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Original Research Article

Low dose-extended letrozole versus double dose-short letrozole protocol for ovulation induction in polycystic ovary syndrome

Shirin Jahan*, Farzana Deeba, Shakeela Ishrat, Jesmine Banu, Chalontika Rani, Sumaiya Akter, Sohely Nazneen, Nishat Jahan

Department of Reproductive Endocrinology and Infertility, Bangabandhu Sheikh Mujib Medical University, Dhaka, Bangladesh

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***Correspondence:**
Dr. Shirin Jahan,
E-mail: shirin.dr@gmail.com

ABSTRACT

Background: Letrozole, an aromatase inhibitor has been regarded as the first line drug for ovulation induction in anovulatory PCOS patients because of its monofollicular growth and there is no chance of hyperstimulation by letrozole. Traditional protocol of letrozole includes administration of letrozole for 5 days in first half of follicular phase which induces ovulation in 61.7% cases. Few recent studies have shown that extended letrozole protocol causes more follicles to grow and induces more ovulation than the traditional protocol. The aim was to compare the effects of low dose-extended letrozole protocol and double dose-short letrozole protocol for ovulation induction in infertile PCOS patients.
Methods: A randomized controlled trial (RCT) was conducted in the department of reproductive endocrinology and Infertility at Bangabandhu Sheikh Mujib Medical University (BSMMU) on seventy infertile polycystic ovary syndrome patients. Low dose-extended letrozole group or experimental group received tablet letrozole 2.5 mg daily for 10 days and double dose-short letrozole group or control group received tab. Letrozole 5 mg daily for 5 days starting from the 2nd day of menstrual cycle or withdrawal bleeding. The ovarian response was assessed by folliculometry on day 12 of menstrual cycle by transvaginal sonography for measurement of total number of growing follicles, biggest follicle size and endometrial thickness. Mid luteal serum progesterone was measured on day 21-23 to confirm ovulation.
Results: The mean number of growing follicle was 1.44±0.95 versus 0.99±0.65 in low dose-extended letrozole group and double dose-short letrozole group respectively generating p value of 0.001. The mean size of the dominant follicle at day 12 was greater in low dose-extended letrozole group than the other displaying 17.69±3.63 mm and 16.6±3.49 mm respectively but the difference was not statistically significant. The number of ovulating patients was greater in low dose-extended letrozole group (76.5% versus 71.9%), but without significant statistical difference. Pregnancy rate was insignificantly greater in low dose-extended letrozole group (23.5% versus 18.8%) as well.
Conclusions: Low dose-extended letrozole protocol produces more multifollicular growth and larger size dominant follicle with a trend to raise the ovulation rate and pregnancy rate, though insignificantly.

Keywords: Letrozole, Ovulation induction, Polycystic ovary syndrome

INTRODUCTION

Polycystic ovary syndrome (PCOS) is the most common endocrinopathy affecting reproductive aged women. Diagnosis of polycystic ovary syndrome is based primarily on the clinical history and physical examination. The main clinical features of polycystic ovary syndrome are menstrual dysfunction and hyperandrogenism.[1] Five (5%)

to ten (10%) percent of the female population suffers from this disease.[2] It is the largest single cause of anovulatory infertility (80%).[3]

The underlying cause of this disorder is still unknown.[4] Genetic and environmental contributors to hormonal disturbances combine with other factors, including obesity, ovarian dysfunction, and hypothalamic pituitary abnormalities contribute to the etiology of PCOS.[5] It is a complex disease with reproductive and metabolic disorders.[6] Insulin resistance might ensue contributing to PCOS and hyperandrogenemia.[7] Theca of ovary secrete high levels of androgens due to an intrinsic activation of steroidogenesis even in the absence of trophic factors.[8] Oxidative stress can itself induce insulin resistance and hyperandrogenism in patients with PCOS.[9] Many patients seek medical treatment for acne, hirsutism, irregular or absent menses and infertility.[10]

Criteria used for diagnosing polycystic ovary syndrome (PCOS) are the Rotterdam criteria (2003) of which a woman must have two out of three criteria: oligo- or anovulation, clinical and/or biochemical signs of hyperandrogenism and polycystic ovaries (with the exclusion of related disorders).[11] The treatment of anovulatory infertility in PCOS includes lifestyle changes (diet and exercise), pharmacological therapies (oral agents such as clomiphene citrate, letrozole, metformin or injectable agents such as gonadotrophins), surgical therapy (laparoscopic ovarian drilling) or IVF.[12]

Letrozole, an aromatase inhibitor has been recommended as the first line drug for induction of ovulation in anovulatory infertility in terms of monofollicular ovulation, better endometrial thickness and no cases of lag endometrium.[13,14] Letrozole acts peripherally by blocking conversion of androgens to estrogens; thereby it decreases the estrogen synthesis and releases the HPO axis from estrogen negative feedback. This increases gonadotropin (both FSH and LH) secretion and causes stimulation of ovarian follicle. It facilitates monofollicular growth and ovulation keeping the HPO axis intact without depleting the estrogen receptors throughout the body.[14] Because of the shorter half-life, the effect of letrozole also decreases during late follicular phase and therefore estradiol produced by growing follicles increases. The elevated estradiol level suppresses the release of FSH. The drop in FSH levels causes atresia of small follicles and even selection of dominant follicle is impaired.[15]

Inhibition of estrogen synthesis by AIs is dose dependent.[16] They may be subdivided into steroidal (type I) and nonsteroidal (type II) inhibitors, which interact with the aromatase enzyme.[17] Letrozole is a type II non-steroidal AI which exert their function through reversible binding to the heme moiety of the cytochrome P-450 enzyme. Acting locally in the ovary, letrozole increases the follicular sensitivity to FSH by increasing the intraovarian androgens, as conversion of androgen substrate to estrogen is blocked. Recent data support a stimulatory role for androgens in early follicular growth by increasing the expressions of FSH receptors.[18,19]

Letrozole has no adverse effect on endometrium. Due to its short half-life (45 hours) and the lack of down-regulation of estrogen receptors letrozole has less negative effects on the endometrium and cervix in the late follicular phase.[20] Bao et al found that clomiphene citrate suppressed the expression of the markers of endometrial receptivity [HOXA10 and integrin alpha (v) beta (3)] in rats but not affected by letrozole.[21] Moreover, another important observation about letrozole was that letrozole administration in infertile ovulatory women was associated with in-phase histological dating of endometrium and normal pinopode expression.[22]

Extended letrozole protocol maintains the continuous production of FSH for a longer duration. This results in recruitment of a greater cohort of small follicles in the early part of the cycle and helps to reach maturity (≥18 mm). Pregnancy rate was more in the extended letrozole group. The reason behind using this extended regimen is based on our understanding of the physiology of follicular growth. Decremented follicular-phase FSH levels (referred to as the FSH window) is crucial for selection of a single dominant follicle from the recruited cohort. All but the dominant follicle (with its increased sensitivity to FSH) lose the stimulus to further development as FSH levels fall. The idea of extending the FSH window by providing exogenous FSH or extending the duration of letrozole treatment in the mid follicular phase would maintain FSH levels above the threshold. This allows multi follicular development and larger size dominant and mature follicles which significantly increases both the ovulation rate and pregnancy rate.[23]

Few recent RCTs were done on extended use of letrozole (10 days) for ovulation induction in patients with polycystic ovary syndrome by Badawy et al, Hassanein et al, Yadav et al, Aziz et al, and Salama et al.[18,23-26] These studies demonstrated better results in terms of multifollicular development, increased size and number of mature follicle, ovulation and pregnancy rate than traditional short letrozole protocol (5 days).

New concepts and new treatment protocols are introduced day by day as ovulation physiology is understood by infertility specialists. Sometimes traditional letrozole protocol (5 days) fails to induce ovulation in PCOS patients while gonadotropins or laparoscopic ovarian drilling might be needed for induction of ovulation. This leads them to bear extra expense which very often becomes a burden for many poor PCOS patients.

So, with this background present study was conducted to evaluate the role of low dose-extended or long letrozozle protocol and double dose-short letrozole protocol in induction of ovulation in polycystic ovary syndrome patients.

METHODS

This was a randomized controlled trial and was conducted after getting the permission from Institutional Review Board (IRB) of Bangabandhu Sheikh Mujib Medical University (BSMMU) bearing registration number: N0/BSMMU/2020/6751, Date: 27.06.2020. This study was done on the diagnosed cases of PCOS patients with subfertility in the department of reproductive endocrinology and infertility of BSMMU, Dhaka, Bangladesh during the period of July 2020 to June 2021. Total 70 patients were recruited.

Inclusion criteria

Inclusion criteria included age between 18-35 years, infertility, PCOS patients diagnosed according to Rotterdam criteria, and body mass index was between 18-<30 kg/m^2.

Exclusion criteria

Exclusion criteria included presence of infertility factors other than anovulation (endometriosis, bilateral tubal block, decreased ovarian reserve, male factor), endocrine disorders (hypothyroidism, hyperprolactinemia), other causes of hyperandrogenism, medical diseases (like diabetes mellitus, hypertension), history of taking insulin sensitizer (metformin, myoinositol), and history of taking ovulation inducing drug (clomiphene, letrozole or gonadotropin) in previous three months.

The randomization was done using computer generated random table. Eligible women who gave their informed consent were randomized to either low dose-extended letrozole group (35 patients) or double dose-short letrozole group (35 patients). serially numbered closed opaque envelops were used for allocation concealment which was done by an allocator (thesis supervisor). Each envelop was labelled with a serial number and had a card noting the intervention type. Allocation that was found after opening the closed envelops was not changed. Four patients (1 from extended letrozole group and 3 from short letrozole group) withdrew themselves from the study before their first follow up visit due to their personal reasons. Treatment was started from the 2nd day of menstruation after the baseline visit and investigations in the remaining patients. Total 34 patients participated in first cycle in low dose-extended letrozole group and 32 patients participated in double dose-short letrozole group. Tablet letrozole 2.5 mg was given in low dose-extended letrozole group for 10 days starting from the 2nd day of menstrual cycle or withdrawal bleeding for consecutive three cycles and tablet letrozole 5 mg daily was given to double dose-short letrozole group of PCOS women for 5 days starting from the 2nd day of menstrual cycle or withdrawal bleeding for three consecutive cycles. All patients were instructed not to take any other medications without consulting us. For the convenience of description, we considered low dose-extended letrozole group as 'group I' and double dose-short letrozole group as 'group II'. Ovarian response was assessed by transvaginal monitoring of follicle growth on 12th day of cycle for the appearance of preovulatory follicle (mean diameter ≥18 mm) and endometrial thickness 7 mm or more. Responders were defined as patients who developed preovulatory follicular size of ≥18 mm following letrozole therapy. After giving ovulation induction, follicle size 10 mm or more detected during TVS folliculometry was regarded as growing follicle in the present study. Addition of number of growing follicles in both ovaries was the total number of growing or developing follicles. The size of the biggest follicle was measured during transvaginal sonography by taking the mean of the two greatest internal follicular diameters measured in two planes perpendicular to each other. Endometrial thickness was measured at the greatest diameter perpendicular to the mid sagittal plane in the fundal region.

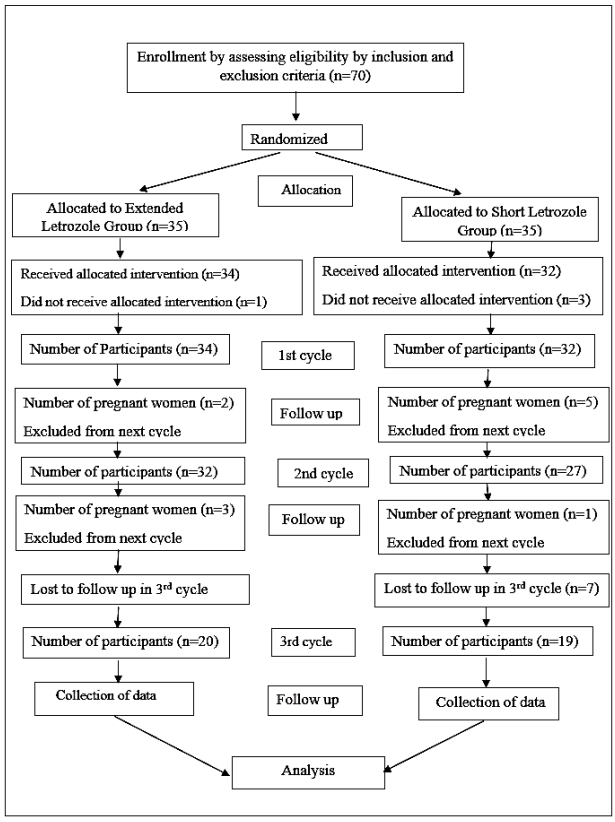

Figure 1: Flow chart of the participants.

Injection HCG 5000 IU was given to women with follicular size ≥18 mm and timed intercourse was advised every other day from the day of HCG administration. Patients were advised to do serum progesterone within day 21-23 of the cycle. Mid luteal serum progesterone was measured on day 21-23 of the cycle by chemiluminescence immunoassay method in SIEMENS ADVIA Centaur XP autoanalyzer by using Alinityi Progesterone Reagent Kit 08P36 in department of Biochemistry of Bangabandhu Sheikh Mujib Medical University. Typically, day 21 to 23 serum progesterone concentrations of ≥3 ng/ml indicate

normal ovulation.[1,27] Ovulation was confirmed by mid luteal serum progesterone ≥ 3 ng/ml at follow up visit.[1,27] Pregnancy was diagnosed either by serum beta HCG, urinary pregnancy test kit or by ultrasonography. The primary outcome measures were number of growing and mature follicles, biggest follicular size, serum progesterone (ng/ml), endometrial thickness (mm). Primary outcome was ovulation rate and secondary outcome were pregnancy rate and miscarriage rate.

During periodic lockdown in pandemic situation, as face to face visit to hospital was not always possible, some patients consulted with us over telephone and sent their documents through private communication system (WhatsApp). The women who got pregnant in first or second cycle were excluded in next cycle/cycles. As this study was conducted during COVID-19 pandemic period, all patients could not complete all three cycles due to the adverse situation of lockdown or personal illness or due to some other reasons. Total eighty-six and seventy-eight cycles were completed by group I and group II respectively.

Statistical analysis

Statistical analyses were carried out by using the Statistical Package for Social Sciences version 23.0 for Windows (SPSS Inc., Chicago, Illinois, USA). The mean±SD values were calculated for continuous variables and percentages for qualitative data. Data were tested using the t-test, chi-square test as appropriate. Comparison of quantitative variables between the study groups were done using Student's t-test for independent samples when normally distributed and Mann Whitney U test when not normally distributed. For comparing categorical data, the Chi-square (χ^2) test was performed. P values <0.05 was considered as statistically significant.

RESULTS

A total of 66 women participated in the study and were included in analysis. Table 1 shows important baseline characteristics of both groups which revealed that most of the patients about 80% belonged to similar age group that is less than 27 years in both extended (group I) and short letrozole (group II) group. There was no significant difference in mean duration of infertility and BMI. The hormonal status of the patients revealed normal mean FSH and LH values that supported PCOS patient's normogonadotropic characteristics. There was no significant difference between mean LH levels of both groups. All the patients of both groups were in euthyroid state and had normal prolactin level (Table 2). Table 3 displays that the number of patients that developed more than one follicular growth in first and second cycle were more or less double in extended letrozole group compared to short letrozole group but this difference could not meet the statistical significance level. On the other hand, 55% versus 15.8% patients attained multifollicular growth in extended and short letrozole group respectively in third cycle which bore statistically significant difference

Table 1: Baseline characteristics of study participants.

Variables	Extended letrozole group I (n=34)		Short letrozole group II (n=32)		P value
	Frequency/Mean±SD	Percentage	Frequency/Mean±SD	Percentage	
Age (years)	24.85±2.96		25±3.62		0.86^{ns}
18-24	12	35.3	13	40.6	0.86^{ns}
24-27	15	44.1	12	37.5	
>27	7	20.6	7	21.9	
BMI (kg/m^2)	24.49±1.99	-	24.61±2.36	-	0.53 (M)^{ns}
BMI (kg/m^2)					0.50^{ns}
18-<25 (normal)	18	52.9	16	50	
≥25 (overweight)	16	47.1	16	50	

Data were expressed as frequency, percentage and mean±SD. Mann Whitney U (M) test for quantitative variables and Chi-square test for qualitative variables were done to analyse the data, ns = not significant

Table 2: Hormone profile of study participants.

Variables	Extended letrozole group (n=34)	Short letrozole group (n=32)	P value
	Mean±SD	Mean±SD	
FSH (mIU/ml)	5.46±1.37	5.77±1.64	0.83^{ns}
LH (mIU/ml)	5.99±3.07	6.45±2.48	0.23(M)^{ns}
TSH (mIU/ml)	2.31±0.83	2.26±1.15	0.76(M)^{ns}
Prolactin (ng/ml)	15.65±5.63	13.31±5.48	0.84^{ns}

Data were expressed as mean±SD. Unpaired t-test was done to analyze the normally distributed data and Mann Whitney U test was done to analyse the data that were not normally distributed; ns= not significant

Table 3: Total number of growing follicles in study population.

Number of growing follicles	Extended letrozole group (group I)		Short letrozole group (group II)		P value
	Frequency	Percentage	Frequency	Percentage	
1st cycle	**(n=34)**		**(n=32)**		
0	8	23.5	7	21.9	
1	10	29.4	18	56.3	0.05^{ns}
>1	16	47.1	7	21.9	
2nd cycle	**(n=32)**		**(n=27)**		
0	7	21.9	8	29.6	
1	12	37.5	13	48.1	0.32^{ns}
>1	13	40.6	6	22.2	
3rd cycle	**(n=20)**		**(n=19)**		
0	0	0	2	10.5	
1	9	45	14	73.7	0.02^{s*}
>1	11	55	3	15.8	

Table 4: Comparison of development of biggest follicle in both groups.

Variables	Extended letrozole group (group I)		Short letrozole group (group II)		P value
	Frequency	Percentage	Frequency	Percentage	
1st cycle	**(n=34)**		**(n=32)**		
<14 mm	12	35.3	11	34.4	
14 to <18 mm	14	41.2	11	34.4	0.75^{ns}
≥18 mm	8	23.5	10	31.3	
2nd cycle	**(n=32)**		**(n=27)**		
<14 mm	12	37.5	12	44.4	
14 to <18 mm	5	15.6	8	29.6	0.20^{ns}
≥18 mm	15	46.9	7	25.9	
3rd cycle	**(n=20)**		**(n=19)**		
<14 mm	5	25	7	36.8	
14 to <18 mm	4	20	4	21.1	0.68^{ns}
≥18 mm	11	55	8	42.1	

Data were analysed by Chi-square (χ^2) test. ns=not significant

Table 5: Comparison of ovarian response on day 12 and mid luteal serum progesterone between two groups.

Variables	Extended letrozole group (group I) (n=34)	Short letrozole group (group II) (n=32)	95% CI		P value
	Frequency Mean±SD	Frequency Mean±SD	Lower	Upper	
No. of growing follicle	1.44±0.95	0.99±0.65	0.20	0.70	0.001*(M)^{s}
Mean size of dominant follicle (mm)	17.69±3.63	16.6±3.49	-0.13	2.32	0.08*^{ns}
Endometrial thickness (mm)	7.32±1.47	7.63±1.80	-0.82	0.19	0.22(M)^{ns}
Mid luteal serum progesterone (ng/ml)	14.69±9.62	14.16±11.57	-2.83	3.91	0.51(M)^{ns}

Data were expressed as mean±SD, Unpaired t-test was done to analyze the normally distributed data and Mann Whitney U test was done to analyse the data that were not normally distributed; ns= not significant, s=significant

Table 4 shows that the development of dominant follicle (18 mm) was more in group II than group I in first cycle but in second and third cycles, greater number of patients of group I developed dominant follicle (18 mm) than the other. However, none of the cycles showed significant difference. The summary of ovarian response was displayed in Table 5 that revealed that there was significant difference between group I and II in terms of mean number of growing follicles displaying the higher rate in group I. So, group I developed comparatively more multifollicular growth than group II. However, there were no significant differences in mean size of the dominant follicle, mean endometrial thickness and mean levels of mid luteal serum progesterone between two groups. The ovulation rate was greater in group I than group II in second and third cycles but the result was reverse with a little difference in first cycle. Cumulative ovulation rate was almost similar in both groups with RR 1.06. However, none of the differences proved significant statistically (Table 6). There was no significant difference between the two groups in terms of pregnancy rate in any of the cycles and in cumulative pregnancy rate as well (Table 7). There was one miscarriage (1 out of 8 pregnancies, 12.5%) in extended letrozole group and two out of six pregnancies (33.33%) ended up in miscarriage in short letrozole group

in this study. Concerning the adverse effect, letrozole was well tolerated by most of the patients in both extended and short letrozole group; 88.2% and 96.9% patients had no adverse effects in two groups respectively. Headache was the mainly complained adverse effect in both groups that occurred in a very small portion of patients. Other complaints like hot flush and cramp were negligible (Table 8).

Table 6: Comparison of ovulation rate in study participants in three cycles.

Ovulation rate	Extended letrozole group (group I)		Short letrozole group (group II)		RR	95% CI		P value
	Frequency	Percentage	Frequency	Percentage		Lower	Upper	
1st cycle	**(n=34)**		**(n=32)**					
Yes	23	64.7	23	71.9	0.94	0.68	1.29	0.70^{ns}
No	11	35.3	9	28.1				
2nd cycle	**(n=32)**		**(n=27)**					
Yes	24	68.8	18	66.7	1.12	0.81	1.57	0.48^{ns}
No	8	31.3	9	33.3				
3rd cycle	**(n=20)**		**(n=19)**					
Yes	15	75.0	13	68.4	1.10	0.74	1.63	0.64^{ns}
No	5	25.0	6	31.6				
Cumulative	**(n=34)**		**(n=32)**					
Yes	26	76.5	23	71.9	1.06	0.79	1.41	0.67^{ns}
No	8	23.5	9	28.1				

Data were analysed by Chi-square (χ^2) test. ns=not significant, RR=Relative Risk

Table 7: Comparison of pregnancy rate in study participants in three cycles.

Pregnancy rate	Extended letrozole group (group I)		Short letrozole group (group II)		RR	95% CI		P value
	Frequency	Percentage	Frequency	Percentage		Lower	Upper	
1st cycle	**(n=34)**		**(n=32)**					
Yes	2	5.9	5	15.6	0.38	0.08	1.8	0.19^{ns}
No	32	94.1	27	84.4				
2nd cycle	**(n=32)**		**(n=27)**					
Yes	3	9.4	1	3.7	2.53	0.28	22.95	0.37^{ns}
No	29	90.6	26	96.3				
3rd cycle	**(n=20)**		**(n=19)**					
Yes	3	15	0	0	0.85	0.71	1.02	0.13^{ns}
No	17	85	19	100				
Cumulative	**(n=34)**		**(n=32)**					
Yes	8	23.5	6	18.8	1.33	0.54	3.24	0.43^{ns}
No	26	76.5	26	81.3				

Data were analysed by Chi-square (χ^2) test. ns=not significant, RR=Relative Risk

Table 8: Adverse effects of letrozole ovulation induction in both groups.

Variables	Group I		Group II		P value
	Frequency	Percentage	Frequency	Percentage	
1st cycle	**(n=34)**		**(n=32)**		
No effect	30	88.2	31	96.9	0.51^{ns}
Headache	2	5.9	1	3.1	
Cramp	1	2.9	0	0	
Abdominal pain	1	2.9	0	0	
2nd Cycle	**(n=32)**		**(n=27)**		
No effect	26	81.3	25	92.6	0.38^{ns}
Headache	5	15.6	2	7.1	
Cramp	1	3.1	0	0	
3rd cycle	**(n=20)**		**(n=19)**		
No effect	16	80	18	94.7	0.48^{ns}
Headache	2	10	1	5.3	
Hot flush	1	5	0	0	
Abdominal pain	1	5	0	0	

Data were analysed by Chi-square (χ^2) test. ns=not significant, RR=Relative Risk

DISCUSSION

This study suggested that low dose-extended letrozole regimen leads to a significant increase in the number of growing follicles with a higher trend to raise the dominant follicle size, ovulation rate and pregnancy rate compared to double dose-short letrozole protocol.

In our study, mean age of extended letrozole group (group I) was 24.85±2.96 years and that of short letrozole group (group II) was 25±3.62 years (Table 1). There was no significant difference between two groups. Similar age group patients were selected by Badawy and Hassanein.[23,24]

Mean body mass index (BMI) was 24.41±1.99 kg/m^2 in group I and 24.61±2.36 kg/m^2 in group II in our study which was more or less normal BMI. There was no significant difference between two groups. About half of the patients carried normal body mass index (BMI) and rest half was of overweight in both groups (Table 1). Similar to this, patients included in Yadav et al study, were 50% and 56.25% overweight in short and extended letrozole group respectively. Contrary to this, in Badawy et al study, mean BMI of both extended and short letrozole group was about 34 kg/m^2 and it was about 28 kg/m^2 in both groups in the study done by Hassanein. In these studies, mean BMI was in obese and overweight range in both groups which were higher than our study. However, there were no significant differences between two groups in any of the study.[23-25]

The study participants were normogonadotropic in the present study with no significant difference between two groups (Table 2). The mean LH levels were around 6mIU/ml in Extended and Short letrozole group respectively. Some other study participants had relatively higher luteinizing hormone level than this. Salama et al, Badawy et al and Hassanein et al studies included patients with LH level of >10 mIU/ml; around 12 mIU/ml and around 9 mIU/ml in both groups respectively.[23,24,26] These higher levels of LH might be the reasons for lower rates of ovulation in those studies compared to this one.

In the present study, greater number of study participants had multifollicular growth in low dose-extended letrozole group in all three cycles with significant difference in third one (Table 3). The mean number of growing follicle was 1.44±0.95 versus 0.99±0.65 in group I and group II respectively generating p value of 0.001 (Table 3). These results came in agreement with the results of study done by Badawy et al, Aziz et al, Hassanein et al, Yadav et al, and Salama et al.[23-26] But the total number of growing follicles were more in these studies than this study in both groups.

The total number of growing follicles was significantly higher in Badawy et al study revealing this number as 6.7±0.3 versus 3.9±0.4; in Aziz et al study showing this number as 6.48±0.68 versus 4±0.91; in Salama et al study exhibiting this number as 4±1 versus 3±1 in extended and short letrozole group respectively. Hassanein et al study manifested the highest number of follicles as 8.2±2.19 and 8.17±2.04 in extended and short letrozole groups with no significant difference between them. The probable reason of lower number of growing follicles in this study might be that we considered ≥10 mm size follicle as the growing follicle during stimulation. However other authors did not define 'growing follicle' for their respective study.[18,23-26]

The number of patients that developed dominant follicle (≥18 mm) was more in group I than group II in second and third cycles except the first one where this was more in group II than group I. So, greater number of patients of group I developed dominant follicle (≥18 mm) than the other. However, none of the cycles showed significant difference (Table 3). This result of our study was in agreement with the result of Aziz et al study which showed slightly higher percentage of patients of extended letrozole group developed follicle ≥18 mm than the other (53.3% versus 46.7%) with no significant difference. Hassanein et al study is consistent with this finding as well which revealed significantly greater number of patients develop dominant follicle ≥18 mm (66% versus 54%) in long letrozole group than the short letrozole group (p value <0.05).[18,24]

The mean size of the dominant follicle was insignificantly greater in group I than group II displaying 17.69±3.63 mm and 16.6±3.49 mm respectively (Table 5). This result is consistent with Aziz et al and Salama et al studies. Mean diameter of dominant follicles was one mm and two mm greater in both groups in Salama et al and Aziz et al study respectively. However, none of the study had significant difference.[18,26]

There was no significant difference in mean endometrial thickness on day 12 between two groups in present study but it was slightly lower in extended letrozole group than short letrozole group (7.32±1.47 mm versus 7.63±1.8 mm) (Table 5). Similar to this study, there were no significant differences in mean endometrial thickness at HCG between two groups in Badawy et al, Aziz et al, Salama et al and Yadav et al studies. However, in contrast to the present study, these studies showed greater endometrial thickness at HCG in extended or long letrozole group than short letrozole group revealing more or less 11 mm in Badawy et al and Yadav et al study and around 9 mm in Aziz et al study.[18,23,25,26]

This decrement of endometrial thickness in this study might be due to considering the endometrial thickness by transvaginal sonography at day 12 of menstrual cycle irrespective of follicular size instead of taking the endometrial thickness at HCG by following the patients by serial transvaginal sonography. Another discrepancy of this result is that extended letrozole group had slightly lower endometrial thickness. One of the explanations of this deviation might be that in extended letrozole group, the antiestrogenic effect of letrozole continues up to day

12 or 13 which lowers the intrafollicular estrogen level as well as estrogen production in other sites that might affect the endometrial development. Contrary to this, in short letrozole group, due to shorter half-life of letrozole, this antiestrogenic influence cannot persist for long enough to affect endometrial development adversely. However, serum estradiol measurement which was not done in this study might be helpful in clarifying this issue. Another observation was that this slight decrement of endometrial thickness did not affect pregnancy outcome adversely in extended letrozole group which had been shown in Table 7.

Comparing two groups with regard to mean mid luteal serum progesterone, it was 14.69±9.62 ng/ml and 14.16±11.57 ng/ml in extended and short letrozole group respectively without any significant difference (Table 5). Badawy et al study showed serum progesterone of around 10 ng/ml in both groups without significant difference.[23]

Drawing a comparison on ovulation rate between two groups figured out that the ovulation rate was greater in extended letrozole group than short letrozole group in second and third cycles but the result was reverse with a little difference in first cycle. Cumulative ovulation rate was higher in group I as well [76.5% in extended letrozole group versus 71.9% in short letrozole group; (Table 6)]. However, none of the differences proved significant statistically. This outcome came in similar with the consequences of Badawy, Aziz, Hassanein and Salama. Nonetheless, the ovulation rates were lower in both groups in Badawy et al (65.7% in extended letrozole group versus 61.8% in short letrozole group), Aziz et al (63.3% versus 56.7%in extended and short letrozole group respectively) and Salama et al (65% in extended versus 60% in short letrozole group) study than this one. This lower rate of ovulation might be due to higher mean body mass index, higher mean luteinizing hormone level, or due to inclusion of clomiphene citrate resistant PCOS women as their study participants. In Hassanein et al study, ovulation rate in extended letrozole group (74%) was nearly similar to this study while stating much lower rate in short letrozole group (56%). However, none of the studies showed significant differences in ovulation rate of both groups.[18,23,24,26]

In this present study, pregnancy occurred in 8 out of 34 patients in extended letrozole group and in 6 out of 32 patients in short letrozole group (Table 7). Comparing two groups in terms of cumulative pregnancy rate, it was manifested that pregnancy rate was somewhat higher in group I (23.5%) than group II (18.8%). However, cumulative pregnancy rate between two groups were not significant statistically. This result came in parallel with Badawy et al (17.4% versus 12.4%; p value-0.03), Aziz et al (20% versus 13.3%), Salama et al (25% versus 15%), Hassanein et al (24% versus 14%) and Yadav et al (18.3% versus 12.5%) studies that displayed higher pregnancy rate either cumulative or per cycle in extended letrozole group than the other. However, none of the studies found significant difference regarding pregnancy rate except Badawy et al where p value was <0.05. That study showed that pregnancy occurred in 28 of 225 cycles in the short group (12.4%) and 38 of 219 cycles (17.4%) in the long letrozole group. The probable reason of this significant difference might be the increased number of patients (218) and cycles (444) of Badawy et al study.[18,23-26]

CONCLUSION

Low dose-extended letrozole protocol can be a better alternative to double dose-short letrozole protocol in respect to significant increase in the number of growing follicles with a higher trend to raise the ovulation rate and pregnancy rate though there were no significant differences in dominant follicle size, endometrial thickness, ovulation rate and pregnancy rate between two groups. Studies with large sample size and involving multiple centers can be done in future for having more statistically significant result.

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