

Effect of metformin and antioxidant agents on oxidative stress status and follicular maturation in women with polycystic ovary syndrome

Sulaiman R. Waleed
Al-Omari R. Wafa
Taher A. Mohammed*

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الخلاصة

في هذه الدراسة شاركت أثنان وأربعون امرأة عراقية مصابة بمتلازمة المبيض متعدد الأكياس وقد قسمن إلى ثلاثة مجموعات حسب نوع العلاج:
(1) المجموعة الأولى : وتشمل (14) مريضة عولجن بعقار المتفورمين وبجرعة 500 ملغم ثلاث مرات يوميا.
(2) المجموعة الثانية : وتشمل (14) مريضة عولجن بمركب مكون من المتفورمين بجرعة 500 ملغم ثلاث مرات يوميا وفيتامين (C) بجرعة 250 ملغم ثلاث مرات يوميا.
(3) المجموعة الثالثة :- وتشمل 14 مريضة عولجن بمركب مكون من عقار المتفورمين بجرعة 500 ملغم ثلاث مرات يوميا مع عقار فيتامين (E) بجرعة 200 ملغم ثلاث مرات يوميا. كل هذه المجموعات عولجت لمدة ثلاثة اشهر.
تم اخذ نماذج من دم سحبت المريضات والصحيحات الصائمات على الأقل لمدة 12 ساعة وبصورة دورية لمدة ثلاثة اشهر بحيث يؤخذ نموذج الدم مرة واحدة في الشهر وهي الفترة العلاجية للمريضات ، لغرض قياس وتحديد مستوى المالون ثنائي الالديهيد في مصل الدم.
وجد في هذه الدراسة أن أكثر المريضات بمتلازمة المبيض متعدد الأكياس يعانين الضغط التأكسدي الذي يجب أن لا يهمل عند العلاج. وجد كذلك إن فيتامين E وتأريضه له دور مهم في تخفيف الضغط التأكسدي ويؤازر فعالية المتفورمين في انضاج الجريبات.

ABSTRACT

In this study, forty-two Iraqi Arab Women with PCOS were involved and they were classified into three groups according to type of treatment:

- 1) Group I: fourteen patients who administered metformin (500mg t.i.d).
- 2) Group II: fourteen patients who administered combination of metformin (500mg t.i.d) with vitamin C (250mg t.i.d)
- 3) Group III: fourteen patients who administered combination of metformin (500mg t.i.d) with vitamin E (200mg t.i.d). All groups were treated for three months .

The present study includes the measurement of serum malondialdehyde, Vitamin E and its metabolite (tocopheronic acid) play an important role in alleviation of oxidative stress(MDA) and may potentiate the metformin activity in maturation of follicles

INTRODUCTION :

Polycystic ovary syndrome (PCOS) is characterized clinically by a history of chronic anovulation in combination with some evidence of androgen excess, such as hirsutism and acne. The classic picture of PCOS is a string of small follicles (2-8mm in diameter) arranged like a necklace completely encircling the cortical surfaces of the ovary or in other word there are multiple follicles arrested at prophase of their development⁽¹⁾.

* Clinical Biochemistry Department, College of Pharmacy, Baghdad University, Baghdad – Iraq.

Factors such as insulin resistance, elevated ratio of luteinizing hormone (LH) to follicle stimulating hormone (FSH) and ovaries morphology on ultrasonography were considered to be possible criteria of diagnosis of PCOS. However, participants at the 1990 National Institute of Health Conference on PCOS supported a definition of PCOS that included three key features:

- 1) Oligomenorrhea
- 2) Hyperandrogenism (clinical examination or laboratory measurement)
- 3) The absence of other endocrine disorders includes non-classical adrenal hyperplasia, androgen-secreting tumors, and hyperprolactinemia and thyroid dysfunction⁽²⁾.

Reactive oxygen species are implicated in many disease⁽³⁾. Recent studies indicate that in hypertensive patients, there is an elevated free radical generation and increased lipid peroxidation⁽⁴⁾, and there is an imbalance in antioxidant status⁽⁵⁾. Several studies hypothesized that in hypertension there is an impaired endothelial vasodilator response related to reduced bioactivity of endothelium derived relaxing factor mainly nitric oxide by the free radical superoxide anion, thereby decreasing its vasodilator action which lead to an increase in peripheral vascular resistance and hypertension⁽⁶⁾.

There is no single drug to treat PCOS, because of the presence of more than one etiological factor of PCOS. But there is new strategy of treatment that depends on correcting insulin resistance and hyperinsulinemia, which have the effect to treat all the presentations of PCOS. Recently some workers used the combination of insulin sensitizing agent (rosiglitazone) with clomiphene as an effective therapeutic regimen for correcting the insulin resistance in patients with PCOS and by modifying the hyperandrogenic intrafollicular milieu that occur in PCOS⁽⁷⁾.

Aim of the study:

To compare the advantages of the use of antioxidants as an adjuvant therapy to metformin in PCOS patients on mature follicular size.

PATIENTS AND METHODS:

Patients:

Forty-two Iraqi Women with PCOS mean age (28.1±4.50) were involved in this study. The patients were attending the out patient clinic at both Baghdad Teaching Hospital (Department of Obstetric & Gynecology) at medical city and Al- Elwea Hospital for Obstetric & Gynecology. Diagnosis was based on clinical symptoms, ultrasonography of the ovaries, and on the some biochemical and hormonal changes. Patients with the following conditions were excluded:

1. Patients with serum creatinin level more than 0.8 g/dl.
2. Patients with thyroid dysfunction.
3. Patients with hyperprolactenemia.
4. Patients with plasma glucose >115mg/100ml

The patients were classified according to the type of treatment as follow:

Group 1: fourteen patients who were using metformin (500 mg t.i.d).

Group 2: fourteen patients who were using the combination of metformin (500mg t.i.d) with vitamin C (250 mg t.i.d) as an antioxidant agent.

Group 3: fourteen patients who were using the combination of metformin (500mg t.i.d) with vitamin E (200 mg t.i.d.) as an antioxidant agent.

The patients in all these groups are maintained on the treatment for a period of twelve weeks.

Group 4: normal eleven healthy volunteers of women with regular menstrual cycle (interval between 21 and 35 days) were selected as controls The age was comparable to that of the patients. All subjects were free of medical illness and had not received any medications for ≥3 months before enrollment.

Sample Collection:

Five mls of venous blood sample were drawn from each patient and control after 12 hours fasting and this process was done before taking the drug and after each 4 weeks of treatment for a period of 12 weeks. The sample was transferred into a clean plain tube, left at room temperature for 30 minutes for clotting, centrifuged, and then serum was separated and transferred into plain tube, which was used for measuring the malondialdehyde (MDA)

Methods:

Determination of malondialdehyde

MDA formed from breakdown of polyunsaturated fatty acids, serves as a convenient index of peroxidation reaction. The thiobarbituric acid (TBA) method of Buege and Aust (8) was used to measure the MDA, which react with TBA to give a pink color that is read at 535 nm. The MDA concentrations were calculated using molar extinction coefficient of 1.5×10^5 . The results were expressed as $\mu\text{mol MDA/L serum}$.

Ultrasound study

An ultrasound scan is performed for each patient at about day 12 of the cycle to monitor follicular development and, if necessary, repeated at 3-days intervals to detect a delayed response. This procedure is carried out before taking the drugs and after starting the administration monthly for a period of three months.

Statistical analysis

The results were expressed as mean \pm SD.

Student T- test was used to examine the difference in the mean of parameters tested.

P – Value of less than (0.05) was considered significant.

RESULTS AND DISCUSSION:

An ultrasound scan is used usually to follow up follicular events as an indicator of improvement of fertility in PCOS. It can be said that because of mature follicles are prerequisite for an ovulation, so it is the best to show the effect of metformin and antioxidant agents by the changes occur on the immature follicular cysts in the ovaries of PCOS patients. The data presented in this study revealed that the effect of metformin alone or in combination with vitamin C on the arrested follicles was an improvement in just two cases of each group (table 2) to get follicular size of diameter equal or more than (1.7 cm); this means that 14.4% of patients may get an ovulation when treated with metformin either alone or in combination with vitamin C.

Table 2 . Dominant follicles size in patients after 12 weeks of treatment for all groups.

Groups	Number of patients	Number of patients with follicle size < 1.7 cm.	Number of patients with follicle size \geq 1.7 cm.
I	14	12	2
II	14	12	2
III	14	4	10

After treatment with a combination of metformin and vitamin E, the results showed a remarkable improvement because 10 patients (71%) developed follicles size equal or more than (1.7cm) (table 2 Fig. 1 and 2). Although these results do not mean that all patients who get mature follicles are able to ovulate but this means at least that oxidative stress may play an important role in the pathogenesis of PCOS. However, this study may be the first one that refers to oxidative study and consequently using the antioxidant agents in the treatment of PCOS.

Ultrasound study:

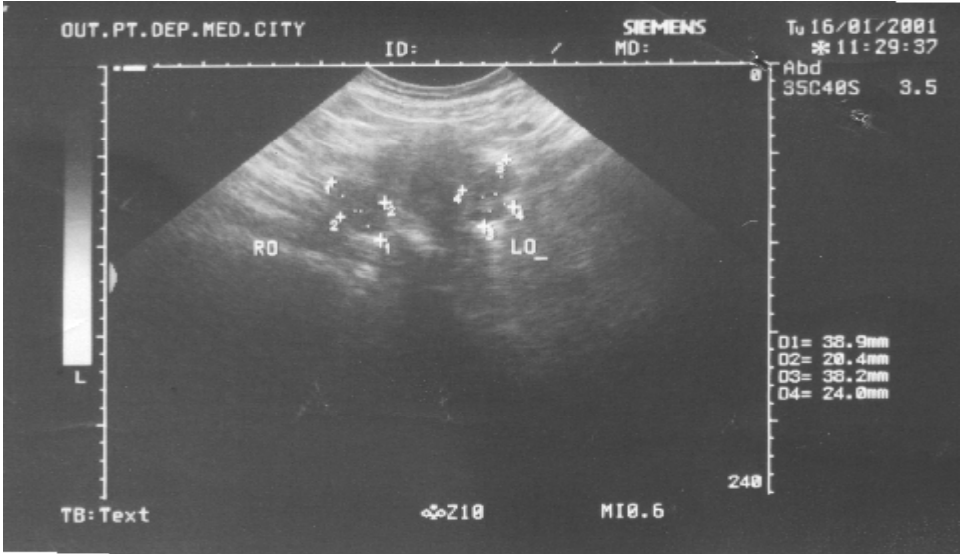


FIG1 . ULTRASOUND SCAN (BEFORE TREATMENT) SHOWING BOTH OVARIES CONTAIN MULTIPLE TINY CYSTIC SHADOW (BILATERAL POLYCYSTIC OVARIES).



FIG 2 . ULTRASOUND SCAN (AFTER 12 WEEKS TREATMENT WITH METFORMIN AND VIT.E) SHOWING RIGHT OVARY CONTAINS A DOMINANT FOLLICLE OF (1.7 X 1.7) CM.

Serum Malondialdehyde (MDA)

Table – 1 reveals elevated mean value for MDA in the sera of patients complained to the control group.

Table 1 . Concentration of MDA in patients with PCOS and control group before treatment.

Variables	Control value	Base line value of patients		
	(n=11)	Group 1 (n=14)	Group II(n=14)	Group III(n=14)
MDA($\mu\text{mol/l}$)	0.42 \pm 0.06	0.75 \pm 0.15**	0.79 \pm 0.46 *	0.98 \pm 0.80 *

* P \leq 0.05

** p \leq 0.005

n= number of subjects

However, most patients participated in this study, showed reduction in serum MDA after the course of treatment (table–3) suggesting that additions of antioxidant agents may at least in part are of value as an adjuvant therapy to insulin sensitizer.

Table 3 . Effect of metformin and antioxidant agents on serum malondialdehyde (MDA) levels in patients with PCOS.

Groups	Patients No.	Base line value	After 4 weeks	After 8 weeks	After 12 weeks
I	14	0.75 \pm 0.15	0.70 \pm 0.42	0.52 \pm 0.09**	0.50 \pm 0.17*
II	14	0.79 \pm 0.46	0.73 \pm 0.44	0.52 \pm 0.06*	0.48 \pm 0.06*
III	14	0.98 \pm 0.8	0.68 \pm 0.21	0.55 \pm 0.1	0.42 \pm 0.05*

* P $<$ 0.05

** P $<$ 0.005

The progressive and significant reduction in MDA with combination of metformin and vitamin E is a further support for this suggestion. Although the reduction of MDA was in all groups but response of other parameters were not at the same way, this may enhance the believing that oxidative stress is part of pathogenic factors that lead to heterogenic symptoms of PCOS. The clinical advantage of vitamins C and E as antioxidants were well established⁽⁹⁾; but the action of metformin in reduction of MDA is not clear and need further investigations. It is possible that metformin act indirectly and as a consequence of alleviation in insulin resistance and by improving insulin sensitivity may cause a reduction of MDA levels in PCOS patients.

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