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A Randomized Trial of Combined Letrozole with Metformin Versus Clomiphene Citrate with Metformin in Polycystic Ovary (PCOS) patients

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ABSTRACT

Background: Polycystic Ovary Syndrome (PCOS) is one of the most common causes of anovulatory infertility. Currently, clomiphene citrate (CC) is considered the first-line therapy for ovulation induction for women with PCOS and infertility. Aromatase inhibitors (AIs) have been introduced as a new treatment option that could challenge CC for ovulation induction. **Aim of the Work:** The present study was designed to compare the efficacy of combined aromatase inhibitor (Letrozole) with metformin versus CC with metformin in PCOS patients. **Patients and Methods:** This study was done on 100 documented PCOS cases. They were divided into two groups, The 1st group received CC 50 mg twice daily from the 3rd day of cycle for 5 days and repeated for 3 cycles with metformin 500 mg 3 times daily as an adjunct with CC and continued for 3 cycles. The 2nd group received aromatase inhibitor (Letrozole) 2.5 mg twice daily from 3rd day of cycle for 5 days and repeated for 3 cycles with metformin 500 mg 3 times daily as an adjunct with letrozole and continued for 3 cycles. These cases were followed up for three cycles by transvaginal ultrasound folliculometry to document ovulation (size and number of follicles). **Results:** The results of the present study revealed both lines of treatment were effective in treatment of PCOS patients, with slight favorability in letrozole group but without statistically significant difference founded between CC group and letrozole group as regard ovulation rate, number of follicles at the end of first, second or third cycles, or as regard the diameter of follicles, i.e., both regimens showed efficacy to the same extent. Conclusion: both CC and letrozole are equally effective in treatment of infertility in PCOS patients, when combined with metformin treatment.

Key Words: PCOS, clomiphene citrate, letrozole, metformin

INTRODUCTION

Infertility has been attributed to various factors, amongst which anovulation is the cause in about 40% of all female infertility. Polycystic Ovary Syndrome (PCOS) is one of the most common causes of anovulatory infertility, affects 4-7% of women [(1)].

Criteria used for diagnosing Polycystic Ovary Syndrome (PCOS) are the Rotterdam Criteria of which a woman must have two out of the followings: 1: Oligo- or anovulation. 2: Clinical and / or biochemical signs of hyperandrogenism 3: Polycystic ovaries (with the exclusion of related disorders).

With exclusion of other conditions with similar signs such as androgen-secreting tumors or Cushing's syndrome and thyroid dysfunction and hyperprolactinemia.

Anti estrogenic drug, such as Clomiphene citrate (CC) is considered the first line treatment for induction of ovulation in women with Polycystic Ovary Syndrome (PCOS). CC is given orally at a dose of 50-100 mg/day from 3rd day of cycle for 5 days. If patients fail to respond to 150 mg/day, they are considered as CC resistant.

Aromatase inhibitors (AIs) have been introduced as a new treatment option that could challenge CC for ovulation induction [(2)].

Aromatase is a cytochrome P-450 hem protein containing enzyme complex (the product of the CYP19 gene) that catalyzes the rate-limiting step in the production of estrogens which is the conversion of androstenedione and testosterone via three hydroxylation steps to estrone and estradiol.

Aromatase activity is present in many tissues such as the ovaries, adipose tissue, muscle, liver, breast tissue, and in malignant breast tumors. The main sources of circulating estrogens are the ovaries in premenopausal women and adipose tissue in postmenopausal women [(3)].

AIs can be applied for ovarian stimulation as its administration early in the follicular phase can induce ovulation by releasing the hypothalamus or pituitary from estrogen negative feedback on GnRH and gonadotropin secretion, leading to an increase in gonadotropin production which would stimulate ovarian follicular development [(4)]. AIs prevent the Androgen-Estrogen conversion and therefore interfere with the negative feedback at the level of the hypothalamus-pituitary. The increased pituitary gonadotropin output will in turn stimulate the ovaries [(5)]. In addition, they act locally in the ovary to increase follicular sensitivity to FSH. This may result from accumulation of intraovarian androgens, since conversion of androgen substrate to estrogen is blocked. Recent data support a stimulatory role for androgens in early follicular growth [(6)].

In some studies, letrozole in contrast to CC is better as it increases endometrial thickness by upregulation of estrogen receptors. So, it increases pregnancy rate and also it decreases incidence of multiple pregnancy [(5,7)].

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AIs reported to be effective in inducing ovulation, increased pregnancy rate, improve uterine environment, endometrial development with favorable cervical mucus [5].

Insulin-sensitizing agents for example (Metformin)

Metformin is an oral anti-diabetic drug from biguanides class used for treatment of type II diabetes mellitus. It is a safe and effective drug that is used for the treatment of PCOS patients [8]. Metformin improves peripheral insulin sensitivity by reducing hepatic glucose production and increasing target tissue sensitivity to insulin. It also decreases androgens in both lean and obese women, leading to increased rates of spontaneous ovulation [9].

Metformin may be used alone or in concert with other medications such as clomiphene citrate, it has been shown to increase the ovulatory response to clomiphene citrate in patients who were previously clomiphene-resistant.

PATIENTS AND METHODS

1- Patients:

This prospective study was done on 100 infertile women from the Infertility Outpatient Clinic of Qena General Hospital. The study was approved by the Ethics Board of Al-Azhar University.

Inclusion Criteria: A. 18-35 years old women that had been already diagnosed as primary infertility due to PCOS according to Rotterdam consensus criteria. B. Women with BM I between 18 & 30. C. Uterus is normal and tubes are patent by hystero-salpingography. D. Normal serum prolactin level (5-20 ng/ml). E. Normal semen analysis of the husband.

Exclusion Criteria: Uterine and adnexal pathology e.g. leiomyomata. Ovarian cysts > 6cm. Hyper- or Hypothyroidism. Previous surgery related to genital tract as per history. Impaired hepatic /renal function. Diabetes mellitus. Hyperprolatinaemia.

Drugs likely to interfere with ovulation especially chronic use of non-steroidal anti-inflammatory drugs, hormonal or chemotherapeutic agents.

2- Methods:

For all women, the following was done:

a) Verbal consent after explanation of nature of study. b) Complete history: At the first visit, detailed history was taken. It included; Personal history: age, marital status, special habits, occupation and duration of marriage. Menstrual history: menarche, menstrual cycle (length and duration), dysmenorrhea, mid cycle pain or discharge, intermenstrual bleeding and date of last menstrual period. History of chronic diseases: including diabetes mellitus and hypertension symptoms and signs suggestive of endocrine disorders. Surgical history: that includes laparoscopy e.g. (ovarian drilling) and laparotomy. Sexual history: frequency of intercourse and coitus problems. Women were also asked about previous investigations that had been done for infertility and any previous treatment that has been given for induction of ovulation.

c) Clinical examination: General examination, for signs of endocrine disorders. Breast examination for galactorrhea. Abdominal examination. Pelvic examination for uterine size and mobility, tenderness, uterine or adnexal masses.

d) Investigation: 1) Semen analysis. 2) Serum FSH and LH. 3) Serum prolactin. 4) Thyroid function tests. 5) Pelvic ultrasound was performed

After enrollment, participants were randomly divided into two groups by using identical sealed envelopes technique into:

1- First group: included 50 women that took clomiphene citrate (Clomid; Aventis pharma S.A.E, Global Napi pharmaceuticals,Cairo, Egypt) 50mg twice daily from 3rd day of cycle for 5 days and repeated for 3 cycles with metformin, (cidophage; chemical industries development C I D, Cairo, Egypt), 500 mg three times daily as an adjunct with CC and continued for 3 cycles for all women.

2- Second group: included 50 women that took letrozole (Femara; Novartis pharma AG, Basle, switzerland) 2.5mg twice daily from 3rd day of cycle for 5 days and repeated for 3 cycles with metformin, (cidophage; chemical industries development C I D, Cairo, Egypt), 500 mg three times daily as an adjunct with letrozole and continued for 3 cycles for all women.

Follow up:

These cases were followed up for 3 cycles by transvaginal ultrasound folliculometry to document ovulation, Folliculometry was done on day 11, 13 and day 15 of the cycle to detect number of growing follicles and size of follicles. Follicles measure more than 18mm were considered mature follicles.

Also measurement of endometrial thickness in (mm) at the greatest diameter perpendicular to the midsagittal plane in the fundal region including both layers of the endometrial cavity.

HCG (10.000 IU) was given IM to trigger ovulation when at least one mature follicle of 18 mm or more was detected.

Patient was advised to have intercourse 24 to 36 hours after HCG injection.

Patients with no follicles or less than 15 mm were not considered as non responders and followed up in next cycles

Serum β-HCG was measured if the menses was delayed for one week for diagnosis of pregnancy chemically then followed up by TVS to detect gestational sac at (4 weeks of gestation) and fetal pulsation at (7 weeks of gestation).

Ultrasound after 48 hours of HCG administration to confirm ovulation

RESULTS

In the present study we had 100 patients equally divided into two groups. Each group included 50 patients, the 1st group is CC with metformin group and the 2nd group is letrozole with metformin group as previously mentioned.

In the study, there was no statistically significant difference between CC group and letrozole group as regards age or duration of infertility (mean age was 27.43 ± 2.91 years and mean duration of infertility was 4.80 ± 1.90 years in CC group and mean age was 28.4 ± 3.1years and mean duration of infertility was 4.90 ± 1.80 years in letrozole group). As regards BMI mean BMI was 21.1 ± 2.80 in CC group and mean BMI was 22.3 ± 1.90 in letrozole group

Table (1): Comparison between CC and letrozole groups as regards age and duration of infertility and BMI.

Item	CC N=50		Letrozole N=50		p Value
	Mean	SD	Mean	SD	
Age	27.43	2.91	28.4	3.1	0.218 (NS)
Duration of infertility in years	4.80	1.90	4.9	1.8	0.835 (NS)
BMI	22.1	2.8	22.3	1.9	0.220 (NS)

In the present study, there was no statistically significant difference between CC group and letrozole group as regard pregnancy rate

There is only 2 cases get pregnant in CC group which represent (4%) of the group and only 3 cases in Letrozole group which represent (6%). All cases that got pregnant showed both chemical and clinical pregnancy.

Table (2): Comparison between CC and letrozole groups as regards pregnancy rate.

	CC N=50	Letrozole N=50	P Value
Pregnancy rate	2 (4.0%)	3(6.0%)	0.592 (NS)

As regards to effect of treatment on ovulation, CC reported 27 cases (54.0%) ovulation at the first cycle after treatment, increased to 35 cases (70.0%) at the second cycle and to 45 cases (90.0%) at the third cycle.

On the other hand, letrozole reported 30 cases (60.0%) ovulation at first cycle after treatment, which increased to 40 cases (80.0%) at the second cycle and 43 cases (86.0%) at the end of the third cycle. Similarly, CC achieved a cumulative rate of 71.33% while letrazole achieved 75.33%. In conclusion both drugs affected ovulation nearly to the same extent (P >0.05).

Table (3): Effect of treatment on ovulation in studied groups.

Ovulation	CC N=50	Letrozole N=50	P value
Ovulation after one month treatment	27 (54.0%)	30 (60.0%)	0.780 (NS)
Ovulation after two months treatment	35 (70.0%)	40 (80.0%)	0.661 (NS)
Ovulation after three months treatment	45 (90.0%)	43 (86.0%)	0.762 (NS)
Cumulative ovulation cycles	107/150 (71.3%)	113/150 (75.3%)	.500 (NS)

As regard mean number of follicles in CC group, it was reported to be 1.25 ± 0.58 at first cycle, elevated to be 1.32 ± 0.95 at the second cycle, and to 1.44 ± 0.70 at the third cycle. In letrozole group, mean number of follicles was 1.36 ± 0.61 at the first cycle, elevated to 1.39 ± 0.99 at the second cycle and finally to 1.43±0.83 at the third cycles. No statistically significant difference was observed between both groups at the first, second or third cycles, i.e. both drugs increased number of follicles nearly to the same extent.

Table (4): Effect of treatment on the number of follicles in studied groups.

Item	CC N=50			Letrozole N=50			Statistics
	Mean	SD	No	Mean	SD	No	P Value
No. of follicles in the 1st month	1.25	0.58	26	1.36	0.61	30	0.594 (NS)
No. of follicles in the 2nd month	1.32	0.95	35	1.39	0.99	40	0.799 (NS)
No. of follicles in the 3rd month	1.44	0.70	45	1.43	0.83	45	0.946 (NS)

As regard mean follicular diameter in CC group, it was 18.41 ± 1.82 mm, 17.61 ± 2.10 mm and 19.36 ± 3.32 mm at the first, second and third cycles respectively, while in letrozole group, it was 17.8 ± 1.91 mm, 17.9±2.3 mm and 19.2±3.4 mm at first, second and third cycles respectively and there was no statistically significant difference between both groups at first, second or third month.

Table (5): Effect of treatment on the mean follicular diameter (MFD) in studied groups.

Item	CC N=50			Letrozole N=50			Statistics
	Mean	SD	No	Mean	SD	No	P value
Mean follicular diameter in the 1st month	18.41	1.82	26	17.8	1.91	30	0.349 (NS)
Mean follicular diameter in the 2nd month	17.61	2.10	34	17.9	2.3	40	0.658 (NS)
Mean follicular diameter in the 3rd month	19.36	3.32	46	19.2	3.4	45	0.861 (NS)

As regards number of follicles >18mm, there was no statistically significant difference between studied groups at the first, second or third cycles.

Table (6): Effect of treatment on the number of follicles >18mm.

Item	CC N=50			Letrozole N=50			Statistics
	Mean	SD	No	Mean	SD	No	P value
No. Fol.>18mm in the 1st month	1.00	0.52	26	1.1	0.55	30	0.590 (NS)
No. Fol.>18mm in the 2nd month	0.91	0.53	35	1	0.59	40	0.591(NS)
No. Fol.>18mm in the 3rd month	1.26	0.45	45	1.12	0.51	45	0.295 (NS)

No statistically significant difference was found between CC group and letrozole group per three cycles as regard the final situation of P4, cumulative number of follicles, cumulative number of mean follicular diameter, and endometrial thickness, i.e., the results proved that, both drugs had nearly similar effects. There were no complications detected in both groups (no ovarian hyperstimulation syndrome).

Table (7): Effect of treatment on, cumulative number of follicles, mean follicular diameter, and endometrial thickness between the study groups.

Item	CC			Letrozole			Statistics
	Mean	SD	No	Mean	SD	No	P
Cum. no. of follicles	1.42	0.75	106/150	1.51	0.82	115/90	0.512
Cum. mean follicular diameter	18.53	2.70	106/150	17.9	2.9	115/150	0.197
Cum. no. of Fol .>18mm	1.08	0.51	106/150	1.1	0.6	115/150	0.813
Cum. Endometrial thickness	9.6	1.37	104/150	10	1.31	114/150	0.089

DISCUSSION

PCOS is a common endocrine disorder that primarily affects women of reproductive age, with prevalence rates ranging from 5% to 10% [(10)].

In 2013, a consensus panel established a controversial definition (the Rotterdam criteria) for PCOS, to include at least 2 of the following criteria: Oligo- or anovulation (menses less than once every 35 days), Hyperandrogenism (laboratory-confirmed or clinical symptoms), or Polycystic ovaries on ultrasound.

With exclusion of other conditions with similar signs such as androgen-secreting tumors or Cushing's syndrome and thyroid dysfunction and hyperprolactinemia [(11)].

Given these endocrine abnormalities, infertility is a common complication of PCOS. Studies have reported PCOS as the major cause of infertility in up to 20% of couples [(12)].

Clomiphene citrate has been considered first-line therapy for ovulation induction for women with PCOS and infertility [(11)].

Third-generation aromatase inhibitors (anastrozole, letrozole) have ovulation-inducing effects by inhibiting androgen-to-estrogen conversion. Centrally, this effect releases the hypothalamic/pituitary axis from estrogenic negative feedback, increases gonadotropin secretion, and results in stimulation of ovarian follicle maturity. Moreover, peripherally, aromatase inhibitors may increase follicular sensitivity to follicle-stimulating hormone (FSH). These aromatase inhibitors have relatively short half-lives (~2 days), so estrogen target tissues (e.g., endometrium) are spared adverse effects [(13)].

Because of these mechanisms, it is postulated that aromatase inhibitors may have superior ovulation induction properties in terms of follicular growth and endometrium development, which is important for embryo implantation [(14)].

The results of the present study revealed that all cases were homogenous in age, duration of infertility and BMI and showed that the pregnancy rate which is the secondary outcome of the study was more favorable in letrozole group than CC group as it represent only (4%) in CC group and (6%) in letrozole group but without statistically significant difference between both group.

As regards ovulation rate which is the primary outcome of the study CC group showed ovulation rate (54%, 70% and 90%) of cases in (1st, 2nd and 3rd) cycles respectively and letrozole group showed ovulation rate (60%,80% and 86%) of cases in (1st,2nd and 3rd)cycles respectively, with no statistically significant difference between both groups (P >0.05).

As regards total number of follicles and number of follicles >18mm there was slight increase in 2nd and 3rd cycle without statistically significant difference between both groups.

As regards mean follicular diameter, both regimens showed efficacy to the same extent with no statistically significant difference between both groups.

The present study showed cumulative endometrial thickness more favorable in letrozole group than CC group which was (9.2 ± 1.37) in CC group and (10 ± 1.31) in letrozole group. But without statistically significant difference between both groups.

The following are some studies which agreed with the results of our study. The study of Abo ***Hashim et al.***[15] which was done on 250 anovulatory women (582 cycles) with CC resistant PCOS. The patients received 2.5 mg of letrozole daily (123 patients, 285 cycles) or combined metformin-CC (127 patients, 297 cycles) for 3 treatment cycles. No statistically significant difference regarding the pregnancy rate was observed between both groups.

The study of ***Badawy and colleagues***[16] who studied 438 infertile women (1063 cycles) with PCOS. Patients were randomized to treatment with 5 mg of letrozole daily (218 patients, 540 cycles) or 100 mg of CC daily (220 patients, 523 cycles). In this study, advantage to the use of letrozole over CC as a first-line treatment for induction of ovulation in women with PCOS was not observed as significant differences in ovulatory cycles, pregnancy rates or miscarriage rates were not found. In contrast to previous studies, endometrial thickness at the time of HCG administration was significantly greater in the CC group (9.2 versus 8.1 mm).

The study of ***Hu et al.*** [17], in this clinical trial, 107 infertile patients with PCOS received 100mg CC (n=57) or 5mg letrozole (n=50) daily since day 3-7 of their menstrual cycles. The results showed that the number and the size of mature follicles were similar between both groups and the pregnancy rate in letrozole group was higher than CC group (20% versus 14%) but the difference was not significant (P=0.286).In letrozole group, 86% of patients developed mature follicles, whereas 72% of patients in CC (P=0.07) which was not significant.

The study of ***Kar***[18] in which 103 infertile PCOS women were randomized to treatment with 5mg letrozole (51 patients) or 100mg CC (52 patients) daily starting day 2 to day 6 of menstrual cycle. Timed intercourse or intra uterine insemination (IUI) was advised 24 to 36 hours after (HCG) injection. The ovulation rate was 73.08% in letrozole group and 60.78% in CC group, which was not statistically significant (P=0.398). There was no statistically significant difference between endometrial thickness (CC 7.61 ± 1.96, letrozole 7.65 ± 2.10) days to ovulation (CC 14.2 ± 3.41, letrozole 13.13±2.99) and monofolliculogenesis (CC 54.84 %, letrozole 79, 49 % P=0.027) and pregnancy rate was (CC 7.84%, letrozole 21.56% P=0.0125).

The study of ***Angel et al.***[19] which done on 50 women with ovulatory dysfunction as a cause of infertility were enrolled and randomized into 2 groups of 25 each. Group 1 received CC in incremental dose from 50mg up to 150mg and group 2 patients received letrozole in incremental dose from 2.5mg to 7.5mg, depending upon the ovulation response. The results showed no statistically significant difference between both groups as regards the number of follicles (1.89±0.9 vs. 1.18 ± 0.393) while follicular diameter (20.67 ± 0.970 mm vs. 20.76 ± 0.903 mm) and endometrial thickness (8.5mm vs.7.4mm).

On the other hand there were some studies that showed different results for example:

The study of ***Roy et al.***[20] which was prospective randomized clinical trial included 204 PCOS patients divided into 2 groups 98 patients (294 cycles) received 2.5- 5 mg letrozole, 106 patients (318 cycles) received 50-100 mg CC both orally from day 3-7 of menstrual cycle and the treatment continued for 3 cycles in both groups. The pregnancy rate was more favorable in letrozole group that was (43.8%) 43 patients and (26%) 28 patients in CC group. And showed also endometrial thickness 9.1 ± 0.3 mm in letrozole group and 6.3 ± 1.1 mm in CC group (P=0.014) these differences between the results of this study and our results may be due to the large number of cases participated in this study.

The study of ***Sohrabvand et al.***[21] in which 59 infertile women with PCOS were randomly divided into metformin-letrozole (29 patients) and metformin –CC (30patients). After an initial 6-8 weeks of metformin, they received either letrozole 2.5mg or CC (100mg) from day 3-7 of their menstrual cycle. Moreover, the results showed that the mean number of mature follicles> 18mm and ovulation rate and endometrial thickness was significantly higher in letrozole group. The pregnancy rate in letrozole group was 10 patients (34.5%) as compared with CC group 5 patients (16.67%). Whereas full term pregnancies were higher in letrozole group 10 patients (34.5%) versus 3 patients (10%) in CC group. Over all this study showed a good response with letrozole more than CC, which may be due to the period of

metformin administration (6-8 weeks) before the start of treatment.

In one trial, ***Rahmani and colleagues***[22] evaluated the efficacy of high-dose letrozole in 64 women with PCOS. If ovulation did not occur when women took CC 100 mg/day for 2 consecutive cycles, they were randomized to receive high-dose letrozole 7.5 mg/day or high-dose CC 150 mg/day. Similar to previous studies, letrozole therapy resulted in significantly greater endometrium thickness compared with clomiphene citrate (10.37 ± 1.2 vs. 9.03 ± 0.89 mm; $p < 0.001$). Although ovulation occurred in a higher percentage of letrozole-treated patients (62.5% in letrozole vs. 37.5% in CC; $p < 0.05$), pregnancy rates were significantly different (40.62% in letrozole vs. 18.75% in CC; $p > 0.05$). These different results may be due to the high dose used in both regimens.

The study of ***Rahmani et al.***[22] was a double blind, multicenter trial, in which 750 PCOS patients randomly assigned in a 1:1 ratio, to receive letrozole or CC for up to 5 treatment cycles. The results showed that women who received letrozole had more cumulative live births than those who received CC (103 of 374{27.5%} versus 72 of 376{19.1%} P=0.007). The cumulative ovulation rate was higher with letrozole than with CC (834 of 1352 cycles {61.7%} versus 688 of 1352 cycles {48.3%}, P< 0.001). Twin pregnancy was higher in CC group (7.4% in CC group versus 3.4% in letrozole group). There were no significant differences between both groups in pregnancy loss (49 of 154 pregnancies in letrozole group {31.8%} and 30 of 103 pregnancies in CC group {29.1%}).

These differences in this study may be due to the large number of patients participated in this study and the long duration of treatment which represent 5 treatment cycles.

The study of ***Roque et al.***[23] was 7 prospective RCTs comparing the use of letrozole to CC in PCOS patients. It included 1833 patients (906 in letrozole group and 927 in CC group) Five of included studies reported data on live birth and pregnancy rate which showed statistically significant increase in the letrozole group when compared to the CC group. In addition, these differences may be due to the huge number of patients used in these studies.

CONCLUSION

The present study showed that both CC and letrozole are equally effective in treatment of infertility in PCOS patients, when combined with metformin treatment. No statistically significant difference was found between CC group and letrozole group as regards pregnancy rate, ovulation rate, number of follicles in the end of first, second or third cycles, or as regards the diameter of follicles, both regimens showed efficacy to the same extent.

Limitation of the study:

The small number of patients participated in the study. The short duration of treatment. The doses of drugs used in the study.

Therefore, it is advised to design future prospective studies on women with PCOS with larger number of patients and with longer duration of treatment for 6 cycles or more and with higher doses of treatment which may give different results.

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