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Letrozole Therapy versus Extended Clomiphene Citrate Therapy for Induction of Ovulation in Resistant Patients with Polycystic Ovarian Syndrome in Low Resource Communities

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ABSTRACT

Background: many drugs can be used in ovulation induction in patients with clomiphene-resistant polycystic ovarian syndrome (PCOs).

Objective: to evaluate the effect of letrozole therapy versus extended clomiphene citrate therapy for ovulation induction in clomiphene-resistant polycystic ovarian syndrome patients

Patients and Methods: The study included 100 infertile patients who had failed induction by clomiphene for three cycles. They were randomly allocated to receive either letrozole or extended clomiphene therapy. The effects of these drugs on follicular volume, ovulation, endometrium thickness and occurrence of pregnancy were evaluated.

Results: No significant difference was found between both groups regarding age, body mass index, basal FSH level, LH level, prolactin level, thyroid function and semen analysis of the husband. Use of letrozole or extended clomiphene led to improvement of number of growing follicles, ovulation rate and pregnancy rate after 3 cycles of ovulation induction.

Conclusion: The results of this study revealed that use of letrozole or extended treatment with clomiphene did not show better outcomes over each other in number of growing follicles, ovulation rates, occurrence of pregnancy and pregnancy outcomes. Letrozole group showed a significant increase in the endometrium thickness than extended clomiphene group but this did not affect pregnancy rate.

Keywords: Letrozole, extend clomiphene therapy, PCOs, clomiphene resistance.

INTRODUCTION

Polycystic ovarian syndrome (PCOs) is a common endocrinal problem, which estimated that PCOs affects 5 % to 10 % of females between 18 and 44 years old [(1)]. Moreover, PCOs is the commonest cause of anovulation-induced infertility (around 75% of the cases) [(2)]. The Rotterdam consensus workshop concluded that PCOS is a syndrome of ovarian dysfunction along with the cardinal features of hyperandrogenism and polycystic ovary morphology on ultrasonography [(3)]. PCO patients present with variety of manifestations [(4)] and many affected patients have decreased fertility due to chronic anovulation [(5)]. Infertility can have direct psychological and social burdens among infertile women, which decreases the life quality for these patients [(6)].

Clomiphene citrate (CC) is considered to be traditional drug for ovulation induction in PCOs because of its low cost and oral route of administration with limited side effects [(7)]. CC induces ovulation by competing with estrogen (E2) on its receptor in the hypothalamus which prevents the negative feedback effect of estrogen and stimulates the production of follicular stimulating hormone from the pituitary gland that induces ovulation [(8)]. The CC starting dose should be around 50 mg/ day for 5 days, starting on day 2 to 5 of menstrual cycle following spontaneous or progestin- induced withdrawal bleeding. The dose can be increased up to 250 mg/day with most effective dose that is between 100-150 mg/day, as ovulation occurs in more than 75% of cycles treated with these doses. After six to nine cycles of CC therapy, the cumulative pregnancy rates are 70-75 % [(9)].

Letrozole is a specific aromatase inhibitor that was used to treat patients with breast cancer [(10)]. Letrozole blocks estrogen production from androgen by inhibiting aromatization. By decreasing circulating E2, the secretion of FSH and luteinizing (LH) hormone increases [(11)]. Letrozole is considered better drug than CC because of its lesser antiestrogenic effect on endometrium [(12)].

There is no specific starting dose for letrozole, however, it is better to start letrozole in low dose (2.5 mg daily), and the dose is successively increased according to the patient' response [(13)].

About 15 to 40 % of PCOs patients are not responding to ovulation induction by clomiphene therapy. Clomiphene resistant PCOs is defined by ovulation failure after receiving 150 mg/day for 5 days for at least 3 cycles [(14)].

Patients with obesity, insulin resistance and hyperandrogenism are more susceptible for failure of induction by clomiphene; however, presence of clomiphene resistance is unexplainable or unpredictable in most of the cases [(8)].

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PATIENT AND METHODS

This prospective comparative study was carried out in the Infertility Clinic in Sohag General Hospital from March 2019 to March 2020 for patients who had failed induction by clomiphene citrate for three cycles.

Ethical approval: The study was approved by the Ethical Committee, Faculty of Medicine, Menofyia University and written informed consent was obtained from all cases. The study included 100 infertile patients.

Inclusion criteria:

1- Infertile women with patent fallopian tubes proven by hysterosalpingography (HSG), their partner has normal semen parameters according to modified criteria of the World Health Organization (2010).
2- Normal TSH, prolactin and 17-hydroxyprogesterone level.
3- Written consent was obtained from patients after explaining the method and the aim of the study.

Exclusion criteria:

Women with associated male factor, poor ovarian reserve, tubal factor, endometrial factor and mullarian anomalies by hystosalpingogram. Additionally, associated endocrinal problem as thyroid dysfunction, hyperprolactinemia, congenital adrenal hyperplasia and women with contraindications for pregnancy.

The women included in the study were assessed by comprehensive history taking, body mass index (BMI) and gynecological ultrasound by (Samsung SonoAce R3 Ultrasound with mean frequency of 5 to 7.5 MHz) to evaluate uterus, endometrium, antral follicles count and to exclude presence of uterine anomalies or presence of any ovarian cysts.

The patients were allocated into two groups contained 50 cases each: Group I, received letrozole 5 mg (Letrozole 2.5 mg ®; Techno Pharma for Investment and Development, Giza, Egypt) that started from day 3 until day 7 of menstrual cycle. Group II: received 100 mg of clomiphene citrate (Clomid®; Hoechst Marion Russel, Cairo, Egypt) daily starting from day 2 of menses for 10 days.

The mean follicular volume and thickness of the endometrium were assessed on days 7, 9, 11 and up to day 15 of the cycle till one follicle reaches 17 mm.

HCG injection (5000 IU IM) was given when at least one follicle measured at least 17 mm. Patients were advised to have intercourse 24–36 hours after HCG injection. Serum progesterone (ng/ml) was measured on days 21–23 of the cycle by radioimmunoassay using an antibody-coated tube method (Coat-A-Count; Diagnostic Product Corporation, Los Angeles, CA, USA). Serum pregnancy test was required in the absence of menstruation to diagnose pregnancy.

Outcome measures: Number of growing follicles, mean follicular volume, endometrial thickness, progesterone level, presence of pregnancy.

Statistical analysis

Results were statistically analyzed by SPSS version 20. Paired t test was used for parametric data. Chi-Squared ($\chi 2$) test was used for qualitative variables. P value ≤ 0.05 is considered significant.

RESULTS

The study included 100 infertile patients with clomiphene-resistant PCO divided into two groups comprised of 50 patients each, the first one received letrozole for 3 cycles but the second received extended clomiphene therapy for 3 cycles.

There were no significant differences between the two groups regarding the demographic data (age, body mass index and regularity of menstrual cycle) as shown in table (1).

Table (1): Comparison between letrozole group and clomiphene group as regards demographic data

		Letrozole group (No. = 50)		**Clomid group (No. = 50)**		**Independent t test**	
		Mean	**SD**	**Mean**	**SD**	**t**	**p value**
Age (years)		24.90	2.92	25.90	2.46	1.852	0.067
BMI (weight in kg/height in m^2)		26.34	3.04	26.58	2.97	-0.399	0.691
		No	%	No	%	Chi-square	
Regularity of the cycle	Amenorrhea	4	8.00%	4	8.00%		
	Oligomeonorrhea	29	58.00%	30	60.00%		
	Regular	17	34.00%	16	32.00%	0.047	0.977

BMI: body mass index

In addition, there were no significant differences between the two groups regarding type of infertility, parity and number of abortions (Table 2).

Table (2): Comparison between letrozole group and clomiphene group as regards infertility, parity and abortion

		Letrozole group (No. = 50)		Clomid group (No. = 50)		Chi square test	
		No	%	No	%	x^2	p value
Infertility	Primary	35	70.0%	38	76.0%	0.457	0.499
	Secondary	15	30.0%	12	24.0%		
Parity	Zero times	44	88.0%	44	88.0%	0.000	1.000
	One time	3	6.0%	3	6.0%		
	Two times	3	6.0%	3	6.0%		
Abortion	Zero times	38	76.0%	44	88.0%	5.639	0.131
	One time	6	12.0%	3	6.0%		
	Two times	2	4.0%	3	6.0%		
	Three times	4	8.0%	0	0.0%		

There were no significant differences between the two groups regarding prolactin level, TSH level, hysterosalpingography (HSG) and semen analysis findings (Table 3).

Table (3): Comparison between letrozole group and clomiphene group as regards prolactin, HSG, semen analysis and TSH

		Letrozole group (No. = 50)		Clomid group (No. = 50)		Chi square test/ Independent t test*	
		No	%	No	%	x^2/t*	p value
Prolactin	Normal	50	100.0%	50	100.0%	NA	NA
HSG	Normal	50	100.0%	50	100.0%	NA	NA
Semen analysis	Normal	50	100.0%	50	100.0%	NA	NA
TSH	Mean ±SD	2.20 ± 0.771		2.34 ± 0.86		-0.882	0.380

HSG: hysterosalpingography TSH: thyroid-stimulating hormone

Basal FSH and LH levels did not show significant differences between the two groups (Table 4).

Table (4): Comparison between letrozole group and clomiphene group as regards basal FSH and basal LH

	Letrozole group (No. = 50)		Clomid group (No. = 50)		Independent t test	
	Mean	SD	Mean	SD	t	p value
Basal FSH	4.34	0.68	4.36	0.64	-0.167	0.868
Basal LH	10.43	0.84	10.67	0.83	-1.435	0.155

FSH: follicular stimulating hormone LH: luteinizing hormone

Regarding the first cycle, there were no significant differences between the two groups regarding number of growing follicles, ovulation rates, occurrence of pregnancy and pregnancy outcomes. The endometrium thickness was significantly greater in letrozole group than in extended clomiphene group (Table 5).

Table (5): Comparison between letrozole group and clomiphene group as regards first cycle

		Letrozole group (No. = 50)		Clomid group (No. = 50)		Chi square test/ Independent t test	
		No	%	No	%	x^2/t*	p value
Number of follicles ≥16 mm	Zero	36	72.0%	37	74.0%	4.680	0.096
	One	9	18.0%	3	6.0%		
	Two	5	10.0%	10	20.0%		
HCG trigger	No	36	72.0%	37	74.0%	0.051	0.822
	Yes	14	28.0%	13	26.0%		
Endometrial thickness (mm)	Mean ± SD	10.90 ± 1.90		8.74 ± 1.82		5.801*	<0.001
Day 21 Progesterone (ng/ml)	Mean ± SD	7.11 ± 4.96		7.15 ± 4.52		-0.042	0.967
Ovulation rate	No	36	72.0%	37	74.0%	0.051	0.822
	Yes	14	28.0%	13	26.0%		
Missed period	No	44	88.0%	44	88.0%	0.000	1.000
	Yes	6	12.0%	6	12.0%		
Pregnancy test after missed period	Negative	3	6.0%	3	6.0%	6.000	0.112
	Positive	3	6.0%	3	6.0%		
Pregnancy outcomes	Missed abortion	0	0.0%	1	2.0%	6.000	0.112
	Positive pulse	3	6.0%	2	4.0%		

***significant** HCG: human chorionic gonadotropin

Regarding the second cycle, there were no significant differences among the two groups regarding number of growing follicles, ovulation rates, occurrence of pregnancy and pregnancy outcomes. The endometrium thickness was significantly greater in letrozole group than in extended clomiphene group **(Table 6)**.

Table (6): Comparison between letrozole group and clomiphene group as regards second cycle

		Letrozole group (No.=47)		Clomid group (No.=47)		Chi square test/ Independent t test	
		No	%	No	%	x^2/t*	p value
Number of follicles ≥ 16mm	Zero	36	76.6%	33	70.2%	0.507	0.776
	One	6	12.8%	8	17.0%		
	Two	5	10.6%	6	12.8%		
HCG trigger	No	36	76.6%	33	70.2%	0.490	0.783
	Yes	11	23.4%	14	29.8%		
Endometrial thickness (mm)	Mean ± SD	10.47 ± 1.83		8.89±1.41		4.682*	<0.001
Day 21 progesterone (ng/ml)	Mean ± SD	6.12 ±4.34		6.48±4.73		-0.382	0.704
Ovulation rate	No	36	76.6%	33	70.2%	0.490	0.783
	Yes	11	23.4%	14	29.8%		
Missed period	No	44	93.6%	38	80.9%	3.439	0.179
	Yes	3	6.4%	9	19.1%		
Pregnancy test after missed period	Negative	0	0.0%	6	12.0%	6.409	0.041
	Positive	3	6.4%	3	6.4%		
Pregnancy outcomes	Missed abortion	1	2.1%	0	0.0%	1.200	0.549
	Positive pulse	2	4.3%	3	6.4%		

***significant** HCG: human chorionic gonadotropin

Regarding the third cycle, mature follicles were significantly increased in clomiphene group than in letrozole group, while ovulation rates, occurrence of pregnancy and pregnancy outcomes showed no significant differences. The endometrium thickness was significantly greater in letrozole group than in extended clomiphene group (Table 7).

Table (7): Comparison between letrozole group and clomiphene group as regards third cycle

		Letrozole group (No.=44)		Clomid group (No.=44)		Chi square test/ Independent t test	
		No	%	No	%	x^2/t*	p value
Number of follicles ≥16mm	Zero	32	72.7%	33	75.0%	13.415*	0.001
	One	12	27.3%	3	6.8%		
	Two	0	0.0%	8	18.2%		
HCG trigger	No	32	72.7%	33	75.0%	0.059	0.971
	Yes	12	27.3%	11	25.0%		
Endometrial thickness (mm)	Mean ± SD	9.95 ± 1.74		8.52 ± 1.57		4.367*	0.001
Day 21 Progesterone (ngm/ml)	Mean ± SD	6.62 ± 4.73		6.28 ± 4.44		0.346	0.730
Ovulation rate	No	32	72.7%	33	75.0%	0.059	0.971
	Yes	12	27.3%	11	25.0%		
Missed period	No	40	90.9%	38	86.4%	0.451	0.798
	Yes	4	9.1%	6	13.6%		
Pregnancy test after missed period	Negative	1	2.3%	2	4.5%	7.378	0.061
	Positive	3	6.8%	4	9.1%		
Pregnancy outcomes	Missed abortion	1	2.0%	2	4.0%	4.344	0.227
	Positive pulse	2	4.0%	2	4.0%		

***significant** HCG: human chorionic gonadotropin

DISCUSSION

Comparing the effect of letrozole and clomiphene on ovulation in PCO patients was addressed in many studies [15, 16, 17, 18, 19]. In some of these studies, higher ovulation rate was reported in patients treated by clomiphene [15, 16]. Other studies reported higher ovulation rate in women received letrozole in comparison to patients received clomiphene [17, 18, 19].

This study was performed to compare the effectiveness of letrozole versus extended clomiphene citrate in women with clomiphene resistant PCO and evaluate benefits of these drugs on ovulation induction and achieving pregnancy.

Our study found that ovulation rates in letrozole-treated group were 28%, 22% and 24% in first, second and third cycles respectively. They were not statistically significant form extended clomiphene group where they were 26%, 28% and 22% in first, second and third cycles respectively. These results are consistent with **Badawy *et al.*** [20] and **Bayar *et al.*** [21] who found a comparable ovulation rates between letrozole and clomiphene. Other studies showed higher ovulation rates among patients received letrozole in comparison with patients received clomiphene [17, 18, 22]. Five-day clomiphene therapy was used in these studies. Therefore, the higher ovulation rates that detected in our study may be due to the use of extended clomiphene therapy.

The number of mature ovarian follicles (greater than 16 mm) during the third followed cycle was significantly higher in women received clomiphene than in those received letrozole, but it was comparable between both groups in first and second cycles. Multi-folliculogenesis was reported to be higher after ovulation induction by clomiphene than letrozole [20]. Mono-folliculogenesis of letrozole is a consequence of smaller rise in the follicular phase FSH, which is shorter in duration compared to clomiphene [16].

In our study, statistically significant increase was reported in endometrial thickness in patients received letrozole compared to those received clomiphene. Compared with clomiphene, letrozole doesn't have negative effects on cervix mucus and has no antiestrogenic effects on the endometrium, which contributes to a better chance of implantation and pregnancy as well [23]. Many studies showed that women treated with letrozole had a better endometrium thickness in comparison with women received clomiphene [16, 24, 25] or clomiphene and metformin [22].

The effectiveness of these drugs to achieve pregnancy was studied. The chemical and clinical indicators of pregnancy and abortions were not significantly different between clomiphene-resistant patients in the letrozole and clomiphene groups. These findings are comparable to the results of **Liu *et al.*** [18] who reported no significant difference in the pregnancy rate, abortion rate, and live birth rate in women with PCOS who received letrozole or clomiphene. In a systematic review and meta-analysis on patients with unexplained infertility treated with letrozole compared to clomiphene citrate, there were no significant differences in pregnancy test (24% vs 23%), clinical pregnancy, live birth, spontaneous

miscarriage, or twin gestation [(23)]. Other studies concluded that treatment with letrozole was associated with a higher pregnancy rates in comparison with clomiphene [(26)]. In a recent Cochrane review, showed that use of letrozole in PCOS patients was associated with higher pregnancy rates than clomiphene citrate. There are little or no differences in rates of miscarriage and multiple pregnancies [(27)]. However, all these studies did not use an extended clomiphene therapy and experienced a higher significant rate of ovulation in letrozole groups than clomiphene groups, which may be the reason for decreasing pregnancy rate.

CONCLUSION

Our study showed comparable effectiveness of letrozole and extended clomiphene therapy in the treatment of clomiphene-resistant PCOS. Extended clomiphene therapy, compared to letrozole, was associated with an equal ovulation rates, pregnancy rate and pregnancy outcome.

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