

Estimation of catalase activity and Malondialdehyde levels in blood groups ABO of PCOS patients

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Abstract

The present study has been carried out at the Women's and Educational Children's Hospital in the city of Diwaniyah to determine level of oxidative stress indicators

(57) samples of patient women with PCOS were collected and observation of some clinical signs were followed, in addition to confirming the results of ultrasonic ultrasound tests, the group of women patient with PCOS was divided into four subgroups according to the type of blood group, (8) samples of blood group A, (21) sample Of the blood group B, (7) of the AB blood group and (21) of the blood group O. 30 samples of the non-polycystic ovarian syndrome women were selected. They did not suffer from chronic diseases and were considered a control group to measure the level the activity of Catalase enzyme (CAT) and concentration levels of malondialdehyde MDA as oxidative stress indicators by using the Spectrophotometer

The results showed a significant decrease ($p < 0.05$) in the efficacy of catalase and a significant increase ($p < 0.05$) in the level of the MDA in women with PCOS compared to control group, in addition to decrease of CAT enzyme activity and an increase in the level of MDA were more in the blood group O compared with the other blood groups.

Keywords: PCOS, CAT, MDA, ABO blood groups.

Introduction

Polycystic ovarian syndrome (PCOS) is a disorder of the endocrine and metabolic processes and is more common in women of childbearing age (1), where it occurs by 17.8% and is characterized by irregular menstrual with increase Hyperandrogenism, non-ovulation, polycystic ovaries (2), in addition to disorders of metabolism, leading to aberrant metabolic profile, Obesity, insulin resistance (IR) and hyperinsulinism (3).

The causes of PCOS are unclear and can be shared by several factors. There is a common relationship between hyperinsulinemia and excessive hyperandrogenism in women with PCOS resulting from a change in the ratio of LH/FSH due to disorder in the secretion for the GnRH (4)

PCOS is a condition that shows a significant reduction in antioxidants and serum vitamin levels and these women are at increased risk of developing oxidative stress (5),

which increases their rate of hypertension and high cholesterol levels, which leads to a condition known as dyslipidemia (6) Which causes metabolic syndrome (7). PCOS may lead to ovarian cancer, which results from increased gene expression of TRAF2 genes (8).

PCOS syndrome is responsible for the failure to complete the growth of normal ovarian follicles during the menstrual cycle. There is a change in the level of hormone LH / FSH due to the presence of inappropriate amounts of FSH produced by the pituitary gland as well as a high level of LH and thus do not occur ovulation and remain follicles The lower the level of the enzyme aromatase, which converts the testosterone (T) to an estrogen (E) and therefore the production of T and when the increase in its production leads to increased positive feedback of the E-secretion ,which lead to increase of LH from pituitary gland, which results from an increase in the formation of ovarian hormones Which in turn lead to an increase in the secretion of androgen as the secretion of the androgen by stimulation of the hormone lutein and in this case leads to a relative decrease in the FSH (9).

Materials and Methods

Study design

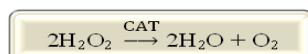
The present study was conducted at the Women's and Children's Educational Hospital in Diwaniyah city and included (57) samples of women with PCOS (ages between 22-34 years) followed by note that some clinical signs such as obesity, irregular menstrual and acne, as well as confirmation of tests ultrasonic. women patient with PCOS was divided into four subgroups depending on the type of blood group , included (8) sample of blood group A , (21) samples of blood group B , (7) samples of blood group AB (21) samples of the blood group O.(30) samples of women without PCOS were selected.

Collection of blood samples

Intravenous blood was withdrawn during the second or third day of the menstrual cycle. In the absence of the menstrual blood samples are collected on any day, blood samples left for 20 minutes at room temperature then, placed in a centrifuge with speed of 3000 rpm / minutes for 3-5 minutes after which, serum was withdrawn and placed in new test tubes and kept in deep freezing until use for the purpose of measuring oxidative stress indicators.

Determination of catalase activity (CAT) (u\ml)

Catalase activity was estimated by spectronic method ((Aebi, 1974). This method depends on the effectiveness of catalase in the decomposition of hydrogen peroxide, and its effectiveness is measured by the low absorption of hydrogen peroxide (Mueller et al., 1997) as in the following equation:



Testing Procedure: 50 µl of the sample was taken and diluted with 50 µl of Phosphate buffer solution (PBS) pH 7 condition, and then two sets of test tubes were prepared as follows:

Reagents	Sample	Blank
Serum	2 ml	2 ml
PBS (pH 7.0)	-	1ml
H ₂ O ₂	1 ml	-

The reaction was started after the addition of H₂O₂ to each test tube. After the mixture was mixed, a reading of the sample was read after 15 seconds. The first reading was recorded (d1) and after 30 seconds the second reading (t2) Samples at a wavelength of 240 nm by spectral spectrometer Apple PD-303 UV spectrometer, then efficiency of the catalase enzyme was calculated using the following equation:

$$\text{Catalase activity (U/ml)} = \frac{(\Delta A_s - \Delta A_o) \times df}{\epsilon \times Tv \times \Delta t \times L}$$

ΔA_s : The difference between the two readings of the sample

ΔA_o : The difference between two readings of a cell , Df : mitigation factor

ϵ : Absorption factor (0.0436 M⁻¹ cm⁻¹ , Tv : Overall size

L : Path of Light , Δt : The difference between the reading time

Determination of malondialdehyde (MDA) concentration

The level of Malondialdehyde was estimated in the serum using the modified method used by the Guidet and Shah (1989) based on this method, serum lipid peroxide was estimated by measuring the amount of malondialdehyde, which is one of the main products of lipid peroxidation. This method depended on The interaction between serum malondialdehyde and the thiobarbituric acid (TBA). This reaction is done in acidic condition and the reaction product was colored. Absorption intensity was measured at a wavelength at 539 nm.

Add 1ml of (0.6%) TBA and 1 ml of trichloroacetic acid 17.5% (TCA) to 150 µl of sample, mix well and then put in a water bath for 15 minutes then leave to cool down, then add 1ml (70%)TCA, all tubes left at room temperature for 20 minutes , then placed in a centrifuge at 4500 rpm for 15 minutes. Supernatants from each tube was Transfer and put in new test tubes. Absorption intensity was measured at a wavelength 539 nm by using the Spectrophotometer (PD-303uv).

To Calculate concentration of MDA used following equation

$$D \times \text{MDA Con} = \frac{A \text{ of sample at } 532 \text{ nm}}{L \times \epsilon}$$

L = track length (1cm) ,

ϵ = absorption coefficient (1.56 X 10⁵ M⁻¹ cm⁻¹)

D= dilution factor

Volume used in reference = D Total size / sample size

D = 0.15 + 1 + 1 + 1 \ 0.15 = 21

Statistical Analysis

To analysis of results, Graph Prism 7 has been used to determine the significant differences in the criteria included in the current study, the unpaired t-test was used to comparison between the control groups and PCOS group. Whereas ANOVA one way were used at level of 0.05 to comparing between blood groups ABO of PCOS patients.

Results

Estimation of CAT activity and MDA concentration in women patient with PCOS compare with control.

The results of the statistical analysis of the efficacy of the catalase enzyme (table 1 and figure 1A) showed a significant decrease $P < 0.0001$ in the efficacy of catalase in women with PCOS compared with women in the control group. On other hand, the results proved significant increased $P < 0.0001$ in the level of MDA in women with PCOS compared to women in the control group (Table 1 and Figure 1B).

Table (1) The level of efficacy of CAT (u \ ml) and the concentration of MDA (μ mole / L) in women with PCOS compared to control group

oxidative stress indicator	group	mean \pm SE	p value	t-test	Confidence period 95% CI
CAT activity(u\ml)	C	3.823 \pm 0.3622 a	****<0.0001	8.224	-3.4 to -2.044
	PCOS	1.101 \pm 0.1508 b			
MDAconcen (μ mole /L)	C	1.102 \pm 0.06898 ^a	****<0.0001	7.436	1.282 to 2.257
	PCOS	2.871 \pm 0.1634 b			

Values represent the averages \pm standard error. The different letters indicate a significant difference in the probability level $P < 0.0001$ between control groups, women with PCOS, t test values and confidence interval at 59%

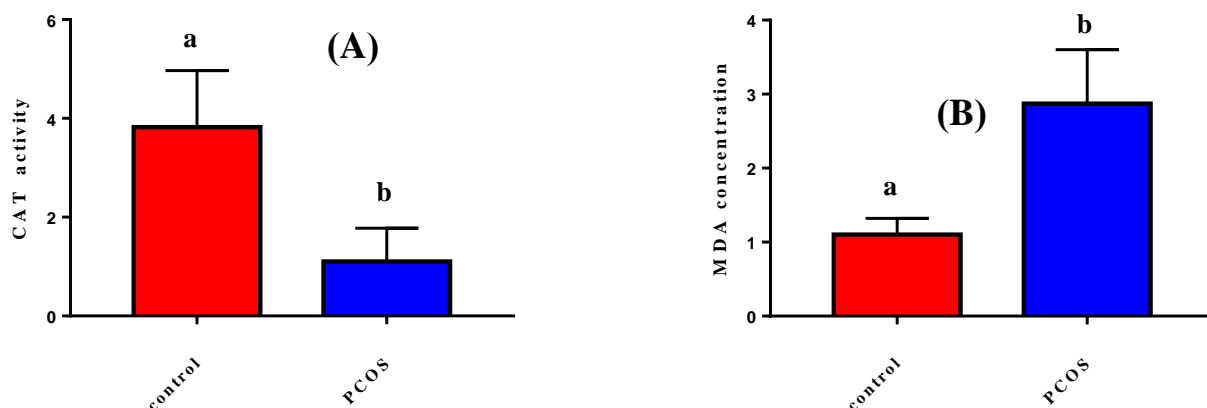


Figure (1) shows the level of efficacy of the CAT (A) and serum MDA (B) concentration in infected women compared with the control group. represents the group without PCOS and PCOS: represent the women with PCOS and the letters indicate a significant difference $P < 0.0001$.

Comparison of CAT activity and MDA concentration between blood groups ABO of women patient with PCOS

The results of CAT enzyme activity (table 2 ,figure 2A) recorded significant decreased $p < 0.05$ in CAT enzyme activity of women patient with PCOS for blood groups A, B and O compared to AB group . This reduction was more pronounced in the blood group O compared with the other blood groups, while no significant differences between groups A and B ($P > 0.05$).

data analysis of the levels of serum MDA (table 2 , figure 2B) indicated the presence of significant increases $p < 0.05$ of MDA of women patient with PCOS for blood groups A, B and O compared to AB group . This increment was more in the blood group O compared with the other blood groups, while no significant differences were recorded between groups A and B ($P > 0.05$)

Table (2) The level of efficacy of CAT (μml) and the concentration of MDA ($\mu\text{mole / L}$) between the ABO blood groups of women with PCOS

ABO groups (pcos)	mean \pm SE	
	CAT activity (μml)	MDA concen ($\mu\text{mole / L}$)
Group A	1.293 \pm 0.114 a	2.482 \pm 0.1503 a
Group B	1.304 \pm 0.1199 a	2.632 \pm 0.17103 a
Group AB	1.845 \pm 0.1131 b	1.779 \pm 0.143 b
Group O	0.4723 \pm 0.1399 c	3.579 \pm 0.1677 c

The values represent the averages \pm the standard error and the different letters indicate a significant difference ($p < 0.05$) while the similar letters indicate no significant difference ($p > 0.05$)

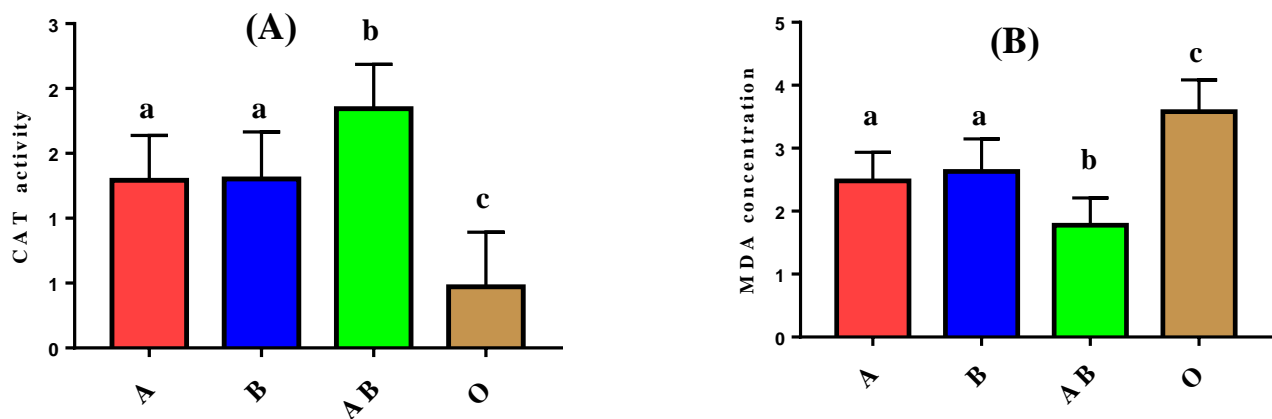


Figure (2) shows the level of efficacy of the CAT (A) and serum MDA (B) concentration in infected women compared with the control group. represents the group without PCOS and PCOS: represent the women with poly ovarian poly cyst e syndrome and the letters indicate a significant difference $P < 0.0001$.

Discussion

ROS and antioxidants are key factors involved in the physiological metabolism of the ovary. Several studies have demonstrated the presence of antioxidants in the female reproductive system (10). The balance of ROS and antioxidants has been found to significantly affect reproductive activity Such as changes in the endometrium of the luteal phase, follicular development, ovulation, fertilization, placental growth, and fetal development (11), which is reflected in reproductive processes. Reproductive processes are affected by stress conditions which are characterized by impaired ovarian function and the amount of eggs (12).

The results of the present study showed a significant decrease in the level of the catalase enzyme activity and a significant increase in serum MDA concentration in women with PCOS compared to the control group. This difference in both indicators (CAT and MDA) was significantly higher in blood group type O of patients with PCOS than Other blood groups.

The decreasing in the level of catalase enzyme activity may be due to the defect in the hypothalamic-pituitary axis as the level of catalase enzyme varies during the stages of the ovarian cycle and hormonal regulation of it, gonadotropin hormones play an important role in stimulating follicular maturity and differentiation (13), in addition to Promote increased catalase and estrogen activity after stimulation of FSH (14). This may indicate the role of catalase in the selection of follicles and prevent apoptosis after ovulation , as catalase is significantly more effective in the predominant follicle (15)., which is supported by the

results of the present study, The level of efficacy of the catalase enzyme with low serum FSH level (Unpublished).

the increasing of MDA level especially in blood group type O may be a compensatory adaptive response to increase the demand for the removal of superoxide roots, or may be the result of hyperglycemia, insulin resistance or chronic inflammation as oxidative stress and chronic inflammation which leads to ROS generation, while oxidative stress emphasizes inflammation (16), Our results correspond to the studies conducted by Sabuncu et al (2001)(17)

Inflammations are associated with insulin resistance (18). Therefore, hyperglycemia stimulates the generation of ROS, thus increasing the concentration of MDA. As a result, NFκB is activated, which one of the inflammatory signals (19) and therefore, increase levels of TRAF2 gene expression (20) this is confirmed by the results of the present study (Unpublished)

Endogen et al. (2008)(21) found no statistically significant differences in the level of lipid peroxidation in women with PCOS and control group (22). The results of the current study are consistent with Kazem (2014)(23) who's noted a high level of MDA and a low level of catalase enzyme in women with PCOS compared to control group.

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