Effect of antioxidants as adjuvant therapy to clomiphene citrate in controlled ovarian stimulation: a comparative study

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Abstract

Aim of the work: This work aims to evaluate the value of adding L-carnitine to clomiphene citrate in ovulatory dysfunction according to ovulation and pregnancy rate.

Patient and method: This study was conducted at the obstetric and gynaecology clinic, Mansoura University Hospital, from May 2021 to August 2022. The study included 186 participants presenting with infertility; Participants were allocated into two groups. Group A: received clomiphene citrate(100mg) plus L-carnitine (2gm). Group B: received clomiphene citrate (100mg) alone. Patients were allocated to groups by case order. The outcomes include ovulation, estradiol level, endometrial thickness, and pregnancy rate.

Results: Group A had significantly higher follicle count, dominant follicle size, endometrial thickness, serum E2 and earlier day of HCG injection ($P < 0.001$) compared to group B. regarding the response to induction in both groups, there were non-significant differences between them regarding ovulation rate, pregnancy rate and early miscarriage rate ($P = 0.071, 0.336, 0.502$ respectively). Regarding the induction in Poly Cystic Ovary Syndrome (PCOS) patients in both groups, the patients with PCO in group (A) had an earlier day of HCG injection ($P = 0.035$). However, there were non-significant differences between PCO patients in both groups regarding other variants.

Conclusion: L-carnitine as an adjuvant therapy to clomiphene citrate in the controlled ovarian stimulation was associated with higher follicle number, better endometrial thickness, and earlier days of HCG injection in PCO patients.

Keywords: L-carnitine, induction of ovulation, infertility, clomiphene citrate.

INTRODUCTION

Globally, 15% of couples of reproductive age suffer from subfertility (1), and ovulatory dysfunction represents 30 to 40 % of infertility in women (1). According to the World Health Organization, ovulatory disorders are divided into four classes:

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1. Hypogonadotropic hypogonadal anovulation
2. Normogonadotropic normal-estrogenic anovulation
3. Hypergonadotropic hypoestrogenic anovulation
4. Hyperprolactinemic anovulation (2)

Anti-estrogenic drugs such as Clomiphene Citrate have been used for ovulation induction since the 1960s in 50–150 mg (3). The conception rate in clomiphene citrate-treated cycles is about 40% only; however, the ovulation rate is between 80-85% (4). Repeated ovulation stimulation affects the quantity of mitochondrial DNA in oocytes, leading to a decline in egg quality and the number of oocytes (5).

L-Carnitine (LC) is one of the conditionally essential nutrients called quasi-vitamins (6). L-carnitine intake can promote β-oxidation, endurance, burn fat and shorten post-workout recovery (7,8). LC increases the GnRH secretion from the hypothalamus by causing K+-induced depolarisation in the hypothalamic neuronal (9).

Treatment with LC increases serum levels of estradiol, progesterone, and LH and decreases prolactin levels (10). Its indirect endocrine effect prevents Poly Cystic Ovary Syndrome (PCOS), amenorrhea and other problems related to the female reproductive cycle (11).

From the previously mentioned benefits, we added L-carnitine to clomiphene citrate in ovulation induction to study the impact on ovulation pattern and pregnancy rates.

Patients and Methods :

The IRB of faculty medicine Mansoura University approved this study (MS.21.04.1447); it was done at the obstetrics and gynaecology clinic of Mansoura university from May 2021 to August 2022. patients were allocated to groups by order. Females with a history of infertility (primary or secondary) with ovulatory dysfunction (WHO class II & 1V), aged 20-35 years, have a body mass index(BMI) of 18-30 kg/m2. Hormonal profile (serum prolactin and TSH levels, FSH, LH levels) was investigated. A semen analysis of the husband and hysterosalpingogram should be okay.

Exclusion criteria:

Females over 35 years refuse to participate, with hypersensitivity to one of the used drugs cannot participate. Females with known causes of infertility (such as abnormal semen analyses - pelvic adhesions - endometriosis - large submucous fibroid, premature ovarian insufficiency and Hypogonadotropic hypogonadal anovulation) were excluded.

The entire history was taken, a general examination was done, and the study was explained to each participant. Transvaginal ultrasound (TVUS), abdominal ultrasound (TAUS), and hysterosalpingogram were done, and the husband's infertility was excluded from the semen analysis. This study included 186 cases, with 93 participants in each group.

Group A(n=93): received clomiphene citrate (Tecnovula 50mg oral tab Techno _ pharma Borg Al Arab algadida city Alexandria - Egypt) 100 mg every day in the form of two oral tablets, starting from day1 to day 5 of the menstrual bleeding for five days Plus L.carnitine 2gm (Carnivita Fort 1gram oral tab Eva pharma, 6th of October City Giza Egypt) every day starting in concomitant with Clomiphene citrate till the day of the positive pregnancy test.

Group B (n=93): received clomiphene citrate (100mg)) (Tecnovula 50mg oral tab Techno _ pharma Borg Al Arab algadida city Alexandria - Egypt) 100 mg every day in the form of two oral tablets, starting from day1 to day 5 of the menstrual bleeding for five days.

The first follow-up visit was on day ten of the menstrual bleeding. TVUS was used to measure the number and size of the growing follicles and the endometrial thickness. Serum estradiol level was measured on day 10. Then TVUS was done every other day until the mature follicle reached (18-24) mm, and ovulation was triggered by 5.000 IU of hCG (Choriomon 5000 I u amp IBSA Switzerland imported by the scientific habit office). Participants were advised to have Intercourse every other day and 36 hours of hCG administration. Ovulation was confirmed when the corpus luteum was seen (collapsed dominant follicle) then a pregnancy test (β-hCG level in blood) was done two weeks after ovulation to confirm or exclude pregnancy. TVUS was done 2-3weeks after a positive pregnancy test to ensure a gestational sac. In cases where the pregnancy was confirmed, participants were followed up till 13 weeks of pregnancy. The same protocols were repeated for three consecutive months in cases where the pregnancy was excluded. In instances where ovulation not occurred or menstruation was delayed, withdrawal bleeding by Dydrogesterone 20 mg was done for five days (Duphaston 10mg, Penta pharma_Egypt, Abu sultan industrial zone Ismailia Egypt), and ovulation induction was repeated.

Outcome Measures

The Primary outcome of this study was to include the ovulation response (Number and size of follicles), Pre-ovulatory endometrial thickness, and serum estradiol level. The pregnancy rate and early miscarriage rate were reported.

Sample size calculation:

The sample size was based on ovulation rate among females with clomiphene citrate only and females with clomiphene citrate with L.Carnitine retrieved from previous research (Kortam et al., 2019) Using G*power version 3.0.10 to calculate sample size based on the difference of 25.5%, 2-tailed test, α error = 0.05 and power = 90.0% the total calculated sample size was 154. By adding 20% to compensate for possible dropout then, the total estimated sample size was 186 (93 in each group).

The cumulative clinical data of the studied groups and results were collected, tabulated, and statistical analyses were done.

RESULTS

The participants were assigned into two groups; group (A) included 93 patients who received clomiphene citrate with a dose of 100 mg per day plus L-Carnitine with a dose of 2 grams per day. Group (B) included 93 patients who received clomiphene citrate alone with a dose of 100 mg per day.

Table (1): Demographic characteristics of the studied groups:

		Group A (n= 93)	Group B (n= 93)	95% CI	P
Age		28.20 ± 4.434	28.90 ± 4.884	-2.05, 0.65	0.308
BMI		26.31 ± 2.882	26.03 ± 2.680	-0.53, 1.08	0.494
Residence	Rural	38 (40.9%)	25 (26.9%)	-	0.044
	Urban	55 (59.1%)	68 (73.1%)		
Gravidity		2.13 ± 1.469	2.12 ± 1.265	-0.39, 0.41	0.963
Parity		1.57 ± 1.067	1.87 ± 1.154	-0.62, 0.02	0.066

Data are expressed as mean and standard deviation or as percentage and frequency—95% CI: 95% confidence interval of the mean difference between both groups. P is significant when < 0.05.

There were non-significant differences between both groups regarding age, BMI, gravidity and parity (P = 0.308, 0.494, 0.963 and 0.066, respectively). There was a statistically significant difference between both groups regarding the residence (P = 0.044), as shown in table (1).

Table (2): Response to induction in both groups:

		Group A (n= 93)	Group B (n= 93)	95% CI	P
Infertility type	Primary	16 (17.2%)	15 (16.1%)	-	0.844
	Secondary	77 (82.8%)	78 (83.9%)		
Infertility duration (month)		21.78 ± 11.505	22.34 ± 9.341	-3.61, 2.50	0.720
Follicle Count		3.44 ± 2.184	1.65 ± 1.515	1.24, 2.33	< 0.001
Dominant follicle size day 10		15.05 ± 2.637	13.58 ± 1.702	0.69, 2.25	< 0.001
Endometrial thickness day 10		8.26 ± 2.110	6.59 ± 1.562	1.13, 2.20	< 0.001
Serum E2 day 10		210.75 ± 109.375	119.84 ± 75.689	63.70, 118.13	< 0.001
Day of HCG injection		11.31 ± 1.071	13.89 ± 1.573	-3.12, -2.06	< 0.001
Ovulation rate		63 (67.7%)	51 (54.8%)	-	0.071
Side effects	None	90 (96.8%)	93 (100.0%)	-	0.218
	Gastric upset	2 (2.2%)	0 (0.0%)		
	Dizziness	1 (1.1%)	0 (0.0%)		
Pregnancy rate		30 (32.3%)	20 (21.5%)		
Number of sacs by US	1	26 (86.7%)	14 (93.3%)	-	0.502
	>1	4 (13.3%)	1 (6.7%)		
Early miscarriage		2 (6.7%)	3 (15.0%)	-	0.336

Regarding the response to induction in both groups, there were non-significant differences between them in infertility type, duration, history of ovulation induction cycles, ovulation, side effects, outcome, number of sacs by US and early miscarriage (P = 0.844, 0.720, 0.648, 0.071, 0.218, 0.098, 0.502 and 0.336 respectively) as shown in table (2).

Group (A), who received clomiphene citrate plus L-Carnitine, had significantly higher follicle count, dominant follicle size day 10, endometrial thickness day 10, serum E2 day ten and earlier day of HCG injection (P < 0.001) as shown in table (2).

Table (3): Demographic characteristics of PCO patients (WHO class II) in the studied groups:

		PCO in Group A (n= 13)	PCO in Group A (n= 13)	95% CI	P
Age		29.77 ± 4.419	28.36 ± 5.988	-3.00, 5.82	0.516
BMI		27.38 ± 2.873	26.00 ± 2.966	-1.09, 3.86	0.259
Residence	Rural	9 (69.2%)	4 (36.4%	-	0.107
	Urban	4 (30.8%)	7 (63.6%		
Gravidity		1.77 ± 1.166	1.45 ± 1.293	-0.73, 1.36	0.537
Parity		1.38 ± 0.870	1.45 ± 1.293	-0.99, 0.85	0.876

Data are expressed as mean and standard deviation or as percentage and frequency. 95% CI: 95% confidence interval of the mean difference between both groups. P is significant when < 0.05.

Table (3) shows that regarding the comparison between the PCO patients in both groups, there were non-significant differences regarding age, BMI, residence, gravidity and parity (P = 0.516, 0.259, 0.107, 0.537 and 0.876, respectively).

Table (4): Response to induction in PCO patients in both groups:

		PCO in Group A (n= 13)	PCO in Group B (n= 11)	95% CI	P
Infertility type	Primary	2 (15.4%)	4 (36.4%	-	0.237
	Secondary	11 (84.6%)	7 (63.6%		
Infertility duration (month)		16.69 ± 5.559	23.27 ± 13.454	-15.04, 1.88	
Follicle Count		1.46 ± 1.808	0.73 ± 1.009	-0.54, 2.01	
Dominant follicle size day 10		14.42 ± 2.417	14.13 ± 0.479	-2.59, 3.17	
Endometrial thickness day 10		8.38 ± 3.283	6.27 ± 1.489	-0.15, 4.35	
Serum E2 day 10		121.31 ± 89.100	83.00 ± 27.695	- 19.81, 96.42	
Day of HCG injection		11.60 ± 0.894	14.00 ± 1.826	-4.58, -0.22	
Ovulation rate		5 (38.5%	6 (54.5%	-	
Side effects	None	12 (92.3%)	11 (100.0%	-	0.347
	Gastric upset	-	-		
	Dizziness	1 (7.7%)	0 (0.0%		
Outcome	Failure	9 (69.2%	9 (81.8%	-	
	Pregnancy	4 (30.8%	2 (18.2%		
Number of sacs by US	1	3 (100.0%)	2 (100.0%)	-	
	2	-	-		
Early miscarriage		0 (0.0%)	0 (0.0%)	-	-

Data are expressed as mean and standard deviation or as percentage and frequency—95% CI: 95% confidence interval of the mean difference between both groups. P is significant when < 0.05.

Table (4) shows that regarding the response to induction in PCO patients in both groups, the patients with PCO in group (A) had an earlier day of HCG injection (P = 0.035). However, there were non-significant differences between PCO patients in both groups regarding other variants.

Discussion

Adding antioxidants to clomiphene citrate in ovulatory dysfunction will improve ovulation and pregnancy rate. The present study has demonstrated that administration of L-carnitine as an adjuvant therapy to the controlled ovarian stimulation by clomiphene citrate was associated with higher follicle count, dominant follicle size, endometrial thickness, serum E2 and earlier day of HCG injection. However, all these improvements in cycle parameters did not lead to a significant increase in the ovulation and pregnancy rates, which may be explained by the small sample size and the diversity of factors that affect the occurrence of pregnancy.

CC has been used to induce ovulation by blocking estrogen receptors, thus increasing follicle-stimulating hormone and ovulation (12).

Carnitine may be either the L-carnitine, the biologically active form, or the biologically

inactive D-carnitine (13). L-Carnitine is essential in energy production, oxidative stress and glucose metabolism; it can stabilise mitochondrial membranes, increasing the organelle's energy supply, and protect the cell from apoptotic death(10,14,15).

Miyamoto et al. 2010 stated that repeated ovulation induction decreased the amounts of mitochondrial DNA and increased 8-hydroxydeoxyguanosine in oocytes (5).

In our study, Group (A), who received clomiphene citrate plus L-Carnitine, had significantly higher follicle count, dominant follicle size, endometrial thickness, serum E2 day and earlier day of HCG injection ($P < 0.001$). Consistent with our results, El Sharkwy et al. (16) 2019 compared N-acetylcysteine and l-carnitine in ovulation induction; after three months of treatment, there was a significant improvement in menstrual pattern, FSH, LH, free testosterone, and insulin resistance markers.

Gaafar et al. 2007 (17) evaluated the Effect of N-Acetyl-Cysteine (NAC) in the ICSI cycle; the NAC group showed a non-significant increase in retrieved oocytes. The study group showed a significant increase in mature oocytes, a higher fertilisation rate, and an insignificant drop in follicular testosterone. However, these positive effects of NAC did not lead to a significant change in the pregnancy rate.

Sheida et al. (18) 2021 added L-Carnitine to the GnRH-antagonist protocol on assisted reproductive technology in women with polycystic ovarian syndrome. They stated that the duration of stimulation and endometrial thickness were comparable in both groups ($p > .05$). The Serum estradiol level on the day of HCG triggering was significantly higher in the L-Carnitine group compared to the control group ($p < .05$). However, chemical (26.8 vs 30.7%) and clinical (24.3 vs 25.6%) pregnancy rates, were non-significant between L-Carnitine and control groups respectively.

Levels of serum L-carnitine in women with polycystic ovarian syndrome are much less due to hyperandrogenism and hyperinsulinemia (11). The combination of L-carnitine and CC in clomiphene-resistant PCOS significantly improves ovulation and the cumulative pregnancy rates in clomiphene-resistant PCOS (64.4% vs 17.4% and 51.5 % vs 5.8 %)(19).

In the current study, regarding the comparison between the PCO patients in both groups, there were non-significant differences between them regarding the age, BMI, residence, gravidity and parity ($P = 0.516$, 0.259, 0.107, 0.537 and 0.876 respectively).

In the current study, regarding the response to induction in PCO patients in both groups, the patients with PCO in the group (A) had an earlier day of HCG injection ($P = 0.035$). However, there were non-significant differences between PCO patients in both groups regarding other variants, including Infertility duration, Follicle Count, Dominant follicle size at day 10, Endometrial thickness at day 10, Serum E2 at day 10, Ovulation, Side effects, Outcome, Early miscarriage and Number of sacs by TVUS.

Finally, in the present study, L-carnitine was a well-tolerated drug by all patients, with no manifest side effects reported from any case in the study group.

Conclusion

L-carnitine is a well-tolerated drug with minor side effects. Combining L-carnitine and clomiphene citrate in ovulatory dysfunction will significantly improve the follicular count, endometrial thickness and pregnancy rate.

No Conflicts of interest

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