Efficacy of Two Letrozole Protocols for Ovulation Induction in Polycystic Ovary Syndrome Women with Clomiphene Resistance

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ABSTRACT

Background: Inability of clomiphene citrate to induce ovulation, clomiphene resistance, occurs in 20-25% of women with polycystic ovary syndrome. Because of the advantages of letrozole a lot of researches has been done to utilize letrozole in ovulation induction of those women with controversial results.

Objectives: To evaluate the efficacy of short and extended long course of letrozole therapy for ovulation induction in polycystic ovary syndrome women with clomiphene resistance.

Methods: This prospective cross-sectional study conducted between September 2019 and March 2020. It included eighty subfertile women selected from the women attending the outpatient clinic of Kamal Samarae Fertility hospital and private practice. All women were diagnosed as having anovulation due to polycystic ovary syndrome and clomiphene citrate resistance. Women were divided into two groups; one for long course letrozole treatment who received 2.5 mg of letrozole daily for 10 days and the other was for short course letrozole treatment who received 5 mg of letrozole daily for five days. All women were monitored by transvaginal ultrasound for the mean follicular volume and endometrial thickness. The primary outcome measures were number of mature follicles, secondary outcome measures were the occurrence of pregnancy.

Results: There were no statistical significant differences between the two groups regarding age, duration of subfertility, body mass index, hormonal profile and ultrasound criteria. The number of ovulating women was greater in the long letrozole group (64% *vs.* 50%), but without statistical differences. The total number of follicles during stimulation was insignificantly greater in the long letrozole group (8.91±1.34 *vs.* 9.02±0.43). The numbers of follicles ≥18 mm were significantly greater in the long letrozole group (66 % *vs.* 54 %). Pregnancy occurred in 10 (25%) of the short group and 14 (35%) of the long letrozole group, and the difference was statistically insignificant.

Conclusion: In this study, the extended letrozole treatment proved to be more effective than the standard five days treatment, with more mature follicles and more pregnancies.

Keywords: **Clomiphene citrate, Clomiphene resistance, Letrozole, Ovulation induction, Polycystic ovary syndrome.**

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Ovulation dysfunction is one of the most common cause of reproductive failure in infertile couples. The prevalence of this syndrome in subfertile women is about 30 to 40 %[1,2].

Polycystic ovary syndrome (PCOS) is a spectrum of disorders that comprises a heterogeneous group of symptoms and signs with mild presentation in some women but severe disturbance of endocrine, reproductive and metabolic functions in others[3,4].

The definition of the syndrome had a lot controversial. Key features include disturbed menstrual cycle, hyperandrogenism and obesity. In spite of extra-ovarian contribution to the pathophysiology of PCOS, ovarian dysfunction is central[5,6].

Numerous factors influence ovarian function and fertility is adversely affected by an individual being overweight, the degree of hyperandrogenism and having elevated serum concentrations of Luteinizing hormone (LH)[2].

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The high prevalence of PCOS in women and its consequences in subfertility have led to numerous studies aiming new drugs with less or none side effects. In these women, medications such as clomiphene citrate and letrozole are used to induce ovulation and overcome subfertility[7,8].

Currently, clomiphene citrate and letrozole are highly demanded treatments with many controversies on effectiveness and side effects. However, the associated hyperinsulinemia and insulin resistance, in addition to the associated increase in androgen production led to more studies on new medications. Letrozole is a broadly recommended drug that can stimulate follicular stimulating hormone (FSH) secretion and ovarian follicular development[9,10].

Inability of clomiphene citrate (CC) to induce ovulation, (CC resistance), is unexpected and for most an unexplainable condition, however, it is mostly associated with obesity, hyperandrogenism, insulin resistance and women with elevated LH levels[11,12].

Traditional alternatives to CC include weight reduction, higher doses of CC, extended CC therapy, pretreatment with oral contraceptive pills, alternative drugs e.g. dexamethasone, and insulin sensitizing agents, aromatase inhibitors, gonadotropins can be tried in addition to laparoscopic ovarian drilling[7,8].

Achieving weight reduction to control obesity and hormonal imbalance associated with PCOS and subsequently achieving ovulation may be extremely difficult for some PCOS women, gonadotropins are accepted substitute to CC for induction of ovulation; however, they increased risk of multiples, are associated with ovarian hyperstimulation syndrome, are available only as injectable formulations, and are expensive. Dexamethasone, because of its effect on the levels of blood sugar, can worsen diabetic tendencies, and it may not be a suitable option for women with diabetes, insulin resistance or glucose intolerance, common presentation in many PCOS women. Insulin sensitizing agents poorly tolerated by women because of its side effects, and laproscopic ovarian drilling is a surgical procedure requiring anaesthesia and still there is its own drawbacks of reduced ovarian reserve and periovarian adhesions[7-10].

Aromatase inhibitors (AI) have been proposed as an alternative treatment to CC therapy as the discrepancy between ovulation and pregnancy rates with CC has been attributed to its anti-estrogenic action and estrogen receptor depletion especially at the endometrium and cervix[13,14].

Aromatase inhibitors are a newer of drugs that were introduced for ovulation induction in the last two decades. Inhibition of the aromatase enzyme reduces the aromatization of androgens to oestrogens that in turn releases the hypothalamic–pituitary axis from negative feedback of oestrogen. There are reports of accepted pregnancy rates with a low incidence of multiple pregnancies. Over the last years, data from many clinical trials have been collected and there is evidence that the Third generation AI, letrozole, might be as effective as CC, but the reported data are variable. In spite of having encouraging data regarding efficacy and safety, still many results did not reach statistical significance.

Aromatase inhibitors are like CC administered orally, but due to their short half-life elimination time of 48 hours there are fewer adverse effects on oestrogen target tissues such as endometrium and cervix compared to CC[15-17].

Letrozole treatment does not need intensive monitoring by ultrasound compared to CC and gonadotropins. In addition, letrozole was very well tolerated as no women reported any side effects. Because of the letrozole tolerability and its short half-life, many studies has been done to utilize letrozole in ovulation induction in different doses, durations and indications[18-22].

Unfortunately studies in Iraq discussing and comparing clomiphene citrate and letrozole in PCOS women were limited to ovulation induction[23,24], to our knowledge this is the first study comparing the efficacy of AIs in PCOS women with clomiphene resistance.

The aim of this study was to evaluate the efficacy of short and long course of letrozole therapy in induction of ovulation in clomiphene resistant women with PCOS.

Methods

This prospective cross-sectional study conducted between September 2019 and March 2020 included eighty subfertile women who were selected from the women attending the outpatient clinic of Kamal Samaraey fertility Hospital and the private clinic practice, Baghdad, Iraq. All women were diagnosed as having anovulation due to PCOS. The diagnosis of PCOS based on the revised Rotterdam criteria[5]. In addition, all those women with PCOS were diagnosed of having CC resistance (persistence of anovulation after three cycles of clomiphene citrate treatment with maximum dose of 150 mg) in previous cycles of ovulation induction. Women in the study were matched for age and BMI. Written informed consent was obtained from all women.

They were divided into two treatment groups: short letrozole group (40 women) and long letrozole group (40 women).

Women in the short letrozole group received 5 mg of letrozole tablets twice daily for 5 days, starting day 2 of spontaneous or progesterone inducing menstrual bleeding using 10 mg dydrogestrone tab (20 mg/day for 10 days) to induce withdrawal bleeding in the amenorrheic women. Women in the long letrozole group received 2.5 mg of letrozole tablets daily starting day 2 of spontaneous or progesterone inducing menstrual bleeding for 10 days. All women were monitored by transvaginal ultrasound for the mean follicular volume and thickness of the endometrium on days 10, 12, and 14 of menstrual cycle.

Human chorionic gonadotrophin (hCG) injection (5000 IU IM) was given when at least one follicle measured ≥18 mm. Ovulation confirmed with transvaginal ultrasound for free fluid at pouch of Douglas and shrinkage of follicle 24-36 h after hCG injection and couples were advised for timed intercourse. Serum hCG was determined 2 weeks after hCG injection in the absence of menstruation for diagnosis of pregnancy, followed by transvaginal ultrasound for demonstration of the gestational sac two weeks later.

The primary outcome measures were development of dominant follicles, secondary outcome measures were the occurrence of pregnancy.

Data were collected, summarized, analyzed and presented using statistical package for social sciences (SPSS) version 23 and Microsoft Office Excel 2010. Quantitative variables (number and percentage) were presented as mean ± standard deviation values and compared using the Independent Samples t test and Mann–Whitney U test. Qualitative variables (number and percentage) were compared with Fisher's exact or Pearson chi square tests. Statistical significance was assumed with a probability error of $p < 0.05$.

Results

Table 1 shows that there was no statistical difference between short letrozole and long letrozole groups regarding age (23.87 ± 4.36 and 24.67 ± 4.27, p=0.06), BMI (28.86 ± 1.81 and 27.59 ± 2.36, p=0.07), subfertility duration (2.56 ± 0.90 and 2.85 ± 0.96, p=0.06), testosterone (1.54 ± 0.92 and 1.49 ± 0.33, p=0.12), FSH (4.16 ± 1.56 and 5.0 ± 1.04, p=0.19), LH (8.99±1.55 and 9.39±1.34, p=0.21), TSH (2.33±0.24 and 2.01±0.33, p=0.45), prolactin (15.5± 0.67 and 17.2±0.77, p=0.61) levels and positive ultrasound criteria of PCOS 28(70%) and 30(75%), p=0.31.

Table 2 shows that the number of ovulating women was greater in the long letrozole group (64% vs. 50%), but without statistical differences. The total numbers of

follicles in short therapy group was8.91±1.34 vs. 9.02±0.43 in long therapy group, the difference was insignificant between two groups (p=0.623). The cases with follicles size ≥ 18 mm, there was significant differences between two groups, in long therapy group there was more follicles with size ≥ 18 mm, 27(54%) vs. 33(66%), p=0.04.

Endometrial thickness at the day of hCG administration was significantly different (9.90 ±1.55 vs. 11.04 ±0.75 p= 0.037) between the treatment groups.

Pregnancy occurred in 10 women in the short letrozole group (25%) and 14 women of (35%) in the long letrozole group, and the difference was statistically insignificant (p= 0.329).

Table 1: Women characteristics.

	Short letrozole group	Long letrozole group	p-value
Age	23.87 ± 4.36	24.67 ± 4.27	0.06
BMI	28.86 ± 1.81	27.59 ± 2.36	0.07
Subfertility duration	2.56 ± 0.90	2.85 ± 0.96	0.06
Testosterone (nmol/L)	1.54 ± 0.92	1.49 ± 0.33	0.12
FSH(mIU/L)	4.16 ± 1.56	5.0 ± 1.04	0.19
LH(mIU/L)	8.99 ± 1.55	9.39 ± 1.34	0.21
TSH(mIU/L)	2.33±0.24	2.01±0.33	0.45
Prolactin(ng/mL)	15.5±0.67	17.2±0.77	0.61
Positive ultrasound Criteria of PCOS	28(70%)	30(75%)	0.31

Table 2: Outcome in short and long letrozole groups.

	Short letrozole group	Long letrozole group	p-value
Ovulation rate (percentage)	20 (50%)	26 (64%)	0.266
Total number of follicles	8.91±1.34	9.02±0.43	0.623
Cases with follicles ≥18mm number (percentage)	27(54%)	33(66%)	0.04
Endometrial Thickness at day of hCG	9.90 ±1.55	11.04 ±0.75	0.037
Pregnancy Rate	10(25%)	14 (35%)	0.329

——————————Discussion

Ovulation induction was consistently regarded as an essential treatment procedure for subfertile PCOS women. Although clomiphene citrate is still the traditional option used for inducing ovulation in these women, clomiphene resistance, occurs in 20-25% of PCOS women[1-4].

Aromatase inhibitors are medications that suppress the biosynthesis of oestrogen and, therefore, reduce the negative feedback effect on the hypothalamic–pituitary system. This results in increased secretion of FSH that results in follicle selection and maturation. The third-generation agents, letrozole, has been used for ovulation induction in PCOS women resistant to clomiphene citrate or with inadequate endometrial thickness during clomiphene citrate treatment[7].

In the present study, we appraised the novel protocol of extended letrozole therapy to keep the in vivo production of FSH continuous for a longer duration. This allowed a greater cohort of small follicles, recruited in the early part of the cycle, to reach maturity (≥18 mm).

Age, duration of infertility, BMI were statistically similar in both groups. Also, all

basal hormonal profile on day 3 of the cycle including FSH, LH, prolactin, testosterone and TSH were statistically not significant in both groups.

The results of the present study revealed that both types of treatment were effective in treatment of clomiphene resistant PCOS women regarding ovulation rate where there was no statistical significant difference between short letrozole group (62.0%) and long letrozole group (64.0%). However, ultrasound variability is an important confounding factor, particularly if follicle size is the main deciding factor for ovulation induction. This becomes especially true when ultrasonography reveals a collapsed follicle that has already ovulated. Measuring such a follicle can result in a classification error where the diameter of the follicle is assigned to an incorrect category and hence, underestimates the true value.

Pregnancy rate was more in the extended letrozole group. In the present study, pregnancy rate per cycle was significantly higher with long letrozole group (35%) vs. short letrozole group (25%).

The rationale behind using this extended regimen was based on concept of the physiology of follicular growth. The FSH window appear to be crucial for selection of a single dominant follicle from the recruited cohort. As FSH levels fall, all but the dominant follicle, because of its increased sensitivity to FSH, lose the stimulus to further development and become atretic. The concept of extending the FSH window by extending the duration of letrozole therapy throughout the midfollicular phase would maintain FSH levels above the threshold similar to administering exogenous gonadotrophines, allowing multifollicular development to occur[25, 26].

In this study, the results are in agreement with those of El-Aziz et al, Hassanein et al, and Badawy et al regarding the number of ovulating PCOS women, total number of follicles during stimulation, number of cases with dominant and mature follicles and pregnancy rates[27-29].

Regarding this study, the extended therapy caused no cases of twin pregnancies or in ovarian hyperstimulation syndrome. Therefore, long letrozole treatment does not need intensive monitoring compared to CC and gonadotropins.

There were no increased costs for the extended therapy, because the same total dose was utilized over ten days rather than five days.

The limitations of our study include the small group size and the observed response to treatment. It is possible that the women in whom ovulation was not detected by ultrasound method at days of examination, may have ovulated at a later date influencing the primary outcome of the study. No difference was found in the endometrial thickness between the two treatments groups, this reflecting the continued estrogenic activity present in PCOS women.

In conclusion; the extended letrozole treatment proved to be more effective than the standard five days treatment, with more women having mature follicles developed in their first cycle of ovulation induction with letrozole and more pregnancies.

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