B. The difference was not statistically significant (p>0.05) between two groups. Irregular menses was 11(13.9%) and 7(8.8%) in clomiphene citrate and letrozole group respectively. Regular was 5(6.3%) in clomiphene citrate group and 1(1.2%) in letrozole group. Hirsutism was higher in clomiphene citrate group than letrozole group (50.6% vs 38.8%). Acne was 31(39.2%) in clomiphene citrate group and 30(36.2%) in letrozole group. Harira (2018) [30] consisted that oligomenorrhea was 76(71.7%) in CC+letrozole group and 85(80.2%) in Letrozole+hMG group. The difference was not statistically significant (p=0.55). In this current study it was observed that mean LH, FSH, TSH and serum prolactin were not statistically significant (p>0.05) between two groups. Similar study done in our country by Zeba *et al.,* (2018) [31] found mean LH, FSH TSH and serum prolactin were not statistically significant between clomiphene citrate group and letrozone group (p>0.05). Hajishafiha *et al.,* (2014) [4], demonstrated that mean luteinizing hormone (LH) was 10.05±4.19, the mean FSH level was 5.24±1.9, the mean thyroid- stimulating hormone level was 1.76±0.9. Mean FSH 5.1±1.4 iu/I and 5.0±1.6 iu/I in clomiphene citrate group and letrozole group respectively. In this study we found progesterone withdrawal cycle, ultrasound cycle day and day of serum progestone level were not statistically significant (p>0.05) between two groups. In this present study it was observed that formation of dominant follicle per cycle was 52.2% in combined group in comparison to 63% in letrozole group which was not statistically significant. This difference was not statistically significant. The more follicle development in combined group in that study may be due to mature follicle diameter was >15mm and in our study it was >18mm.In letrozole group.The formation of less number of follicle in that study may be due to lower dose of drug (2.5 mg letrozole vs 5 mg in our study). A study done by Hajshafiha *et al.,* (2014) [4], showed its formation in 82.9% cases in combined group. It may be the result of higher doses of both drugs. Harira (2018) [30], reported of dominant follicle formation of 85(80.2%) in CC+letrozole group, which was more in comparison to our study (52.2%) This difference may be due to higher dose of drugs (letrozole 5mg+CC 100mg), In this current study it was observed that the monofollicular growth in letrozole group was 82.8% in comparison to combined group which was 75%. The difference was not statistically significant. Sahu and Rout (2020) [32], demonstrated that in letrozole group, 81.25% and 85% of the cases respectively developed single follicle. Harira (2018) [30], in their study concluded that multifollicular development were more in combined letrozole+cc group which was consistent with my study. In this study it was observed that in 1st cycle mean endometrial thickness was 7.8±2.1 mm in group A and 8.1±1.6 mm in group B. In 2nd cycle mean endometrial thickness was 7.5±1.7 mm and 7.9±1.4 mm in group A and group B respectively. The difference was not statistically significant (p>0.05) between two groups. Harira (2018) [30] reported that the mean endometrial thickness in combined group were 9.6±1.7, 8.17+1.3. Sahu and Rout (2020) [32], Zeba *et al.,* (2018) [31] observed that the endometrial thickness was significantly higher in letrozole group. In this present study it was observed that in 1st cycle mean serum progesterone level was 9.0±7.2 ng/ml in group A and 9.5±6.5 ng/ml in

group B. In 2nd cycle mean serum progesterone level was 8.8±7.2 ng/ml and 9.2±6.6 ng/ml in group A and group B respectively. The difference was not statistically significant (p>0.05) between two groups. Harira (2018) [30] reported that serum progesterone was significantly higher in CC+letrozole group (11±1.1 ng/ml). In this study we found that ovulation rate was 72.0% in group A and 76.0% in group B, P=0.747 with rate ratio for ovulation 0.95 without any significant difference. Hajshafiha *et al.,* (2014) [4] found ovulation rate of 82.9% in combined group. But follicle size alone is not necessarily a reliable way to confirm ovulation. Furthermore, it was not randomized, and used a limited population of PCOS women resistant to both CC and letrozole alone. A study conducted by Harira (2018) [30], showed there was ovulation rate about 80.2% in combined Letrozole + CC group. This may be due to higher doses of both drugs (letrozole 5mg+CC 100mg). Hussain *et al.,* (2013) [33], Sahu and Rout (2020) [32] found the comparable ovulation rate of 78.7%, 76%, 82.4% in letrozole group. In this present study it was observed that pregnancy rate was 4(16.0%) and 6(24.0%) in combined and letrozole group respectively with absolute difference 8.0 and relative ratio 0.67. A study conducted by Harira (2018) [30], showed pregnancy rate was 25.4% in combined Letrozole + CC.This again may be due to higher dose. Sahu and Rout (2020) [32], Badawy *et al.,* (2008) [16], reported that pregnancy rate achieved after ovarian stimulation with letrozole was 24%,15.1%and 21.6% which were comparable to our study. There are a few studies that showed significantly higher pregnancy rates with Letrozole than with Clomiphene group (Kar, 2012, Hendawy 2011) [34, 35]. In a Bangladeshi study conducted by Zeba *et al.,* (2018) [30] showed pregnancy rate per cycle was significantly higher with Letrozole group (44%) vs CC group (24%). Regarding side effects majority 7(28.0%) patients had nausea in group A and 5(20.0%) in group B. Headache was found 5(20.0%) and 4(20.0%) in group A and group B respectively. Fatigue was found 4(16.0%) in group A and 3(12.0%) in group, B. Mood change was 3(12.0%) and 4(16.0%) in group A and group B respectively. Headache, mood change, insomnia, fatigue, hot flash, nausea and nigh sweat were not statistically significant (p>0.05) between two groups. The most commonly reported side effects in the letrozole group included headache (41%), fatigue (22%), and abdominal pain or cramping (19%). The most commonly reported side effects in the letrozole and CC group included: hot flashes (31%), headache (28%), and abdominal pain or cramping (19%). Amer *et al.,* (2017) [36], demonstrated that serious adverse events included two cases of haemorrhagic cysts (one in each arm) and one acute cholecystitis (CC group), requiring hospitalization. Both haemorrhagic cysts resolved spontaneously. Twelve participants on letrozole developed minor adverse events including cyst formation (n = 3), diarrhoea, nausea and vomiting (n=2), hot hands, heavy leg, headache, neck pain, urinary tract infection and skin spots. Eleven women on CC experienced minor adverse events including cyst formation (n=3), hot flushes (n=3), migraine, low mood, elevated lever enzymes and skin rash.

CONCLUSION

This present study concluded that the combination of letrozole and CC was not superior to letrozole alone for inducing ovulation in the setting for infertility treatment in women with PCOS. Although ovulation and pregnancy rate was a little higher in alone letrozole group than combined group it was not statistically significant. The results of this preliminary study suggest that letrozole may have a better role as a first-line treatment for anovulatory patients with PCOS.

LIMITATIONS OF THE STUDY

- The study population was selected from single center, so that the results of the study may not reflect the exact picture of the community.
- The present study was conducted at a very short period of time.
- Small sample size was also a limitation of the present study.
- Only two cycle per participant were performed and there was not a stair step approach for increasing the dose in either treatment group.
- The method we chosen for detection of ovulation was not a definitive sign of ovulation.
- Data collection, follow up was challenging due to COVID 19 situation.

RECOMMENDATIONS

Further community based or multicenter studies can be undertaken by including large number of patients. IT will be important in future studies to compare cumulative ovulatory rate and live birth rate between letrozole alone and the combination of letrozole and CC with the use of multiple cycles and dosing regimens.

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