Original Article

Transvaginal sonographic assessment of follicular development and endometrial thickness in letrozole stimulated cycles of PCOS and non-PCOS infertile women

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Abstract

Background: *Infertility in polycystic ovary syndrome (PCOS) is one of the leading causes of anovulatory infertility. Ovulation induction is indicated for the management of anovulatory infertility in PCOS and for augmentation of ovulation in ovulatory infertility, in unexplained infertility. The aim of this retrospective study was to compare and determine the efficacy of letrozole administration in infertile women with PCOS to that of infertile women without PCOS by transvaginal sonography.*

Methods: *This retrospective study was done at Centre for Assisted Reproduction (CARE), BIRDEM General Hospital 2 from January to December 2011. Fifty six infertile women including 16 diagnosed as having PCOS and 40 infertile women with regular menstrual cycle (non-PCOS) were included in this study. Patients were treated with letrozole 7.5 mg/day from day 2-6 of the menstrual cycle. Subjects were monitored once during the days 11 to 14 of the cycle by transvaginal ultrasound. Main outcome measures were number of ovulatory follicles, dominant follicle diameter and endometrial thickness.*

Results: *Letrozole as an ovulation inducing drug was found equally effective in terms of follicular recruitment, follicular maturation and endometrial development both in PCOS and non-PCOS women, as there was no significant difference regarding mature follicular development and endometrial response between the two study groups. Association of endometrial response particularly with follicular diameter 18 mm or more among the study groups revealed no statistically significant difference.*

Conclusion: *In conclusion, our results indicate that the effect of letrozole on endometrial thickness and follicular development in patients of anovulatory PCOS did not significantly differ compared to non-PCOS infertile women.*

Key words: *Letrozole, infertility, PCOS, non-PCOS, transvaginal sonography, follicular development, endometrial thickness.*

(BIRDEM Med J 2019; 9(3): 234-239)

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Received: August 7, 2019 **Accepted:** August 15, 2019

Introduction

Polycystic ovary syndrome (PCOS) is a common multisystem endocrine disorder in women, with long-term health consequences. Along with polycystic ovaries, infertility, obesity, hypertension, dyslipidemia, insulin resistance and type 2 diabetes mellitus, hyperandrogenism and menstrual abnormalities are the primary clinical features of PCOS. This chronic and heterogeneous endocrine disease affects 5–8% of women of reproductive age.[1]

There are many possible options for treatment of subfertility in women with anovulatory PCOS. Clomiphene citrate (CC) has been the most widely used and standard drug for the treatment of infertility since its introduction into clinical practice in the 1960s. It is known that clomiphene results in an ovulation rate of

60–85%, but a conception rate of only about 20%. About 20–25% of women are resistant to CC and do not ovulate.[2] CC has a long half-life of 5–7 days and this may have a negative effect on the cervical mucus and endometrium. Endometrial thinning due to peripheral anti-estrogenic effects is the possible explanation for low pregnancy rates.

Therefore, a safe, more effective oral drug that could replace CC as a first-line treatment for anovulatory infertility is needed. Letrozole (LE), a third-generation aromatase inhibitor, has been widely used to treat breast cancer. This potent, reversible, highly selective, nonsteroidal aromatase inhibitor suppresses the enzyme responsible for the conversion of androgens to estrogens and therefore could be used to induce ovulation in women with PCOS. By reducing the levels of estrogen in the body, LE promotes the release of follicle-stimulating hormone (FSH) and luteinizing hormone (LH), which support the growth of ovarian follicles. The first report of LE for clinical ovulation induction was published in 2001.[1]

In contrast to clomiphene, letrozole elicits a monofollicular response and does not adversely affect either the endometrium or the cervical mucus, due to an absence of a peripheral estrogen receptor blockage. This retrospective study was carried out to assess the efficacy of letrozole for induction of ovulation in anovulatory PCOS to that of infertile women without PCOS by transvaginal sonography.Main outcome measures were number of growing and mature follicles, leading follicular diameter and endometrial thickness.

Methods

Study Subjects

This retrospective study was carried out in Centre for Assisted Reproduction (CARE), Department of Obstetrics and Gynaecology, BIRDEM General Hospital 2, during the period of 1st January 2011 to 31st December 2011. Considering inclusion and exclusion criteria, 56 patients were screened. From these 56 infertile women, 16 were diagnosed PCOS and 40 were infertile women with regular menstrual cycle (non-PCOS). Patients were treated with letrozole 7.5 mg/day from day 2-6 of the menstrual cycle. Subjects were monitored once during the days 11 to 14 of the cycle by transvaginal ultrasound. Transvaginal ultrasonography (TVS) was performed for follicular diameter tracking and measurement of endometrial thickness. Follicular diameter measurement was done in 2 perpendicular planes and the mean was calculated, while endometrial thickness was measured in the sagittal plane at the widest part of endometrial cavity. A trilaminar diameter of 8 mm was considered a satisfactory response and follicle was considered as mature when it attained 18 mm or more. Growing follicles of less than 10 mm were considered non-significant.

Ethics

The study protocol was approved by the institutional ethical review committee.

Data analysis

Statistical analysis was done through SPSS (ver. 16). Frequency, percentage distribution and mean ± SD were used to describe the result. χ^2 test was used to compare the results between the two study groups. A p-value of <0.05 was taken as statistically significant.

Results

Total of 56 infertile patients, who were treated with 7.5 mg of letrozole, were selected from the hospital records of CARE, BIRDEM General Hospital 2 during the period of 1st January to 31st December 2011. Among them 16 were diagnosed case of PCOS and the rest 40 patients were infertile women with regular menstrual cycle other than documented PCOS. All patients had some sort of past infertility treatment, at various occasions from various parts of the country.

Though the target age group was 20 to 40 years, the subjects in the sample were between 22 and 37 years of age, with a mean of 29.80 years. Among the 16 PCOS women, 13 (81%) were in the <34 age group and 3 (19%) patients were in the >34 age group. Again, among the 40 non-PCOS women, 33 (82%) were in the <34 age group and 7 (18%) patients were in the >34 age group. BMI (kg/m^2) ranged from 23 to 32 with an average of 27.50 (Table I). Thirty two non-PCOS were of primary subfertility and 8 were of secondary subfertility. There was no secondary subfertility in the PCOS group.

Table I Demographic profile of patients undergoing ovulation induction with letrozole (N=56)

	Study Group (Number and Percentage)	
Age Distribution	PCOS(n=16)	Non-PCOS(n=40)
< 34 years	13 (81)	33 (82)
≥ 34 years	3 (19)	12(18)
Sub-fertility		
Primary	16 (100)	32(80)
Secondary	0 (0)	8(20)
Mean BMI (kg/m^2)	27.50 ± 2.92	
Mean Age (Years)	29.80 ± 3.86	

When follicle size profiles were plotted for both the ovaries of study participants, the distribution of mature dominant follicles with a diameter of more than 18 mm were found among 8 (50%) PCOS and 19 (47.5%) non-PCOS patients. The number of dominant follicles (e”18 mm) in both the groups was not statistically different (Table II).

Table II Follicular response among the study group

	Study Group (Number and Percentage)		
Follicular diameter	PCOS (n=16)	Non-PCOS (n=40)	*p* value
< 10.00 mm	5 (31.25)	16 (40.00)	
10.00 mm-17.99 mm	3 (18.75)	5 (12.50)	.758
≥18.00 mm	8 (50.00)	19 (47.50)	

After ovulation induction by letrozole monotherapy, it was observed that an endometrial response of more than 8 mm thickness was seen in 62.5% of PCOS and 52.5% of non-PCOS patients (Table III).

Table III Endometrial response among the study groups

	Study Group (Number and Pe rcentage)		
Endometrial Thickness	PCOS (n=16)	Non-PCOS (n=40)	*p* value
<8.00 mm	6 (37.50)	19 (47.50)	.496
≥ 8.00 mm	10 (62.50)	21 (52.50)	

There was no significant association when subjects with dominant follicles (≥18.00 mm) in letrozole treated groups were correlated with endometrial thickness, in both the groups (Table IV).

Table IV Endometrial response among the study groups with follicular diameter more than 18 mm

	Study Group (Number and Percentage)		
Endometrial Thickness	PCOS (n=8)	Non-PCOS (n=19)	*p* value
<8.00 mm	1 (12.50)	7 (36.80)	.206
≥8.00 mm	7 (87.50)	12 (63.20)	

There were no statistically significant differences in the status ofgrowing follicles between the two groups (Table V).

Table V Follicularstatus among the study groups

	Study Group (Number and Percentage)		
Follicular Response (Absent or Present)	PCOS (n=16)	Non-PCOS (n=40)	*p* value
Present	12 (75.00)	24(60.00)	.290
Absent	4 (25.00)	16 (40.00)	

When the effect of letrozole monotherapy were observed in both the study subjects who developed dominant follicles (>18 mm) and endometrial thickness of more than 8 mm vs. those who did not, a positive response was detected in 13 (81%) PCOS women and 30 (75%) non-PCOS subjects. Neither dominant follicular recruitment nor significant endometrial thickness (≥8mm) were statistically different in the non-PCOS study group in comparison to PCOS group after stimulation with 7.5mg of letrozole (Table VI).

Table VI Endometrial and follicular response among the study groups

	Study Group (Number and Percentage)		
Endometrial and Follicular Response	PCOS (n=16)	Non-PCOS (n=40)	*p* value
Yes	13 (81.25)	30 (75.00)	.617
No	3 (18.75)	10 (25.00)	

Discussion

In our study, we tried to compare the effects of letrozole in patients of PCOS and non-PCOS infertile women with due emphasis on the endometrial response and follicular maturation. PCOS is the most common cause of infrequent periods (oligomenorrhoea) and absence of periods (amenorrhoea), affecting about 4% to 8% of women worldwide in their fertile years.[3]

The diagnosis can be made based on the 'Rotterdam criteria 2003', jointly proposed by the European Society for Human Reproduction and Embryology and the American Society for Reproductive Medicine. The woman must have two of the following three criteria to be diagnosed with PCOS[4]:

1. Oligoovulation (infrequent ovulation) or anovulation (absence of ovulation), or both
2. High male hormone levels (hyperandrogenism) diagnosed either clinically (excessive hair growth, hirsutism) or biochemically (raised serum testosterone levels)
3. Ovaries which appear to be polycystic on vaginal sonogram, defined by the presence of 12 or more antral follicles in an ovary or an ovarian volume of more than 10 mL. Antral follicles are defined as measuring between 2 and 9 mm in diameter.

There are many possible options for treatment of subfertility in women with anovulatory PCOS. Clomiphene citrate (CC) is a selective oestrogen receptor modulator (SERM) and is the most common medication used for treating the condition. It was first introduced in 1960 for treatment of World Health Organization (WHO) type II anovulation (a type of subfertility where hormone levels stay normal) in subfertile women and has been the first line treatment ever since. CC is given orally and is relatively safe and inexpensive, but there are also adverse effects associated with it, such as negative changes in endometrium and cervical mucus due to the down regulation of oestrogen receptors that might impair implantation after successful induction of ovulation.[3]

Aromatase inhibitors (AIs) are a newer class of drugs that were introduced for ovulation induction in 2001 by Mitwally and Casper.[5] Since about 2001 data from many clinical trials have been collected and there is evidence that the AI letrozole might be as effective as CC, but the outcome data vary. AIs are 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 the endometrium and cervix compared with CCs. Despite evidence of effectiveness and safety in well designed large randomised controlled trials (RCTs), letrozole is still used off label for ovulation induction, since it has not been approved by the US Food and Drug Administration (FDA) for this indication.[6] A 2005 study[7], including 150 babies, raised some concerns about the teratogenicity of letrozole, but there were major methodological flaws in this study as the intervention group was not well controlled. Two other large studies, including 911 and 470 infants respectively, compared the use of letrozole to CC and spontaneously conceiving women. Both reported no higher levels of minor or major congenital malformations or cardiac abnormalities in newborns after use of letrozole for ovulation induction.[8]

Aromatase inhibitors increase endogenous FSH production in response to decreased estrogen biosynthesis in the ovary and extraovarian tissues, including the brain. Because they do not deplete estrogen receptors like CC, normal central feedback mechanisms remain intact.[9] As the dominant follicle grows and estrogen levels rise, normal negative feedback occurs centrally resulting in suppression of FSH and atresia of the smaller growing follicles. Therefore, a single dominant follicle, and mono-ovulation, is the rule in most cases with the clear advantage of reducing multiple-gestation pregnancies.[9]

Compared to CC, letrozole has been associated with lower preovulatory estradiol (E2) levels, as well as thicker endometrium and a trend towards higher pregnancy rates. Standard ovarian stimulation protocols often produce high preovulatory E2 levels that could adversely affect the development of the endometrium, the follicles and the embryo. Therefore, the lower E2 when using AIs may lead to an improvement of implantation.[9]

In the evaluation and assessment of the infertile couple, the application of TVS has made significant improvements in modern management of infertility. The TVS depicts accurately the pelvic anatomy of the scanned area safely, quickly and reproducibly. It should be mandatory for the person performing the scan to

know about the female endocrinology and be well versed with the causes and management of infertility.[10]

Till date, there are no known adverse biological effects of TVS on the patients, on the oocytes or on the ultrasound operator according to 'American Institute of Ultrasound in Medicine' (AIMU).[11]

For sonographic evaluation of the endometrium, which is the inner lining of the uterus, measurement of the endometrial thickness is done. Endometrium has receptors for ovarian hormones and in response to the estradiol from the ovaries (or exogenous), the endometrial lining grows in a typical pattern which is recognizable by TVS. The endometrium grows at the rate of 0.5 mm/day in the proliferative phase and 0.1 mm/day in the luteal phase. A thickness of more than 7 mm in the preovulatory period is associated with higher pregnancy rates.[12]

Ovarian study and follicle monitoring by TVS are one of the first steps in the evaluation of an infertile women. A detailed history including menstrual history is very essential in correlating with the TVS findings.

Regarding follicular development, in response to pulsatile GnRH during the early part of menstrual cycle, follicle stimulating hormone (FSH) is released from the anterior pituitary gland which influences the progressive development of few follicles. One or occasionally two follicles will continue to develop into the dominant follicle(s). There is a linear increase in the size of developing dominant follicles at the rate of 2 to 3 mm/day, reaching a diameter 16 to 18 mm before ovulation.[9]

It is generally accepted that the aromatase inhibitor letrozole may be a better alternative in terms of endometrial response in ovulation induction. Because of its rapid elimination and reversibility, letrozole allows the endometrium to respond well to rise estrogen levels in the late follicular phase. Previous systematic review and meta-analysis reported that letrozole could significantly enhance the live birth and pregnancy rates in patients with PCOS.[13,14]

By comparing the TVS scans taken during the study period in this retrospective study, it was attempted to determine whether ultrasonographically detectable changes in endometrium and follicle population were occurring in infertile women with or without PCOS.

The number of dominant follicles (≥18 mm) in both the groups of the present study was quite similar. There was no positive correlation between endometrial thickness and number of dominant follicles in both the study groups.

As there was no significant difference regarding growing follicular development and endometrial response between the two study subjects, letrozole as an ovulation inducing drug could be equally effective in terms of follicular recruitment, follicular maturation and endometrial development both in PCOS and non-PCOS women.

This study has some limitations, the most important of which include that it is retrospective, non-powered, non-randomized and not blinded. These issues are inherent in retrospective studies. Also, the sample size was relatively small, which may affect the results of this study. However, we believe the results are of interest since there are very few studies comparing letrozole-stimulated cycles among PCOS and non-PCOS women.

In this retrospective study, follicle populations and endometrial response was analyzed in a small group of infertile women in the hope of generating some insight into the feasibility of using ultrasonography in women with or without polycystic ovaries.

In summary, the results of this study suggest that the aromatase inhibitor letrozole may be an alternative as a first-line drug for ovulation induction in both ovulatory and anovulatory infertile patients, considering its moderate stimulatory effect over the ovary and its favorable effect on endometrial morphology, in set-ups where intense monitoring is not possible. This may lessen some of the heavy socioeconomic burden of infertility treatment especially at the community levels.

Conflict of interest: Nothing to declare.

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