Annals of R.S.C.B., ISSN:1583-6258, Vol. 25, Issue 6, 2021, Pages. 19312- 19322
Received 25 April 2021; Accepted 08 May 2021.

The Effect of Clomiphene citrate with Estradiol Valerate versus Letrozole on Endometrial Thickness and Pregnancy Rate in Infertile PCOS Women

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ABSTRACT

Background: Polycystic Ovarian Syndrome (PCOS) is a common endocrine disorder in infertile women. Infertility influences about 40% of PCOS women. The aim of this work was to study the effect of Clomiphene citrate plus Estradiol Valerate versus Letrozole on endometrial thickness and pregnancy rate in infertile PCOS women underwent induction of ovulation. **Methods:** A randomized clinical trial carried out in Gynecology and Obstetrics Department, Faculty of Medicine, Zagazig university from March 2019 to September 2019, on 70 infertile PCO women with and were divided to 2 two groups , 35 patients patient received clomiphene citrate plus estradiol valerate and 35 patient received letrozole. **Results:** the results showed that endometrial thickness was high in Letrozole group B (9.7 ± 1.1) than clomiphene plus estradiol group A (8.8 ± 1.2) with a significant difference between both groups. Rate of pregnancy was higher in the Letrozole group B (45.2%) compared to clomiphene plus estradiol group A (22.9%). **Conclusion:** Letrozole increase the thickness of endometrial and pregnancy rate more than the Estradiol Valerate plus Clomiphene citrate in the infertile PCOS women who had abnormal endometrial thickness with Clomiphene citrate, so it is recommended for PCOS infertile women .

Keywords: Letrozole, Clomiphene citrate, PCOS, Endometrial thickness**.**

Introduction

Clomiphene reduces estrogen effect by blocking estrogen receptors (EL-Gharib et al., 2015).

The addition of ethinyl estradiol to Clomiphene have a significant effect on thickness of endometrial more than Clomiphene alone, but without any effect on the rate of pregnancy and abortion (Elsemary et al., 2018).

Unlike clomiphene, letrozole prompts agonistic impacts of estrogen on endometrium than antagonistic effect. It incites ovulation by inhibiting the transformation of androgens to estrogen that establishes an estrogen-lacking environment (Seyedoshohadaei et al., 2016).

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The half-life of Letrozole is much shorter than Clomiphene, for this reason, Letrozol gives better circumstance to ovulation compared with Clomiphene. Letrozole mostly produce one follicle; which decrease the risk of multiple pregnancy and Ovarian Hyperstimulation Syndrome (Ghomian et al., 2015).

The aim of this work was to study the effect of Clomiphene citrate plus Estradiol Valerate versus Letrozole on endometrial thickness and pregnancy rate in PCOS infertile women underwent induction of ovulation

METHODS

The present randomized double blind study has been carried out in Gynecology and Obstetrics Department, Faculty of Medicine, Zagazig university on 70 women were previously received clomiphene citrate alone as management of infertile anovulatory PCOS women, but giving improper endometrial thickness < 7mm during the period from March 2019 to September 2019.

Written informed consent was taken from all patients or their relatives and the study was approved due to the research ethical committee of Faculty of Medicine, Zagazig University (International review board ZU-IRB# 5057/19-12-2018). The study was carried in accordce with the ethical Code of the World Medical Association (Declaration of Helsinki) for studies involving humans.

Inclusion criteria:

1. All patients, aged 18–35 years old, with complete infertility workup and were diagnosed as having PCOs women according to the ESHRE/ ASRM, Rotterdam criteria 2003 demonstrating two of the three criteria:

- oligo or anovulation.
- hyperandrogenism.
- Polycystic ovaries by Ultrasonography (12 or more follicles).

After exclusion other causes of androgen excess. (Wang & Mol, 2017)

2. Normal finding in infertility work up including normal hormonal profile (prolactin and TSH), with anovulatory cycles confirmed by midluteal progesterone ≤ 3ng/ml.
3. Bilateral tubal patency diagnosed by hysterosalpingography, sonohysterography and/or laparoscopy.
4. Normal semen analysis (Patel et al., 2018)
5. Body mass index (BMI >18 and <30 Kg/ m2).

Exclusion Criteria:

1. Patients with male factor infertility, hyperprolactinemia and, thyroid disorder.

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2. Patients with any tubal pathology or uterine pathology.
3. Contraindication of ovulation induction, (Multiple ovarian cysts or allergy to inducing agent "clomid").
4. Known or suspected pelvic infection (PID).

Sample size:

The sample size was calculated to be 70 infertile PCOs women using OPEN-EPI program with CI 95% and Power 80%. During the study period.

randomized was done by a computer-generated randomization and patients were divided into two groups:

Group(A): (35) patient received clomiphene citrate plus estradiol valerate.

Group(B): (35) patient received letrozole.

Methods

Patients were subjected to:

Complete history taking : including (Personal ,Obstetric, Present , Past , Family ,Surgical history, history of allergy to any medication and previous history of induction of ovulation in previous 6 months and drugs used were endometrial thickness < 7mm, follicular growth and ovulation).

Examination: (General, abdominal, local clinical and bimanual pelvic examination).

Investigations:

1) Basal hormonal studies on Day2 of cycle included serum FSH,LH,E2, androgen level, prolactin level and thyroid stimulating hormone was measured on day 2 of spontaneous menstrual cycle.
2) Basal transvaginal U/S on day 3 of the cycle to examine each ovary for detecting criteria of PCOS and count ntral follicles in both ovaries to exclude any basal ovarian cyst which suspected when diameter of follicle >1.5 cm) and to measure basal endometrial thickness.

Patients were allocated into two groups:

a) Group A (clomiphene citrate & estradiol group): included 35 anovulatory PCO patients who treated with clomiphene citrate (Clomid; Aventis pharma S.AE, Global Napi pharmaceuticals, Cairo, Egypt) 100 mg daily from the 3^{rd} day of cycle to the 7^{th} day and estradiol valerate 4-mg (two tablets of cyclopregynova) from the 8^{th} day of cycle to 14^{th} day.
b) Group B (Letrozole group): included 35 anovulatory PCO patients who received letrozole (Femara; Novartis pharma AG, Basle, Switzerland) 5 mg every day from the 3^{rd} day of cycle to the 7^{th} day.

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Follow up continued from the treatment to 3 cycles after.

1) treatment stared with follow up by ultrasound scans started from the 8th day of cycle, scan repeated every day or other day for monitoring growth of follicle and endometrial thickness.
2) Technique of US: Midisson X4, Korea with a frequency of 9 MHz.
3) Each patient is advised to empty bladder before examination.
4) Endometrial thickness was defined as the maximal thickness between the high reflective interface of endometrial myometrial junction.
5) Follicular monitoring carried out for both groups with transvaginal ultrasound with same technique , started from the eight day of cycle till attaining a mature follicle with a mean diameter of 18-22 mm, number and size of Dominant follicles, thickness of endometrial and pattern reported on day of hCG administration,
6) Then, an single hCG 10,000 IU injection was administrated for triggering ovulation
7) intercourse timed from the day of hCG for 4 days.
8) Clinical pregnancy detected by serum pregnancy test and transvaginal U/S which detected IUGS and fetal pulsation.
9) Follow up of both groups was done for 3 cycles.

the endometrial thickness on day of hCG, Pregnancy rate were the Primary outcomes. Secondary outcome was the rate of ovulation.

Statistical Analysis: Analysis of data was carried out using SPSS version 20). Description of quantitative variables was given as mean, and Standard deviation. qualitative variables between groups were compared by Chi square test (χ^2 –test). quantitative variables were compared by t-test. The Z-test used for proportions. P-values < 0.05 e considered significant and P values < 0.01 considered a highly significant.

Results

Table (1) : Comparing age and clinical characteristics among women treated with clomiphene citrate plus estradiol valerate (A) and letrozole (B).

Variable	Group (A) (n =35) mean ± SD (Range)	Group (B) (n =35) mean ± SD (Range)	t- test	p-value
Age (years)	26.7±4.40 (22-31)	25.3±3.90 (21-29)		0.10
Body Mass Index (BMI)	25.5±2.50	24.5±3.00	1.6	

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(Kg/ m^2)	(20-29.5)	(19-29.3)		0.09
Duration of infertility (years)	2.2±1.30 (1-6)	1.9±1.40 (1-7.5)		0.30
Variable	**Group (A) No(35) %**	**Group (B) No (35) %**	χ^2	**p-value**
Type of infertility				
Primary	19 54.30%	22 62.90%	0.5	0.60
Secondary	16 45.70%	13 37.10%		

Table (1), showed that **t**here was non statistical significant difference between both groups as regard age, BMI, infertility and duration type.

Table (2): Comparing TSH, Prolactin , FSH, LH and E2 level between women of the two studied groups:-

Variable	**Group (A) (n =35) mean ± SD (Range)**	**Group (B) (n =35) mean ± SD (Range)**	**t-test**	**p-value**
TSH (mu/l)	2.1±0.85 (0.5-3.7)	1.94±0.83 (0.5-3.8)	0.79	0.42
Prolactin (ng/ml)	14.34±5.80 (4.1-25.7)	13.7±5.47 (5.6-26.6)	0.47	0.63
FSH (u/l)	6.53±1.90 (8.43-4.63)	6.49±3.20 (3.29-9.69)	0.06	0.94
LH (u/l)	14.9±6.10 (8.8-21)	14.8±6.90 (7.9-21.7)	0.06	0.94
E2 (pmol)	369.2±120.90 (248.1-489.9)	381.9±110.80 (270.2-492.7)	0.45	0.64

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Table (3): Comparing basal endometrial thickness, stimulation day and endometrial thickness on HCG between the two studied groups:-

Variable	Group (A) (n =35) mean ± SD (Range)	Group (B) (n =35) mean ± SD (Range)		p-value
Basal endometrial thickness	4.0±0.50 (3.5 -4.5)	4.1±0.60 (3.5-4.7)		0.300
Endometrial thickness on day of HCG inj.	8.8±1.20 (7-13)	9.7±1.10 (7-12)	3.3	**0.001****
Number of Stimulation days	14.1±1.70	14.8±1.70		0.0700

Table (3), showed that there was high statistical significant difference between studied groups as regard thickness of endometrial in the day of HCG injection. But regarding basal endometrial thickness and number of stimulation days injection, there was non statistical significant differences.

Table (4): comparing ovulation rate between the two studied groups;:-

ovulation rate	Group (A)		Group (B)		χ^2	p-value
	No.(35)	%	No.(35)	%		
Positive (No=57)	25.0	71.40%	32.0	91.40%	4.6	**0.03***
Negative (No=13)	10.0	28.60%	3.0	8.60%		

Table (4), showed that there was a statistical significant difference between the studied groups as regarding rate of ovulation, where it was high in letrozole group more than clomiphene plus estradiol group.

Table (5): comparing pregnancy rate between the two studied groups:

pregnancy rate	Group (A)		Group (B)		χ^2	p-value
	No.(35)	%	No.(35)	%		
Positive (No=18)	8.0	22.90%	16.0	45.20%	4.1	**0.04***
Negative (No=46)	27.0	77.10%	19.0	54.30%		

Table (5), showed that there was a statistical significant difference between the studied groups as regarding rate of pregnancy, it was higher (45.2%) in letrozole group more than clomiphene plus estradiol group.

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DISCUSSION

The prevalence of polycystic ovaries were recorded by ultrasound in 20–30% of women (range between 18 and 25 years), polycystic ovaries were recorded in 33% by ultrasound, and PCOS prevalence was 26% (Bellver et al., 2018).

This study show that Age distributed as 26.7±4.4yr in clomiphene-estradiol group and 25.3±3.9yr in Letrozole group and that mean BMI distributed as 25.5±2.5 Kg/m2 in clomiphene-estradiol group and 24.5±3.0Kg/m2 in Letrozole group B and that mean duration of infertility (years) distributed as 2.2±1.3yr in clomiphene-estradiol group and 1.9±1.4yr in letrozle group with (P=0.3) with no significant difference among groups

This agreement with study conducted by Harira (2018) who compared letrozole versus clomiphene-estradiol for treating unexplained infertility women which not responding to clomiphene only. the Mean patient age was 24.6 ± 3.8 yr in clomiphene-estradiol group and 23.2 ± 4.1 yr in letrozole group and Mean patient BMI was 24.52 ± 4.02 Kg/m2 in clomiphene-estradiol group and 25.06 ± 3.6 Kg/m2 in letrozole group and that mean duration of infertility (years) distributed as 3.051 ± 1.32yr in clomiphene-estradiol group and 3.71 ±1.21yr in letrozle group with (P=0.13) . There were no significant differences between studied groups as regard Age, BMI and infertility duration.

This study showed that Type of infertility distributed as percentage of Primary infertility was 54.3% and percentage of secondary infertility was 45.7% in clomiphene-estradiol group and percentage of Primary infertility was 62.9% and percentage of secondary infertility was 37.1% in group of letrozole with non significant difference among patients.

A similar study conducted by Seyedoshohadaei et al. (2016) who studied the effect of clomiphene- Estradiol Valerate versuss Letrozole on Endometrial Thickness, Abortion and Pregnancy Rate in PCOS Infertile Women reported that percentage of Primary infertility was 60% and percentage of secondary infertility was 40% in clomiphene-estradiol group and percentage of Primary infertility was 72% and percentage of secondary infertility was 28 % in group of letrozole with non significant difference among patients

This study showed that there was non statistically significant difference between the studied groups as regard basal hormonal profile (TSH and prolactin) (P=0.42), (P=0.63) respectively .These results agreement with study conducted by Harira (2018)who reported that there was non statistical significant difference between the studied groups as regard basal hormonal profile (TSH and prolactin) (P=0.27) (P=0.74) respectively .

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This study showed that there was a high statistically significant difference between the two groups as regard thickness of endometrial on day of HCG injection was 8.8±1.2mm in clomiphene-estradiol group and 9.7±1.1mm in letrozole group with (P=0.001) . thickness of endometrial was higher in Letrozole group than Clomiphene plus Estradiol Valerate group. But regarding basal endometrial thickness (P=0.3) and stimulation days (P=0.07) and mature follicles (P=0.16), there was no statistical significant difference between the studied patients.

Similar finding showed by Harira (2018) who reported that thickness of endometrial was increased after Estradiol Valerate administration plus CC from (5.44 ± 1.64mm) to (8.28 ± 1.7mm) and Letrozole only from (5.75±1.92mm) to (9.2±1.8mm) with a significant difference between the studied patients (P=0.00). Endometrial thickness was higher in Letrozole group than Estradiol Valerate plus CC. But regarding basal endometrial thickness (P=0.55) and stimulation days (P=0.11) and mature follicles (P=0.39), there was no statistical significant difference between the studied patients.

Seyedoshohadaei et al. (2016) reported that the administration of Clomiphene with Estradiol Valerate group improved endometrial thickness from (5.34 ± 1.98mm) to (7.26±1.76mm) and in letrozole group from (5.68±1.98mm) to (8.17±2.08mm) with significant difference between the two groups (P=0.021). The endometrial thickness in Letrozole patients was higher more than CC with Estradiol Valerate.

Hendawy et al. (2016) studied the enfluence of Letrozole plus Clomiphene citrate on ovulation induction and they revealed that Letrozole had a good effect on thickness of endometrial and rate of pregnancy than Clomiphene citrate.

Also Mitwally and Casper (2001) reported that Letrozole increased the thickness of endometrial due to administration of 2.5-mg orally dose for days 3–7 of cycles, to 12 patients with PCOS and 10patients with ovulatory infertility, whom previously received CC with an poor outcome (no ovulation and/or endometrial thickness of ≤0.5cm)..

In a study by Sharief and Nafee (2015) which studied 75 Iraqi women and reported that the mature follicles was significantly lower, but the thickness of endometrial and ovulation were higher in Letrazole patients than in Clomiphene citrate patients ($p<0.05$ each) .

Also, Atay et al. (2006) reported that the number of mature follicles were lower significantly in Letrozole patients; but thickness of endometrial, ovulation and pregnancy rates were higher significantly.

Study of Ghomian et al. (2015) reported no differences between Letrozole and Clomiphene

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citrate regarding endometrial thickness, mature follicles and length of follicular phase.

This study showed that there was a statistical significant difference between the studied patients as regard ovulation rate (P=0.03).

This study showed that there was a statistical significant difference between the studied patients regarding rate of pregnancy, which was (45.2%) in letrozole group than clomiphene plus estradiol group(22.9%) with (P=0.04). Which accordance to the study of Seyedoshohadaei et al. (2016) who reported that the pregnancies number were 24 patients (24%). The pregnancies number in letrozole group (32%) which higher than clomiphene-estradiol patients (16%) and there was a significant differences between studied patients regarding rate of pregnancy (P=0.05).

Different finding showed by Harira (2018) who reported that there was increase in pregnancy rate in Letrozole group and Clomiphene citrate group but without significant difference between studied patients (P=0.42).

This was different from the results of Nahid and Sirous (2012) who found that pregnancy rate in Letrozole plus CC in PCOS infertile female, was almost similar.

The study of Hendawy et al. (2016) found the rate of pregnancy in Letrozole patients was higher than Clomiphene citrate patients. Kar (2012) revealed that Letrozole has excellent rate of pregnancy rates more than Clomiphene citrate .

This study showed that there was no statistical significant difference between the studied patients regarding multiple pregnancy 1 case (2.9%) in clomiphene-estradiol group and 1 case (2.9 %) in letrozole group (P=0.1).These result agreement study by **Harira [8],** showed 2 cases (2.3%) in clomiphene-estradiol group and 0 case(0.00%) in letrozole group. There was no statistically significant difference between the two studied groups

Conclusion

Letrozole increase the thickness of endometrial and pregnancy rate more than the Estradiol Valerate plus Clomiphene citrate in the PCOS infertile women who had abnormal endometrial thickness with Clomiphene citrate, so it is recommended for PCOS infertile women .

No Conflict of Interest
No financial disclosure
-Acknowledgements
Not applicable

Declarations

-Ethics approval and consent to participate

Written informed consent was obtained from all participants and the study was approved by the research ethical committee of Faculty of Medicine, Zagazig University (International review board) ZU-IRB# 5057/19-12-2018

Protocol ID : ZU-IRB# 5057/19-12-2018 (March 2019 to September 2019)

ClinicalTrials.gov ID :NCT04619914 (March 2019 to September 2019)

-Consent for publication

Not applicable

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