Original Article

L-Carnitine and Clomiphene Citrate for induction of ovulation in women with Polycystic Ovary Syndrome : Randomized controlled trial

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ABSTRACT

Aim: The aim of the study was to assess the efficacy of adding L-carnitine to clomiphene citrate for increasing the ovulation and the pregnancy rate in women with PCOS. The primary outcome was the ovulation rate and the secondary outcome was the pregnancy rate.

Materials and Methods: This study was a randomized controlled clinical trial that was conducted on 94 women who attended at the Obstetrics and Gynecology Outpatient Infertility Clinic at Ain-Shams University Maternity Hospital, and they were diagnosed with PCOS according to the Rotterdam Criteria (11). The women were distributed randomly into two equal groups; Group L (n=47) received 100 mg of clomiphene citrate plus 3 gm of L-carnitine orally from day 3 to day 7 of the cycle with continuation of L-carnitine till the day of pregnancy test and Group C (n=47) received 100 mg of clomiphene citrate orally from day 3 to day 7 of the cycle.

Results: There was a significant difference (*P<0.05*) between the two groups regarding the ovulation rate (Group L: 70.2%; Group C: 44.7%). There was no significant difference (*P>0.05*) between the two groups regarding the pregnancy rate (Group L: 8.5%; Group C: 6.4%). There was a highly significant difference (*P<0.01*) between the two groups as regards to the number of mature follicles (Group L: 1.6 ± 1.2; Group C: 0.8 ± 0.7), the days needed till hCG injection (Group L: 12.2 ± 1.5; Group C: 14.0 ± 1.8), the Endometrial thickness (Group L: 10.4 ± 1.2 mm; Group C: 9.1 ± 0.8 mm). There was no significant difference (*P>0.05*) between the two groups regarding the level of progesterone after 8 days from the hCG injection.

Conclusion: L-carnitine added to clomiphene citrate for induction of ovulation in PCOS women had a significant effect on the ovulation rate, but it didn't have a significant effect on the pregnancy rate when compared to clomiphene citrate alone.

Key Words: Clomiphene citrate, induction of ovulation, L-carnitine, polycystic ovary syndrome

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INTRODUCTION

Polycystic ovary syndrome (PCOS) is one of the most popular syndromes and it is considered as one of the most frequent endocrine disorders and the leading reason of infertility in women at their reproductive age. Menstrual abnormalities and chronic anovulation together with polycystic ovarian appearance during ultrasound examination are the criteria that are preferred by gynaecologists, in which their main interest is commonly concentrated on ovarian dysfunction[1]. Menstrual dysfunctions, in particular oligomenorrhea and amenorrhea, can be detected in the majority of women with PCOS[2]. The most popular characteristic of the PCOS is insulin resistance with compensatory hyperinsulinemia and this has a major pathophysiologic role in the hyperandrogenism of the condition[3]. In the majority of situations ovulation can be induced with clomiphene citrate which is considered one of the first-line treatments for induction of ovulation in PCOS women[4]. The most frequently used starting dose of clomiphene citrate for ovulation induction is 50 mg/day for 5 days during the period of the follicular phase[5]. L-Carnitine is a small water-soluble molecule that is obtained from the two amino acids lysine and methionine, and it has an essential duty in fat metabolisms. It also has an important role in the normal mitochondrial oxidation of fatty acids, which increase the supply of energy to the cells[6,7]. L-Carnitine has a significant role in weight loss, glucose tolerance, insulin function and fatty acid metabolism[8]. A study by Celik *et al.*, in 2017 showed that the women with PCOS had significantly lower L-carnitine levels than those of the healthy controls[9]. Another study by Ismail *et al*., depicted that combined L-carnitine and clomiphene citrate resulted in significant improvement in

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both ovulation and cumulative pregnancy rates in women with clomiphene-resistant PCOS[10].

AIM OF THE WORK

This aim of this study was to assess the efficacy of adding L-carnitine to clomiphene citrate for increasing the ovulation and the pregnancy rate in women with PCOS. The primary outcome of the study was the ovulation rate and the secondary outcome was the pregnancy rate.

PATIENTS AND METHODS

This study was a randomized controlled clinical trial that aimed to assess the efficacy of adding L-carnitine to clomiphene citrate for increasing the ovulation and the pregnancy rate in women with PCOS.

This study was conducted on 94 women who attended at the Obstetrics and Gynecology Outpatient Infertility Clinic at Ain-Shams University Maternity Hospital, during the period from July 2018 to February 2019 and they were diagnosed with PCOS based on the European Society of Human Reproduction and Embryology/ American Society for Reproductive Medicine (ESHRE/ASRM) guidelines criteria[11].

In which Fulfillment of at least two out of the following three criteria were sufficient for the diagnosis of PCOS:

- Oligo-menorrhea and/or anovulation.
- Clinical and/or biochemical signs of hyperandrogenism.
- Typical Polycystic ovaries by ultra-sonography.

The 94 women of this study were randomly assigned and distributed into two groups (Group L and Group C) according to the computer-generated randomization sheet:

Group L (47 women): This group received oral clomiphene citrate (Clomid, Global Napi Pharmaceuticals (GNP), Egypt) (50 mg tablet, two times per day) from the third day of the cycle until the seventh day of the cycle plus oral carnitine supplementation (Carnivita Forte, Eva Pharma, Egypt) (1g tablet, three times per day) from the third day of the cycle until the day of the pregnancy test.

Group C (47 women): This group received oral clomiphene citrate only (Clomid, Global Napi Pharmaceuticals (GNP), Egypt) (50 mg tablet, two times per day) from the third day of the cycle until the seventh day of the cycle.

Random allocation and concealment technique was used for these 94 women in which ninety four opaque envelopes were numbered serially, and in each envelope, there was the corresponding letter which denotes the allocated group according to the randomization table. Then all of the envelopes were closed and placed in one box. When the first woman arrived, the first envelope was opened and she was allocated according to the letter inside, and this step was repeated until all of the 94 envelopes were finished.

Inclusion criteria:

- Age ranging from 18-35 years.
- Normal Hysterosalpingography (HSG).
- Normal Semen analysis of the husband according to the levels of the world health organization (WHO)[12].
- Diagnosed with PCOS based on the (ESHRE/ASRM) guidelines criteria[11].

Exclusion Criteria:

- Patient's refusal.
- Male factors of infertility and/or abnormal HSG.
- History of medications that could lead to an increase in Hyperprolactinemia in PCOS women as antihypertensive drugs, antidepressant drugs and anti-nausea medications, or the presence of history of galactorrhea.
- FSH on day 3 > 15 mIU/mL.
- Gross ovarian pathology diagnosed by ultrasound.

Study Tools:

All patients were subjected to the following:

1) Full detailed medical history.

2) Clinical examination: to measure the patient's weight and height (to calculate the body mass index) and to detect the presence of acne and/or hirsutism.

3) Trans-vaginal ultrasonography: to confirm the diagnosis of PCOS, to assess the ovarian volume, to exclude any ovarian pathology, to assess the mean antral follicle count in both ovaries measuring 2-9 mm in diameter.

Study Procedures:

Serum FSH, LH and free testosterone concentrations were measured on day 3 (basal) of the cycle. Trans-vaginal folliculometry was performed on all women on the ninth day of the cycle and then individualized according to the response. When one leading follicle attained a diameter of 17mm or more, the endometrial thickness was measured and 10,000 IU of human chorionic gonadotropin (hCG) was given (im injection; Epifasi; EIPICO, Egypt). Timed intercourse was advised after 36 hours from the night of hCG administration for 2 successive days. Ovulation success was assessed by the use of transvaginal ultrasound after the timed intercourse by 3 days, and ovulation success was confirmed if the leading follicle has collapsed and the corpus luteum has appeared in the ovary, in addition to the presence of some fluid in the Douglas pouch. Another method that was used to confirm the success of the ovulation process was measuring the serum progesterone level after 8 days from the day of hCG injection, and ovulation was confirmed if the serum progesterone level was $\geq$ 5 ng/ml[13]. The adequate level of progesterone in the luteal phase is considered to be at least 10.83 ng/mL[14].

Luteal-phase support was not provided in both groups. Pregnancy test was done in the form of testing the level of beta hCG in the blood after 14 days once the success of the ovulation process has been confirmed.

Ethical Considerations:

An informed written consent was taken from all the patients before the start of the study and every patient had the right to leave the study at any time without being denied of the regular full clinical care.

Sample size calculation:

The required sample size has been calculated using the IBM© Samplepower© version 3.3 (IBM© Corp., Armonk, NY, USA).

The main outcome measures were the ovulation rate and cumulative pregnancy rate. A previous study reported that the ovulation and cumulative pregnancy rates were 64.4% versus 17.4% and 51.5% versus 5.8% in PCO patients receiving L-carnitine with clomiphene citrate or clomiphene citrate alone, respectively[10].

So it was estimated that a sample size of 47 patients in either study group achieved a power of 99% to detect a difference of 47% (64.4% versus 17.4%) between the 2 groups as regards the ovulation rate and a power of 99.6% to detect a difference of 45.7% (51.5% versus 5.8%) as regards the cumulative pregnancy rate. These calculations used a two-sided chi-squared test with a type I error of 0.01.

STATISTICAL ANALYSIS:

Data were collected, tabulated, then analyzed using IBM© SPSS© Statistics version 23 (IBM© Corp., Armonk, NY) and JMP® Version 13.2.1 (SAS© Institute Inc., Cary, NC).

Continuous numerical data were presented as mean and standard deviation and intergroup differences were compared using the unpaired t-test.

Categorical data were presented as number and percentage or ratio and the differences were compared using the Fisher's exact test.

P values <0.05 were considered statistically significant.

P values <0.01 were considered highly statistically significant.

RESULTS

There was no statistical significant difference (*P>0.05*) between the two groups regarding the baseline characteristics (age, body mass index, infertility duration, type of infertility) and basal hormonal profile at the third day of the cycle (Table 1).

The results of the study showed that the ovulation rate was higher in Group L, and there was a significant difference (*P<0.05*) between the two groups (Group L: 70.2% and Group C: 44.7%). There was no significant difference (*P>0.05*) between the two groups as regards to the pregnancy rate (Group L: 8.5% and Group C: 6.4%) (Table 2).

Group L had more number of pre-ovulatory follicles ≥ 17 mm in diameter, and there was a highly significant difference (*P<0.01*) between the two groups (Group L: 1.6 ± 1.2 follicles and Group C: 0.8 ± 0.7 follicles). Also, Group L needed less days till hCG injection was given and there was a highly significant difference (*P<0.01*) between the two groups (Group L: 12.2 ± 1.5 days and Group C: 14.0 ± 1.8 days). Group L also had a thicker endometrial thickness at the day of hCG injection, and there was a highly significant difference (*P<0.01*) between the two groups (Group L: 10.4 ± 1.2 mm and Group C: 9.1 ± 0.8 mm). There was no significant difference (*P>0.05*) between the two groups as regards to the level of serum progesterone after 8 days from the hCG injection (Table 2).

As regards to the side effects reported at the end of the treatment course, there was a significant difference (*P<0.05*) between the two groups, in which Group L had more women reporting side effects than Group C (Group L: 19.1% and Group C: 2.1%) (Table 2). Group L had 3 women complaining of dizziness only, 4 women complaining of abdominal pain only, 1 woman complaining of abdominal pain and dizziness together, and 1 woman complaining of abdominal pain and dizziness with addition to nausea and vomiting, while Group C had only one woman complaining of abdominal pain.

Table 1: Baseline characteristics in both study groups.

Variable	Group L (n=47)	Group C (n=47)	*P-value**
Age (years)	25.2 ± 3.9	25.8 ± 2.6	0.420
Body mass index (kg/m2)	30.0 ± 5.2	30.4 ± 3.0	0.620
Duration of infertility (months)	28.2 ± 18.1	26.4 ± 11.4	0.569
Type of infertility (primary/secondary)	34/13	35/12	1.000 #
Free testosterone (ng/ml)	0.33± 0.19	0.34± 0.10	0.865
FSH (IU/ml)	5.4± 1.1	5.3± 1.8	0.895
LH (IU/ml)	6.2± 1.7	6.4± 1.8	0.644

Data are mean ± standard deviation (SD) or ratio.
*Unpaired t-test unless otherwise indicated.
#Fisher's exact test.

Table 2: Details of the study outcomes in both study groups

Variable	Group L (n=47)	Group C (n=47)	P-value*
Ovulation rate	33 (70.2%)	21 (44.7%)	0.021 #
Pregnancy rate	4 (8.5%)	3 (6.4%)	1.000 #
Number of pre-ovulatory follicles 17 mm	1.6± 1.2	0.8± 0.7	0.0001
Days needed until hCG injection	12.2± 1.5	14.0± 1.8	0.0001
Endometrial thickness (mm)	10.4± 1.2	9.1± 0.8	<0.0001
Serum progesterone (ng/ml)	27.6± 20.5	19.5± 12.0	0.063
Side effects reported	9 (19.1%)	1 (2.1%)	0.015 #

Data are mean ± standard deviation (SD) or number and (Percentage).
*Unpaired t-test unless otherwise indicated.
#Fisher's exact test.

DISCUSSION

Polycystic ovary syndrome (PCOS) is considered as one of the most popular endocrine disorders in women and it is known as the main cause of anovulatory infertility[15]. Methods that are commonly known to help in ovulation induction in women with PCOS include; weight reduction, lifestyle changes, clomiphene citrate, metformin, gonadotropins, laparoscopic ovarian surgery and In vitro fertilization (IVF)[16]. A study by Celik *et al.* showed that the women with PCOS had a significantly lower L-carnitine levels than those of the healthy controls[9]. Another study reported that L-Carnitine has a significant role in weight loss, glucose tolerance, insulin function and fatty acid metabolism[8].

The primary outcome of the study showed that there was a statistical significant difference (*P<0.05*) between the two groups, in which Group L had a higher ovulation rate than Group C.

This was in agreement with the study of Ismail *et al.,* as they reported that there was a highly statistical significant difference (*P<0.0001*) between the two groups, in which the Clomiphene citrate + L-carnitine group (n=85) had an ovulation rate of 64.7% and the group of Clomiphene citrate + placebo (n=85) had an ovulation rate of 17.6%[10]. This was also in agreement with the study of Latifian *et al.,* as they reported that there was a statistical significant difference (*P<0.05*) in the favour of the women that received L-carnitine[6].

As regards to the secondary outcome of the study which was the pregnancy rate, there was no statistical significant difference (*P>0.05*) between the two groups.

This was in disagreement with the study of Ismail *et al.*, as they reported that there was a highly statistical significant difference (*P<0.0001*) between the two groups, in which the Clomiphene citrate + L-carnitine group (n=85) had a pregnancy rate of 51.5% and the group of Clomiphene citrate + placebo (n=85) had a pregnancy rate of 5.8%[10]. This was also in disagreement with the study of Latifian *et al.*, as they reported that there was a statistical significant difference (*P<0.05*) in the favour of the women that received L-carnitine[6].

A possible explanation for this disagreement could be that Ismail *et al.*, and Latifian *et al.*, included women with PCOS that were Clomiphene citrate resistant, while this study included women with PCOS who were not Clomiphene citrate resistant, another possible explanation could be that the timed intercourse between the couples of this study was not done during the suggested period, in addition to the presence of many different variables that have an effect on the pregnancy rate in women, and this could be demonstrated clearly when the studies are in larger sample sizes and includes a lot of women, so the sample size of this study could be one of the factors that had an effect on the pregnancy rate.

As regards to the mean number of pre-ovulatory follicles that were ≥ 17 mm in diameter, there was a highly statistical significant difference (*P<0.01*) between the two groups, in which Group L had more mean number of follicles than Group C.

This was in agreement with the study of Ismail *et al.*, as they reported that there was a highly statistical significant difference (*P<0.0001*) between the two groups, in which the L-carnitine group had a mean number of follicles more than the placebo group[10]. This was also in agreement with the study of Latifian *et al.*, as they reported that there was a statistical significant difference (*P<0.05*) in the favour of the women that received L-carnitine[6].

As regards to the mean number of days needed till hCG injection was given, there was a highly statistical significant difference (*P<0.01*) between the two groups, with Group C needing more days till attaining the desired follicle size.

This was in agreement with the study of Ismail *et al.*, as they reported that there was a highly statistical significant difference (*P<0.0001*) between the two groups, in which the placebo group needed more days than the L-carnitine group to reach the desired follicle size[10].

Regarding the endometrial thickness at the day of hCG injection, there was a highly statistical significant difference (*P<0.01*) between the two groups, with Group L having a thicker endometrium than Group C.

This was in agreement with the study of Ismail *et al.*, as they reported that there was a highly statistical significant difference (*P<0.0001*) between the two groups, in which the L-carnitine group had a thicker endometrium than the placebo group[10]. This was also in agreement with the study of Edris and Barakat; as they reported that there was a highly statistical significant difference (*p<0.0001*) between the two groups in which the L-carnitine group had a thicker endometrium than the placebo group[17]. This was also in agreement with the study of Latifian *et al.*, as they reported that there was a statistical significant

difference (*P<0.05*) in the favour of the women that received L-carnitine[6].

As regards to the mean level of serum progesterone after 8 days from the hCG injection, there was no statistical significant difference (*P>0.05*) between the two groups.

This was in disagreement with the study of Ismail *et al.*, as they reported that there was a highly statistical significant difference (*P<0.0001*) between the two groups, in which the L-carnitine group had a higher mean level of progesterone than the placebo group[10].

A possible explanation for this disagreement could be due to the effect of the high dose of clomiphene citrate that was given by Ismail *et al.*, which was 250 mg per day, another possible explanation could be that the sample size of the study of Ismail *et al.*, was 170 women distributed into two groups while this study had 94 women distributed into two groups, another possible explanation could be that Ismail *et al.*, included women with PCOS that were Clomiphene citrate resistant, while this study included women with PCOS who were not Clomiphene citrate resistant.

As regards to the side effects reported at the end of the treatment course, there was a statistical significant difference (*P<0.05*) between the two groups, in which Group L had more women reporting side effects than Group C.

This was in agreement with the study of Ismail *et al*., as the L-carnitine group had more reports of side effects than the placebo group.

CONCLUSION

L-carnitine added to clomiphene citrate for induction of ovulation in PCOS women had a significant effect on the ovulation rate, but it didn't have a significant effect on the pregnancy rate when compared to clomiphene citrate alone.

CONFLICT OF INTEREST

There are no conflicts of interests.

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