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Combination of alpha lipoic acid and metformin supplement improve assisted reproductive technologies outcomes in polycystic ovary syndrome patients

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Abstract: We aimed to investigating the effects of metformin (MET) in combination with alpha lipoic acid (ALA) on hormonal and biochemical parameters, in polycystic ovary syndrome (PCOS) women undergoing intracytoplasmic sperm injection (ICSI). This experimental pilot study with a randomized design was carried out on 40 PCOS women in two groups: (1) MET group, administered 1,500 mg/day MET, and (2) MET (1,500 mg/day)+ALA (1,800 mg/day) group. Drugs were administered from the third day of the previous cycle until the day of oocyte aspiration (six weeks of treatment in total). MET+ALA significantly increased the number of maturated oocytes and the rate of fertilization when compared to the MET group. Combination MET+ALA could increase significantly the number of oocytes retrieval and the number of good-quality embryos. Also, the malondialdehyde (MDA) level decreased significantly in the MET+ALA group and the total antioxidant capacity (TAC) level increased significantly in the MET+ALA group compared to the MET group. Also, fasting blood sugar (FBS), insulin, luteinizing hormone (LH), and LH/follicle stimulating hormone (FSH) levels were significantly lower in the MET+ALA group. The pregnancy outcomes showed no significant difference in the rates of biochemical pregnancy, clinical pregnancy, miscarriage, and live births between the control and study groups. The combination of MET+ALA treatment could moderate the complications of PCOS and subsequently improve oocyte and embryo quality.

Key words: Polycystic ovary syndrome, Alpha-lipoic acid, Metformin, Assisted reproductive techniques, Ovulation induction

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Introduction

Polycystic ovary syndrome (PCOS) is one of the most common metabolic, endocrine disorders occurring in anovulation, affecting up to 10% of women of reproductive age [1]. Besides, PCOS is an ovarian syndrome that was described with reproductive disorders (menstrual dysfunction, hirsutism, hyperandrogenism, anovulation, and infertility), metabolic disorders (obesity, type II diabetes, hypertension, and dyslipidemia), and psychological features (mood disorders and decreased quality of life) [2, 3]. Despite a long history of studies on PCOS, its etiology is still unknown. Concerning the pathophysiology of PCOS, studies have explained the roles of inflammatory state, endothelial injury, oxidative stress, and genetic mechanisms. Oxidative stress, caused by an imbalance between radicals and antioxidant defense, is a major pathophysiological mechanism in vari-

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ous human diseases [4]. This is due to the overproduction of certain molecules, including reactive oxygen species (ROS), which are produced from nitric oxide and malondialdehyde (MDA) and can completely damage cell components, including proteins, lipids, and DNA [5]. The role of oxidative stress in the pathogenesis of PCOS is not yet fully understood. Studies have suggested that oxidative stress seems to be involved in PCOS by causing altered steroidogenesis in the ovaries, which subsequently contributes to increasing androgen levels, disturbing follicular development, and infertility [6]. Also, PCOS is a condition with a significant decrease in serum antioxidant and vitamin levels, and such women are at an increased risk of oxidative stress [7]. Oocyte quality is one of the most important factors determining the rate of success in assisted reproductive technologies (ART). Besides, PCOS patients are commonly distinguished by an increased number of oocytes during the course of ovulation stimulation. Nevertheless, these oocytes are often of low quality, leading to low fertilization, cleavage, and implantation. In most cases, treatment with some drugs overcomes anovulation associated with PCOS, leading to reductions in risk factors, and improves the response to ovulation induction [8]. Antioxidant supplements have been shown to improve insulin sensitivity and other health-threatening conditions in women with PCOS. Despite the important role of alternative medicine, especially antioxidants, in the management of PCOS in women [8], there are not many well-designed studies reported in the literature, especially in Iran. On the other hand, existing studies on the use of antioxidants in PCOS women have yielded controversial results due to the sample size or variations in the antioxidants prescribed or the outcomes evaluated in them. Alpha-lipoic acid (ALA) is a potent antioxidant, and controlled-release alpha-lipoic acid has been reported to improve glucose control in type 2 diabetic patients presumably by its effects on reducing oxidative stress and insulin resistance [9]. Also, ALA and its reduced form, dihydrolipoic acid, are powerful antioxidant molecules that can act as scavengers of ROS and can regenerate other antioxidant molecules, as well [10]. The present study was designed as a pilot randomized prospective study hypothesize at clarifying whether ALA as an powerful antioxidant in combination with metformin (MET), could moderate PCOS complications by following effect on oocyte quality, fertilization rate, embryo morphology, and pregnancy rate.

Materials and Methods

Study design

This study was a prospective randomized single blinded trial between July 2019 and February 2020. This clinical trial was conducted in the infertility treatment center of the Academic Center for Education, Culture, and Research (ACECR) (Qom, Iran) that was approved by the ethics committee of Islamic Azad University- Qom branch (IR.IAU.QOM.REC.1398.024.) and was registered in Iranian registry for clinical trials (IRCT20191210045681N1), and also informed consent was obtained from all participants. In all, 40 infertile Iranian women with PCOS (aged 25–35 years) that was confirmed by a gynecologist based on the Rotterdam protocol and candidate for intracytoplasmic sperm injection (ICSI). All subjects were asked to avoid any changes in their normal physical activity and diet, and also not to undergo any new pharmacotherapy during the study. Exclusion criteria including: hypothyroidism, fluctuations high blood sugar, smoking, insulin consumption or any supplements of vitamins and minerals during the three months before the start of the study and man infertility. Patients being randomly divided into two groups as follows: (1) MET group, patients received MET (500 mg, Glucophage; Merck, West Drayton, UK) three times daily; (2) MET+ALA group, patients received ALA (600 mg, Batch no. 6N5483; Holzkrichen, Bavaria, Germany) three times daily. All treatments were administered for a period of 6 weeks. Dosage of MET and ALA was prescribed based on previous studies for patients [11, 12].

Assessment of clinical features and baseline biochemical status

Body mass index (BMI), height, and weight were measured and recorded for each patient. Fasting blood samples (15 ml) were collected at baseline (on the 3rd day of menstruation) and the end of the intervention the day of ovum pick-up of the ICSI cycle at the Rooya reference laboratory to measure the hormones. Blood samples were immediately centrifuged (Hettich EBA20, Tuttlingen, Germany) at 3,000 rpm for 10 minutes to separate serum. Then, the samples were stored at –70°C until further analysis. Also following follicular aspiration and oocyte pick-up, follicular fluid (FF) was collected from the first clear sample of follicular fluid with follicles to measure MDA and total antioxidant capacity (TAC). The FF samples were immediately centrifuged at 3,000 rpm for 10 minutes at room temperature and the

supernatants collected and stored at 70°C until analysis. Fasting blood sugar (FBS), insulin, follicle stimulating hormone (FSH), luteinizing hormone (LH), and total T levels were determined by commercially available ELISA kits (Demeditec Diagnostics GmbH, Kiel, Germany). LH and FSH were expressed as mIU/ml while total testosterone (TT) was expressed as ng/ml. Concentrations of MDA a naturally occurring product of lipid peroxidation, were determined by Abnova ELISA Kit (Cat.N.KA3736; Abnova Corporation, Taipei, Taiwan) and TAC was measured using a commercially available kit (Zell Bio GmbH, Wurtemberg, Germany).

Ovarian stimulation and oocyte pick-up

A GnRH agonist or an antagonist treatment was applied for controlled ovarian stimulation according to the patient characteristics. Recombinant follicle-stimulating hormone (GONAL-f, Merck Serono, Geneva, Switzerland) was used in all cases. Human chorionic gonadotropin (hCG) (Ovitrelle; Merck Serono) was administered subcutaneously when the dominant follicle had reached a mean diameter of ~18 mm. Oocyte pick-up was performed 36 hours after hCG administration, under general anesthesia, with a 17-Gdual lumen needle (Swemed, New York, NY, USA) through transvaginal ultrasound guidance. Retrieved oocytes were collected in a MOPS (3 morpholino propanesulfonic acid) buffered medium (G-MOPS; Vitrolife, Gothenburg, Sweden) under oil (Ovoil; Vitrolife), at 37°C. After denudation of the cumulus–oocyte complexes by using two different diameters of denudation pipettes (170–140 μm) and by enzymatic denudation with 40 IU hyaluronidase (Vitrolife) 2 hours after oocyte pick-up, oocytes were placed in a CO_2–O_2-controlled incubator (ESCO, Singapore) for incubation [13].

Preparation of sperm

Ejaculated spermatozoa were obtained by masturbation after 3–5 days of ejaculatory abstinence. After liquefaction of semen at room temperature, sperm samples were prepared by discontinuous density-gradient centrifugation according to WHO criteria [14].

ICSI, embryo culture and pregnancy outcome

Mature (MII) oocytes were identified by the presence of the first polar body under a stereomicroscope (Olympus, Tokyo, Japan). Only those oocytes that had extruded the first polar body (MII oocytes) were used for ICSI. At 16–18 hours after ICSI, fertilization was confirmed by presence of two pronuclei (2PN) and rate of fertilization was calculated. Seventy two hours after ICSI, embryo quality was assessed based on a three-point scoring system: 1) absence or fragmentation of <25% on embryonic surface, 2) equality of blastomere's size and shape, and 3) blastomeres cell number greater or less than 7. Embryos presenting all parameters were scored as "A", embryos having only 2 parameters were scored as "B" and embryos presenting only one of the parameters were scored as "C". By adding number of score, A and B embryos over total number of scored embryos (A+B+C), percentage of top-quality embryos were calculated [15]. Biochemical pregnancy was measured 14 days after transfer by Human B-hCG ELISA Kit (Abnova 100533). Clinical pregnancy was obtained through presenting a gestational sac as well as heartbeat on ultrasound scans conducted six weeks following embryo transfer. The implantation rate (sacs with a heartbeat); miscarriage (pregnancy loss before gestational week 12); and live birth (viable neonate at ≥30 weeks of gestation) were define.

Statistical analysis

The statistical analysis was performed using IBM SPSS version 21.0 (IBM Corp., Armonk, NY, USA). The population normality was verified with the Kolmogorov–Smirnov test, and data are reported as mean±SEM. The differences between the study parameters before and after the treatment have been evaluated with the *t*-test for the paired samples. Statistical analysis between the two treatments groups have been evaluated with independent *t*-test. *P*-values ≤0.05 were considered to be significantly different.

Results

Demographic characteristics and sperm quality

There were no significant differences in age, duration of infertility, BMI and high between two groups (MET and MET+ALA) before treatment (Table 1). Analysis of the man partner's sperm parameters revealed no significant differences among two groups (Table 2).

Evaluation of biochemical and endocrine characteristics

All biochemical and hormonal parameters of patients under study are summarized in Table 3. FBS level significantly reduced in both groups compared to baseline: MET group (*P*=0.04) and in MET+ALA group (*P*=0.01). As expected, insulin level decreased significantly in MET group (*P*=0.03)

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Table 1. Comparison of demographic mean between MET and MET+ALA groups

Parameter	MET group			MET+ALA group		
	Before treat	After treat	*P*-value	Before treat	After treat	*P*-value
Age (yr)	29.54±3.45	29.54±3.45	0.1	28.75±3.39	28.75±3.39	0.1
High (cm)	163.32±1.1	163.32±1.1	0.11	160.53±1.3	160.53±1.3	0.11
BMI (kg/m^2)	27.60±4.20	26.8±4.00	0.09	28.02±5.37	27.1±5.17	0.08
Duration of infertility (yr)	6.34	6.34	0.9	7.23	7.23	0.1

Values are presented as mean±SEM. MET group: administered 1,500 mg/day metformin (MET); MET+ALA group: MET (1,500 mg/day)+ALA (1,800 mg/day). MET, metformin; ALA, alpha lipoic acid; BMI, body mass index.

Table 2. Characteristics of semen parameters between MET and MET+ALA groups

Parameter	MET group	MET+ALA group	*P*-value
Man age (yr)	33.85±4.5	36.25±5.22	0.13
Volume (ml)	2.3±0.19	2.2±0.14	0.16
Sperm concentration (×10^6/ml)	97±6.32	94±8.03	0.43
Total sperm count (×10^6/ejaculate)	184±6.66	181±5.45	0.55
Total sperm motility (%)	60±2.12	58±2.22	0.66
Sperm normal morphology (%)	4.2±0.22	4.8±0.72	0.30

Values are presented as mean±SEM. MET group: administered 1,500 mg/day metformin (MET); MET+ALA group: MET (1,500 mg/day)+ALA (1,800 mg/day). MET, metformin; ALA, alpha lipoic acid.

Table 3. Comparison of biochemical and hormonal parameters from polycystic ovary syndrome (PCOS) patients

Parameter	MET group			MET+ALA group		
	Before treat	After treat	*P*-value	Before treat	After treat	*P*-value
FBS (mg/dl)	98.50±2.93	95.42±2.21	0.04	97.40±2.30	91.30±3.50	0.01
Insulin (mU/L)	6.39±4.62	5.02±3.72	0.03	7.05±2.74	4.14±7.75	0.01
LH (mUL/ml)	10.53±0.38	9.13±0.22	0.09	11.53±0.31	7.3±0.44	0.01
FSH (mUL/ml)	5.18±0.12	5.08±0.22	0.15	6.68±0.22	5.06±0.45	0.08
LH/FSH	2.03±0.12	1.79±0.16	0.09	1.72±0.21	1.38±0.34	0.01
TT (ng/ml)	2.13±0.18	1.9±0.11	0.09	1.13±0.14	0.9±0.11	0.09
E2 (pg/ml)	71.55±4.31	69.23±3.88	0.14	69.53±3.31	67.23±3.37	0.15
MDA (mmol/L)	1.14±0.11	1.11±0.08	0.08	2.70±0.06	2.51±0.04	0.01
TAC (mmol/L)	2.55±0.41	2.88±0.22	0.06	2.04±0.06	3.11±0.11	0.002

Values are presented as mean±SD. MET group: administered 1,500 mg/day metformin (MET); MET+ALA group: MET (1,500 mg/day)+ALA (1,800 mg/day). MET, metformin; ALA, alpha lipoic acid; ALA, alpha lipoic acid; FBS, fasting blood sugar; LH, luteinizing hormone; FSH, follicle stimulating hormone; TT, total testosterone; E2, estradiol; MDA, malondialdehyde; TAC, total antioxidant capacity.

and in MET+ALA group (*P*=0.01) compared to baseline. Analyze other tests such as LH, FSH, LH/FSH, TT, estradiol (E2), MDA and TAC levels in the MET group did not change significantly, but surprisingly, in MET+ALA group, LH (*P*=0.01) and LH/FSH reduced (*P*=0.01), whether there was no significant difference with respect of ALA in FSH level (*P*=0.08), TT level (*P*=0.09), and E2 level (*P*=0.15).

Comparison of level of lipid peroxidation and total antioxidant capacity before and after treatment

MDA levels were significantly lower after treatment with MET+ALA (2.70±0.06 vs. 2.51±0.04 mmol/L; *P*=0.01) but MAD did not significantly differ in MET group (1.14±0.11 vs. 1.11±0.08 mmol/L; *P*=0.08). Levels of TAC significantly increased in MET+ALA group compared to baseline (2.04±0.06 vs. 3.11±0.11 mmol/L; *P*=0.002) while TAC in MET group was approximately similar to baseline (2.55±0.41 vs. 2.88±0.22 mmol/L; *P*=0.06) (Table 3).

Evaluation of oocytes, embryos morphology and pregnancy

Table 4 compares the number of retrieved oocyte, mature (MII) and immature oocytes (GV and MI), viable embryos and their grade (A, AB, B, and C) and pregnancy between the MET and MET+ALA groups. The total number of oocytes retrieved in the MET group were statistically different compared to the MET+ALA group (*P*=0.039). Number of mature oocytes in MET group were approximately similar

Table 4. Distribution of oocytes retrieved, quality of oocytes and embryos outcome in polycystic ovary syndrome (PCOS) patients

Parameter	MET group	MET+ALA group	*P*-value
No. of oocytes retrieval	17.06±0.84	14.86±0.56	0.039
No. of mature oocytes (MII)	12.26±0.75	12.93±0.70	0.7
No. of immature oocyte (GV+MI)	4.08±0.48	1.93±0.39	0.01
No. of fertilized oocyte (2PN)	6.93± 0.63	10.00±0.57	0.04
No. of embryo	8.80±0.62	11.13±0.60	0.01
No. of embryo quality grade A	1.53±0.49	4.00±0.89	0.02
No. of embryo quality grade B	2.4±0.43	5.3±0.57	0.004
Chemical pregnancy rate (%)	31.00	34.88	0.11
Clinical pregnancy rate (%)	25.00	28.80	0.09
Miscarriage rate (%)	7.29	8.52	0.472
Live birth rate (%)	13.37	7.38	0.08

Values are presented as mean±SEM. MET group: administered 1,500 mg/day metformin (MET); MET+ALA group: MET (1,500 mg/day)+ALA (1,800 mg/day). MET, metformin; ALA, alpha lipoic acid; 2PN, two pronuclei.

to MET+ALA group (*P*=0.7). Whereas number of immature oocytes significantly reduced in MET+ALA group compared with MET group (*P*=0.01). With respect to ALA, there was a significantly increase in number of fertilized oocyte (2PN) in MET+ALA group compared to MET group (*P*=0.04). Number of embryo significantly increased in MET+ALA group compared to MET group (*P*=0.01). In general, the quality of embryo in MET+ALA group showed a statistically significant increase compared to MET group (grade A, *P*=0.02 and grade B, *P*=0.004). Also number of embryo quality grade C significantly reduced in MET+ALA group compared to MET group (*P*=0.04). We found no differences in biochemical pregnancy between the groups (MET+ALA group, 34.88%; MET group 31%; *P*=0.11). Percentages of clinical pregnancy in study with MET+ALA and MET groups (28.8% and 25.0%; *P*=0.09). No significant differences between the groups. Finally, no apparent differences were observed between the groups in Live birth rates, and terms of miscarriage rate between two groups (*P*>0.05).

Discussion

Although many studies have been performed on PCOS infertility, it is still one of the most challenging research topics in infertility treatment [16]. This syndrome is associated with complications such as hormonal disorders, increased oxidative stress, and poor ART results [17]. It causes an increased number of low-quality oocytes following ovulation induction, which leads to reduced fertilization, cleavage, and implantation and increased embryonic fragmentation, and a higher abortion rate [18]. In this study, we tried to measure the effects of MET alone and in combination with ALA on endocrine parameters, the quality of oocytes , embryos and ART outcomes in PCOS women undergoing ICSI. The positive effects of MET have been demonstrated in many studies to reduce the output of hepatic glucose, decline insulin concentrations, and decrease the androgen production by theca cells [19, 20]. On the other hand, in very recent times, ALA has been considered a possible therapeutic approach to PCOS. ALA is a powerful antioxidant molecules that can act as a scavenger of the ROS and can regenerate other antioxidant molecules (vitamin E and C) [21]. Considering the important role of MET as an insulin sensitizer and the positive effects of ALA as an antioxidant supplement, we decided to investigate a combination therapy with MET and ALA in the course of ovarian stimulation during the ICSI cycle.

Our results revealed that MET alone significantly decreased glucose and insulin levels in the blood but did not affect significantly LH, FSH, TT, and E2 hormone levels. These findings are consistent with those of other studies [22, 23]. But Cheraghi et al. [24] reported that MET made changes in insulin and LH levels significantly after treatment compared to baseline. The different results reported by other studies are likely due to differences in treatment duration and the dose of MET, as well as genetic differences in the populations studied [25]. PCOS patients treated with MET+ALA consumption, showed positive results on the hormone analysis. MET+ALA consumption was significantly effective in the reduction of insulin, LH, LH/FSH. Also, MET+ALA consumption was significantly effective in the reduction of MDA levels through antioxidant actions. It is possible that ALA could reduce oxidative stress also in the ovary. We may speculate that ALA might contribute to the restoration of a normal environment in the ovary increasing the positive effect of MET.

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These observations are in line with previous reports on ALA, which is considered to play an important role in the pathogenesis of PCOS that influences LH production [21]. Besides, MET+ALA significantly increased the number of oocytes retrieval and maturated oocytes (MII) in compared with MET group and subsequently rate of fertilization significantly increased when compared to the MET group. Modifications in the dose and/or duration of treatment, sample size, and ovulation induction protocols may improve the results. This finding is in line with the results reported by Lei et al. [26], which showed the positive effects of Myo-inositol (MI) and α-lipoic acid on the reproductive outcomes of PCOS patients. Also, Fruzzetti et al. [2] showed that ALA+MI regulated irregular menstrual cycles in women with PCOS. It has been recently reported that the combination of three antioxidants (Acetyl-L-carnitine, N-Acetyl-L-cysteine, and ALA) had a positive effect on the fetal development of embryos in a murine IVF model [27]. Various *in vivo* and *in vitro* studies on protective effect of ALA, introduced ALA as an effective antioxidant in improve follicular development. This improvement might be due to a decrease in ROS levels and an increase in TAC in the follicle [28]. A significant correlation was found between the increased MDA and oxidative stress and the TT and insulin levels in PCOS patients [29]. Consistent with the studies mentioned, we also observed in this study, combination of MET+ALA could decrease elevated oxidative stress in PCOS patients more effectively, which is likely due to its antioxidant and anti-apoptotic properties. In contrast no significant decrease in MDA concentrations was observed following MET administration. Increasing insulin receptor substrate 1 (IRS-1) expression was caused by ALA as a potent antioxidant, which decrease species of ROS, subsequently by phosphorylating IRS-1, caused to reduce insulin resistance [30]. Therefore, it is probably the reason for the increase in the quality of embryos observed in the MET+ALA treated group. In this study, we found that ALA, combined with MET, was able to reduce MDA concentration and subsequently increase fertilization and embryo quality rate, and while level of MDA did not decrease in PCOS patients taking MET alone. The present study is the first study focusing on the effects of MET in combination with ALA on the quality of oocytes and embryos in PCOS patients undergoing ICSI. Although MET+ALA is believed to be useful for oocyte maturation and embryo quality, but the pregnancy rate did not differ significantly among the all groups. However, considering the fact that MET+ALA improves embryo quality, The Pregnancy rate seems to depend on other factors that require further study. To summarize, our data revealed that ALA treatment reduced the number of immature oocytes, increased the number of good embryos formed, and lowered the concentrations of endocrine parameters such as insulin, LH, and MDA levels.

In conclusion, the results of the present prospective randomized single-blinded trial showed for the first time that the use of ALA+MET may have a positive effect on the side effects experienced by PCOS women and ART outcomes. Besides, ALA+MET can reduce FBS and insulin resistance and regulate hormonal disorders. Since alpha-lipoic as a useful antioxidant can regenerate other antioxidants, the TAC increases in these patients. This can increase the quality of the obtained oocytes and eventually, improve the outcomes of assisted reproduction techniques. In the end, it is suggested that further molecular studies be conducted on the role of ALA on metabolic pathways in obese infertile women with PCOS.

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Conceptualization: RJ, HP, SSS, EA. Data acquisition: RJ, HP, SSS, EA. Data analysis or interpretation: RJ, HP, SSS, EA. Drafting of the manuscript: RJ, HP, SSS, EA. Critical revision of the manuscript: RJ, HP, SSS, EA. Approval of the final version of the manuscript: all authors.

Conflicts of Interest

No potential conflict of interest relevant to this article was reported.

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