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Extended Use of Clomiphene Citrate in Induction of Ovulation in Polycystic Ovary Syndrome with Clomiphene Citrate Resistance

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ABSTRACT

Background: Clomiphene citrate (CC) is still holding its place as the first-line treatment for induction of ovulation in polycystic ovary syndrome (PCOS) patients.

Objective: to assess the effect of extended clomiphene citrate on pregnancy outcomes of clomiphene resistant PCOS.

Patients and methods: this study was conducted on 48 PCO patients with CC resistance attended to the Obstetrics and Gynecology Outpatient Clinic at Menoufia University and Al-Bajour General Hospitals, from April 2018 to February 2020. Patients were treated with an extended CC administration (150 mg for 10 days) to overcome anovulation.

Result: There was highly significant change in follicle-stimulating hormone (FSH), luteinizing hormone (LH) and FSH/LH after extended CC treatment ($p<0.001$). Also, serum progesterone and number of follicles were significantly increased after treatment compared to before treatment.

Conclusion: CC extended is a safe, effective with minimal side effect, but low pregnancy rate was observed. Therefore, further studies with large number of PCO patients with CC resistant with addition of other drugs are required to investigate the improvement of the pregnancy rate.

Keywords: Clomiphene citrate resistance, Extended use, Rotterdam criteria

INTRODUCTION

PCOS is the cause of anovulatory infertility; in approximately 75% of the cases [(1)]. It occurs in approximately 4% or 18% of females on childbearing-aged around worldwide [(2)]. So, WHO type II ovulation occurs in 85% of anovulatory patients [(3)]. Women with PCOS should also be evaluated for reproductive function, hirsutism, alopecia, and acne. CC is still holding its place as the first-line of treatment for induction of ovulation in these patients [(4-7)]. CC is able to induce a secretion of follicle stimulating hormone (FSH) from the anterior pituitary and is often enough to reset the cycle of events leading to ovulation. This is achieved through CC action, a non-steroidal compound very similar to estrogen in blocking hypothalamic receptors of estrogen, indication of circulating estrogen deficiency in the hypothalamus and inducing in pulsatile pattern changes release of gonadotropin hormone-releasing hormone (GnRH) [(8)].

Standard practice is to administer CC for 5 days from the second or third day of the menstrual cycle, starting with 50 mg/day and increasing up to 250 mg. However, other studies found the most dosage affective was 100–150 mg/day. After 6 to 9 cycles of treatment with CC cumulative reach 70–75% [(9)]. Another study found that extended of CC is safe, effective with minimal side effect [(10)]. CC resistance defined as failure of ovulation after receiving 150 mg of CC/day for 5 days per cycle, for at least three cycles, is common and occurs in 15-40% in PCOS patients [(11)].

Failure of CC to induce ovulation is unpredicted. This result showed, above all, an inexplicable event. Some studies showed that it is more likely in patients who are obese, insulin resistant and hyperandrogenic to have a genetic predisposition [(12)]. Nevertheless, it is virtually impossible to predict who will respond to which dose of CC, if at all. Also, there was no general agreement on standard CC resistant management. However, these may be costly and sometimes risky. Addition of adjuvants to CC such as N-acetyl cysteine, metformin and glucocorticoids may give a hope for better ovarian response [(13, 14)].

The aim of our study was to assess the effect of extended clomiphene citrate on pregnancy outcomes of clomiphene resistant PCOS.

PATIENTS AND METHODS

A prospective study was conducted on 48 PCOS patients with CC resistance and diagnosed according Rotterdam criteria 2003. They received clomiphene citrate (CC) with maximum dose (150 mg) for 5 days starting from 2nd day of cycle with no evidence of ovulation in the next 3 months preceding recruitment. Patients took 150 mg of CC (Clomiphene Citrate®; Hoechst Marion Russel, Cairo, Egypt) for ten days

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starting from 2nd day of the spontaneous or progesterone withdrawn cycles for (3) cycles.

All patients attended the Obstetrics and Gynecology Outpatient Clinic at Menoufia University and Al-Bajour General Hospitals, from April 2018 to February 2020.

Ethical consideration:

All procedures were carried out in accordance with ethical considerations of the institutional committee.

The study received the approval of Ethical Committee of Faculty Medicine, Menoufia University (No. 12/2018OBSGN). The aim and steps of the study were explained to the participants and written informed consent were obtained from them.

Inclusion Criteria

Infertility with PCOS and history of CC resistant cases diagnosed as failure to ovulate after receiving 150 mg of CC daily for 5 days per cycle for at least three cycles.

Exclusion criteria

Non-ovarian causes of infertility, combined factors of infertility and ovarian cysts or ovarian lumps.

All patients included in the study were subjected to the following:

Complete history: Personal history including name, age, education, occupation, residence, parity, special habits, and present history including symptoms which are usually associated with PCOS and obstetric history (number of prior pregnancies, deliveries and abortions).

Physical examination included anthropometric parameters (height, weight and body mass index, measurement of blood pressure (BP), pulse and temperature, chest and heart examination, measurement of endometrial thickness and vaginal ultrasound examination.

Investigations:

Transvaginal ultrasound (U/S) showing 12 or more peripheral small cysts 2-9 mm in diameter or total ovarian volume of > 10 mm 3, day 3 serum follicle-stimulating hormone (FSH), day 3 serum luteinizing hormone (LH), The ratio of LH to FSH is greater than 1:1 (sometimes more than 3:1), as tested on day 3 of cycle [(15)].

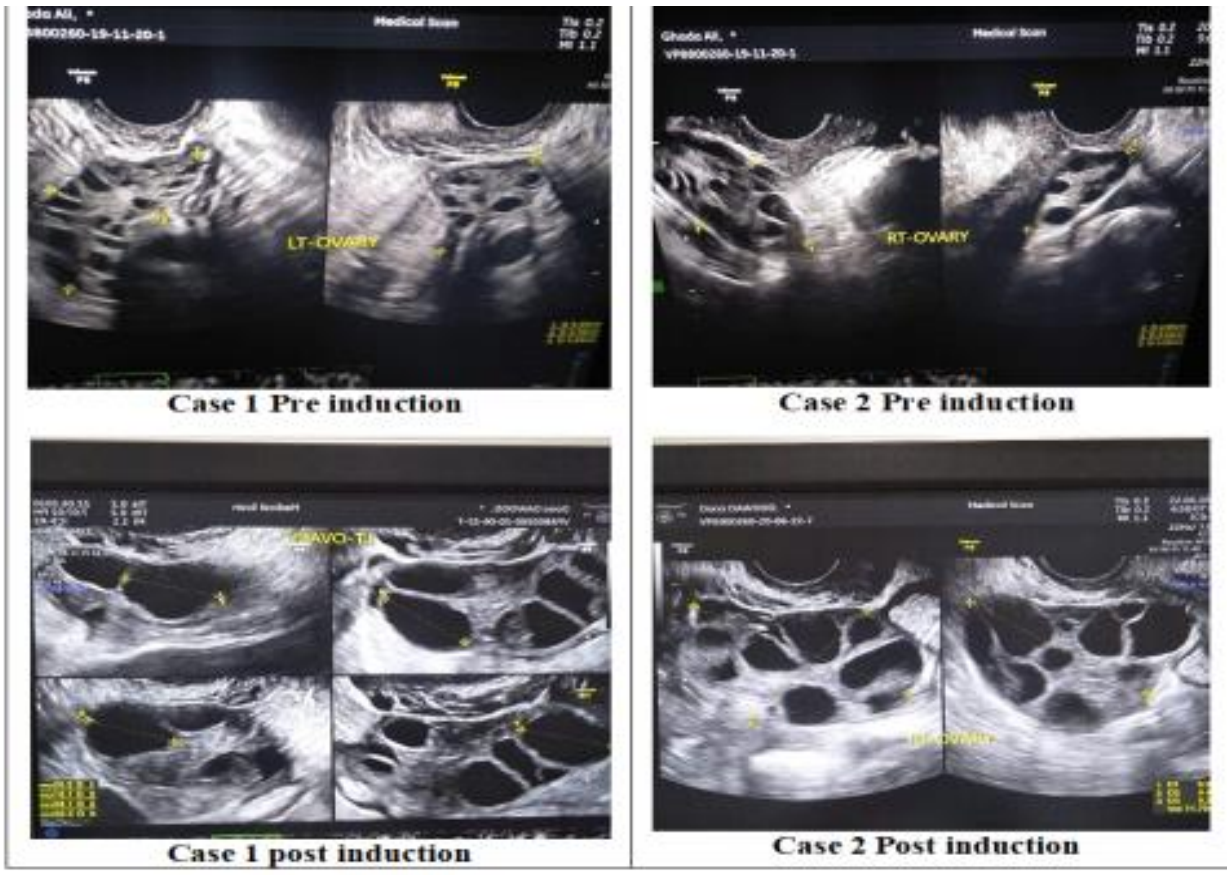
Serum estradiol (pg/ml) was measured at the time of human chorionic gonadotrophin (HCG) injection by radioimmunoassay using dual direct antibody sets. Progesterone was measured on midluteal of the cycle by radioimmunoassay. Serum HCG was determined 2 weeks after HCG injection in the absence of menstruation for diagnosis of pregnancy. Pregnancy was considered when serum HCG concentration was 50 mIU/ml or more. Miscarriage was considered when spontaneous termination of pregnancy occurred before 20 weeks' gestation.

Primary outcome: changes in hormonal profile; (progesterone, glucocorticoids including cortisol, number of growing and follicles growth up to 18 mm size and day 21 progesterone more than 12 ng/m., serum estradiol (pg/ml) and endometrial thickness.

Secondary outcome:

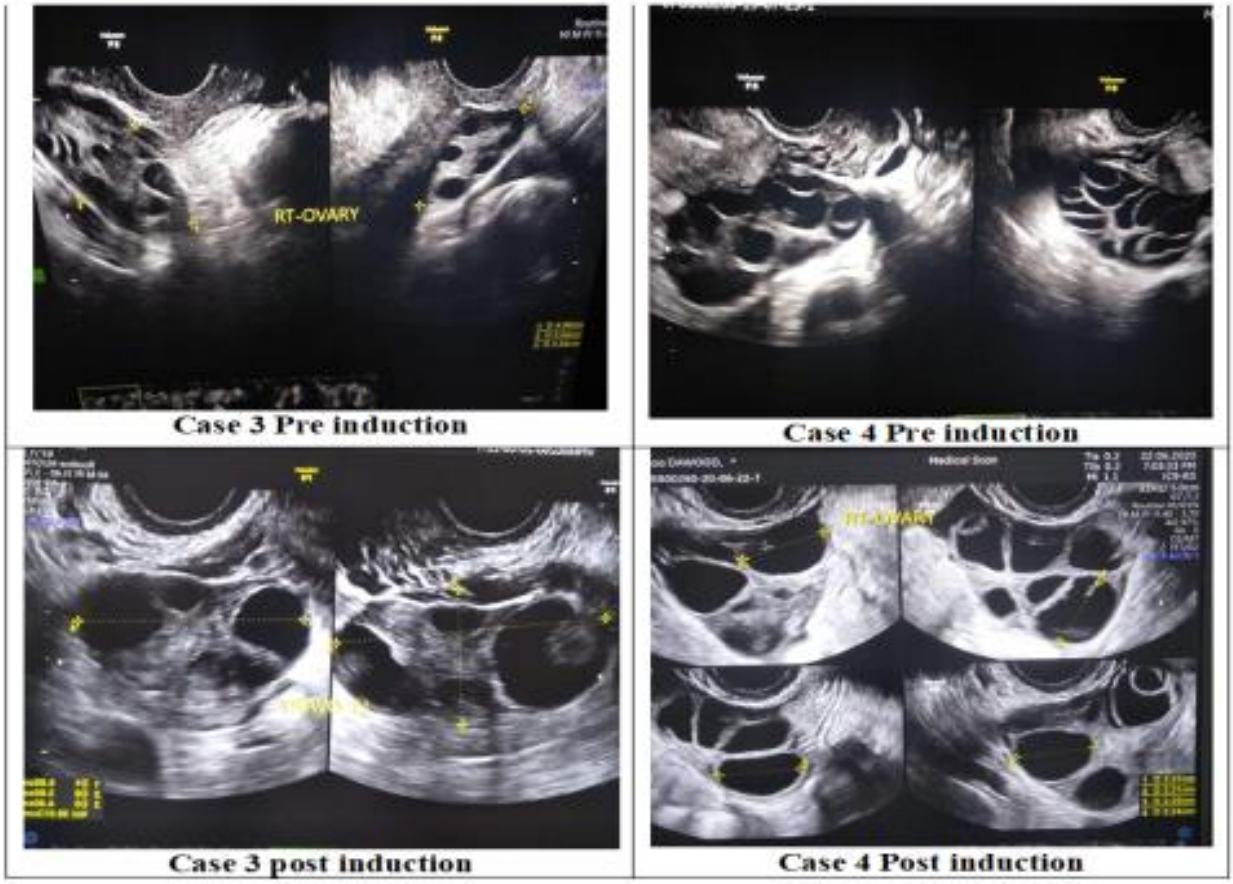
Occurrence of biochemical pregnancy defined by presence of gestational sac with or without pulsating fetal pole. All patients who completed the follow-up period also as the outcome analysis.

Sample size was calculated at 95% confidence interval. Size of about 300 cycles was required to give a test of significance of 0.05 and a power of 0.8.



Case 1 Pre induction | Case 2 Pre induction

Case 1 post induction | Case 2 Post induction



Case 3 Pre induction | Case 4 Pre induction

Case 3 post induction | Case 4 Post induction

Cases (1-3): pre and post induction

Statistical Analysis

The collected data were revised, organized, tabulated and statistically analyzed using statistical package for the social sciences (SPSS) version 22.0 for windows. Data are presented as the Mean ± standard deviation (SD), frequency, and percentage. Continuous normally distributed data were compared by the Student t test (two-tailed). The level of significance was accepted if the P value < 0.05.

RESULTS

The characteristics of the studied PCOS patients are shown in table 1.

Table (1): Distribution of the studied patients regarding their characteristics

Sociodemographic characteristics	PCO patients with clomiphene citrate resistant (N=48)
Age (year)	
Range (year)	25-35
Mean ± SD	29.07±6.20
Infertility: Primary Secondary	 20 (41.67) 28 (58.33)
Body mass index (kg/m^2)	
Range	28-36
Mean ± SD	33.45±7.94
Duration of infertility/year	
Range (year)	2-5
Mean ± SD	3.18±0.92
FSH (IU/L)	
Range	4.5-6.50
Mean ± SD	5.41±0.77
LH (IU/L)	
Range	8.50-11.11
Mean ± SD	10.03±2.91
LH/FSH	
Range	1.89-1.71
Mean ± SD	1.85±0.07

PCO: polycystic ovarian syndrome, Follicle-stimulating hormone (FSH)
LH: Luteinizing hormone, SD: standard deviation

Serum progesterone level among the studied patients ranged from 0 to 5 ng/mL, while mean number of follicles was 3.85±0.95. Regarding estradiol, it ranged from 810-30. Also, 18.75% of PCO patients with CC resistant had regular cycles (Table 2).

Table (2): Serum progesterone level, number of follicles, estradiol, regular cycles, oligomenorrhea, and amenorrhea among PCO patients with CC resistant.

	PCO patients with clomiphene citrate resistant (N=48)	
Serum progesterone (ng/mL)		
Mean±SD	**3.60±0.70**	
Number of primordial follicles		
Mean±SD	3.85±0.95	
Estradiol		
Mean±SD	19.56±2.84	
	No.	**%**
Regular cycles	9	18.75
Oligomenorrhea	27	56.25
Amenorrhea	12	25.00

PCO: polycystic ovarian syndrome **SD:** Stander deviation

There was statistically highly significant difference in FSH, LH and FSH/LH after clomiphene citrate treatment (Table 3).

Table (3): Comparison of FSH, LH and FSH/LH before and after extended clomiphene citrate treatment in the studied women.

	Clomiphene citrate treatment		Paired T-test	
	Before	**After**	**t**	**P value**
FSH (IU/L) Mean±SD	5.45±1.1	8.50±2.40	21.58	<0.001**
LH (IU/L) Mean ± SD	9.13±2.67	4.89±1.60	38.56	<0.001**
LH/FSH Mean ± SD	1.68±0.07	0.58±0.04	14.67	<0.001**

Follicle-stimulating hormone (FSH), Luteinizing hormone (LH) **SD:** standard deviation ****:** high significant differences

Serum progesterone and number of follicles increased significantly after treatment (Table 4).

Table (4): Comparison between the studied women regarding change in serum progesterone, number of follicles and pregnancy rate before and after extended clomiphene citrate treatment.

	Clomiphene citrate treatment		Paired T-test	
	Traditional dose	**Extended dose**	**t**	**P value**
Serum progesterone (ng/ml) Mean ± SD	7.58±1.28	16.93±3.51	37.62	<0.001*
Number of follicles				
Mature follicles Mean ± SD	1.88±0.05	8.72±1.67	4.21	0.030*
Primordial follicles Mean ± SD	5.20±1.82	7.20±1.67	1.45	0.67
	No (%)	**No (%)**		
Pregnancy				
Pregnancy rate	0(0.0%)	3(6.25%)		
Multiply Pregnancy	0(0.0%)	2(4.17%)	NA	---
Ovarian Hyperstimulation				
Mild	0(0.0%)	3(6.25%)		
Moderate	0(0.0%)	2(4.17%)	FET=	1
Severe	0(0.0%)	0(0.0%)	0.006	

SD: standard deviation t: paired t-test FET: Fisher's exact test
*: significant differences NS: non-significant NA: non-comparable

DISCUSSION

In the current study, age of the studied PCOS patients with CC resistant ranged from 25 to 35 years with mean of 29.07±6.20 years, 58.33% of women had secondary infertility while, 41.67% had primary infertility. Also, body mass index (BMI) ranged from 28-36 kg/m^{2}with mean 33.45±7.94 kg/m^2. Our results agreed with **Overbeek *et al.* (16)** who evaluated the role for FSHR receptor polymorphism on position 680 in CC treatment response for induction ovulation in patients with PCOS. They found that age of the studied PCO patients with CC resistant ranged from 20 to 36 years with mean 27.0+4.6 years. Body mass index (kg/m^2) ranged from 17.6–41.6 with mean of 26.3 kg/m^2. Also, **Palomba *et al.* (17)** studied the effect of CC on several ultrasound markers of uterine receptivity in PCOS patients who ovulated under treatment. They found that, the mean age of the studied patients was 25.8±2.8 years. Also, the mean body mass index was 27.2 ± 2.5 kg/m^2. Another study by **Saadia (18)** found that mean age of women was 25.6±7.4 years for the high BMI group and 24.8±5 years for the normal BMI group. The mean BMI was 30.3±4.4 kg/m^2 for the high BMI group and 21.5±1.8 kg/m^2 for the normal BMI group.

In the current study, 18.75% of PCO patients with CC resistant had regular cycles and 25% had amenorrhea, while, 56.25% of patients had oligomenorrhea. Also, number of follicles increased significantly after treatment. In addition, 3 (6.25%) of women had pregnancy rate and 2 (4.17%) had multiple pregnancy. Regarding ovarian hyperstimulation, there were 3 (6.25%) of women had mild hyperstimulation and 2 (4.17%) had moderate hyperstimulation (treated by observation bedrest, increase of oral fluid intake,

ultrasound follow-up, paracetamol when needed as well as anticoagulant to prevent blood clots). Studies with clomiphene citrate found rate of ovulation was 60–85% and rate of pregnancy was 30-40% [19], which was higher than our results. Our results also agreed with **Rashidi-Alavijeh *et al.*** [20] **who** reported the same rate of pregnancy in extended CC patients, while, **Isaacs *et al.*** [19] found high rate of pregnancy rate (46%). Also, **Badawy *et al.*** [21] found the extended CC led to in modest ovulation and rates pregnancy with no side effects, but the pregnancy rate was 11.4% in CC dose of 100 mg for 9 days, which was more than our results of 6.25%, these may be due to our small sample size. Another comparative study by **Rashidi-Alavijeh *et al.*** [20] demonstrated pregnancy rate of 6.52% in group A and 12.21% in group B with no multiple gestation and obvious hyperstimulation syndrome. Which came nearly with our results.

On contrast to our results, **Mathew *et al.*** [22] studied 67 women who completed 221 ovarian stimulation cycles. They found that general population take one dose of 150 IU along with CC to get an ovulation rate of 82% and pregnancy rate of 21%. These results differed from our results because their patients weren't resistant of CC.

In the current study, there was statistically highly significant difference in FSH, LH and FSH/LH after clomiphene citrate treatment. Our results agree with **Kousta *et al.*** [23] who found that LH concentration post CC was significantly higher in the patients who had pregnancy rate (15.5 (4.4) versus 12.0 (7.9); $P = 0.04$). The changes in serum FSH levels were smaller in size as compared with the changes in LH levels. The possibility that the FSH assay may not be sensitive enough to distinguish such subtle changes must be considered. However, the consistent pattern observed indicated that clomiphene citrate induced an increased release of FSH as well as LH. It was shown that clomiphene citrate exerts an increased release and synthesis of LH [24]. on the other hand, FSH release, increased only transiently and FSH synthesis was not affected. These data may explain the relative size of changes in serum LH and FSH concentration during clomiphene citrate treatment [25]. Similarly, **Atwa *et al.*** [26] found a significant improvement in the hormonal profile (serum LH, FSH, and testosterone with a p-value of (0.0001, 0.007, and 0.0001 respectively) in the study group after one month of treatment.

In the current study, serum progesterone was increased significantly after extended treatment. Our results confirmed by the study conducted by **Fritz *et al.*** [27] found that serum progesterone concentrations were also significantly higher ($p<0.05$) after extended clomiphene administration. This difference first became apparent in the periovulatory phase; significant increase in progesterone levels were observed on days LH+0 and LH+1. Mean level of serum progesterone remained elevated during luteal phase but to a widely varying extent among patients; concentrations in one individual rose as high as 166 ng/ml. Another study by **Serafini *et al.*** [28] demonstrated elevated preovulatory serum progesterone levels and their high predictive value for the LH surge in controlled ovarian hyperstimulation induced by clomiphene citrate. These results come to agree with our results.

In the current study, number of follicles increased significantly after CC extended compared to before treatment. Our results in the same line with the retrospective observational analysis by **Isaacs *et al.*** [19] who studied 24 anovulatory patients who failed on 150 mg of CC administration for 5 days. They demonstrated high rate of pregnancy when CC was given daily starting on cycle day 3 of menstruation for 9 days (extended) at a starting dose of 100 to 150 mg/d, along with prednisone 5 mg orally each night throughout the cycle in clomiphene resistant cases. This study presents an alternative before gonadotropins administration or surgery is done to reduce cost and risk.

CONCLUSION

CC resistant PCOS patients were treated with an extended CC administration (150 mg for 10 days) to overcome anovulation. So, CC extended is a safe, effective and minimal side effect, but low pregnancy rate was observed. Therefore, further studies with large number of PCO patients with CC resistant with addition other drugs are required to investigate the improvement of the pregnancy rate.

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