

**Article**

**Investigation of the Oxidative Stress and Trace Elements in Iraqi Female Patients with Polycystic Ovary Syndrome According to Marital Status and Age Variables**

<sup>1</sup>Ashwaq Abood Shenta and <sup>2</sup>Abbas Dawwas Matter Al-Maliki

<sup>1,2</sup>Department of Chemistry-College of Education for Pure Sciences-University of Basrah-Basrah-Iraq

[ashwaq.shenta@uobasrah.edu.iq](mailto:ashwaq.shenta@uobasrah.edu.iq)

**Abstract**

Polycystic ovary syndrome (PCOS) is a complicated condition that infects most adult women, causing various health problems in the reproductive system. So, the current research was performed to estimate and evaluate the oxidative stress caused by this syndrome by measuring the levels of enzymatic antioxidants and trace elements. The blood serum was gathered from 180 women in total (80 healthy and 100 women diagnosed with PCOS). Glutathione peroxidase (GPx), catalase (CAT), and superoxide dismutase (SOD) recorded a significant decrease ( $P^{***} < 0.0001$ ) in patients following age and marital status factor whereas copper and zinc showed decreasing significance ( $P^{**} < 0.0001$ ) in patients for the same variable. Also, there was a clinical correlation between (CAT, SOD, and GPx) and (Cu and Zn).

**Keywords:** PCOS, GPx, CAT, age variable, trace elements, marital status.

**Introduction**

Polycystic ovary syndrome (PCOS) is among several complex syndromes that infect most married and single women, causing many clinical disorders and biochemical changes in the biological work of the reproductive system belonging to females. Also, PCOS is usually associated biochemically with oxidant-antioxidant status so this

syndrome leads to the alteration of the clinical and biochemical systems belonging to the reproductive system which causes many abnormal cases leading to the variation in the levels of enzymatic antioxidants, antioxidant vitamins, lipid profile, malondialdehyde, blood proteins and trace elements (1,2). PCOS is an endocrine disorder that occurs among women of various ages. Most females have PCOS but have not yet been diagnosed, due to the limited knowledge about this syndrome, especially in Iraq (3). Assorted biochemical metabolic mechanisms were suggested to explain the clinical abnormal disorders of the syndrome represented by the pathogenesis state, alteration in hormonal balance, genetic inheritance, and insulin resistance (IR). In PCOS, IR can be partially attributed to the occurrence of obesity in many of the PCOS cases. Therefore, it is still being debated whether IR is originally caused by obesity or is an inherited feature of PCOS (4). Different pre-studies were performed concerning PCOS and its aetiology but most of them focused on the pathophysiology of this syndrome, but its aetiology is still unclear. Therefore, the increasing investigation into the clinical oxidative stress associated with PCOS suggests genomic and DNA damage occurring in the mitochondria, leading to reduced fertility. (5,6). There are assorted variables that have roles in the alteration of the biochemical marker levels represented by age, marital status, pregnancy, blood group, smoking, family history and body mass index (BMI). Also, the biochemical parameters limit the severity of polycystic ovary syndrome, such as fasting glucose, lipid test, fasting insulin, total testosterone, and C-reactive proteins (7,8).

The levels of CAT, GPx and SOD, vitamins (A and C) were estimated in women with polycystic ovarian syndrome in Iran. Significant differences were recorded between polycystic ovarian syndrome patients and healthy women. Simultaneously, there was a significantly negative correlation among CAT, SOD, fasting glucose and vitamin A, while a positive significant correlation was noted within GPx, vitamins A and E. Therefore, these clinical markers are involved as antioxidants that manage polycystic ovary syndrome and these biochemical variables can be used in the woman's body to fight against oxidative damage (9,10). Therefore, the current research was established to estimate and evaluate the oxidative stress of antioxidant enzymes represented by (GPx, SOD, and CAT), copper and zinc (trace elements) in Iraqi females affected by PCOS according to age and marital status variables.

## **Materials and Methods**

### **Sampling and Subjects**

A total number of 100 polycystic ovary syndrome patients and 80 healthy women (control group) underwent this study. They were divided according to their ages to 3 categories, 1<sup>st</sup> (14-24 years), 2<sup>nd</sup> (25-35 years) and 3<sup>rd</sup> (36-45 years). Also, the patients and healthy females were classified as married and single. The whole samples were

obtained from Basrah Teaching Hospital for women and children in Basrah Governorate-Iraq. Patients diagnosed with other diseases were excluded from the study.

### **blood sampling**

The samples of blood were taken from the healthy and PCOS women at 9:00 AM by a trained nurse. Five milliliters of venous blood were drawn from the patients affected by PCOS and healthy women. All samples were then placed in vacutainer tubes and centrifuged at a speed of (5000 rpm) for six minutes. After that, the collected serum was maintained at 22<sup>o</sup> C until the day of estimation of the clinical parameters. The remaining blood was placed in special tubes to separate the blood plasma and then the erythrocytes were carefully washed by using (9% w/v) sodium chloride then the mixture was then lysed with deionized water with a ratio equal to 1:1 (v/v), (11,12).

### **Estimation of clinical biochemical markers**

The activity of the antioxidant enzymes (SOD and GPx) was measured in blood sera for all PCOS and control groups by using the enzyme-linked immunosorbent assay (ELISA) method (13) while the levels of CAT enzyme, were determined spectrophotometrically based on the absorbance of a coloured complex (14). Also, trace elements of (copper and zinc) levels were estimated in all samples of blood serum by the spectrophotometric methods depending on the coloured complex formed at the end of the assessment. Then, the measurement of total absorbances, which were proportional to Zn and Cu levels, was conducted. (15,16).

### **Statistical analysis**

The total data which was obtained in the present research was represented and represented by mean  $\pm$  standard deviation for the total groups of PCOS patients and healthy women according to age and marital status variables. The statistical analysis was conducted according to the statistical program of social science (SPSS, version 25). The analysis was done using the variance univariate program. The regression coefficient (r) was carried out to differentiate between the means belonging to the women affected by PCOS and the healthy women. Also, the accompanying P-value was less than (0.05) for the smallest limit of significance.

## Results

The oxidant-antioxidant defence system is considered the protector against all clinical and biochemical disorders resulting from various diseases including polycystic ovary syndrome. Therefore, some antioxidant enzymes (GPx, CAT, and SOD) and trace elements (Cu and Zn) were estimated in women with PCOS and healthy females. Table (1) indicates the levels of SOD, GPX, and CAT in blood serum of PCOS and healthy women, categorized by age variable into three categories.

**Table (1): Activity of GPx, CAT and SOD enzymes in PCOS and healthy women in accordance with age variable.**

Age category (year)	Women Groups	GPx (pmol/ml)	CAT (k/ml)	SOD (pg/ml)
First (14-24)	Control (no.=28)	97.551±7.938	1.106±0.252	2533.26±363.49
	PCOS (no.=34)	41.682±8.304 <sup>***</sup>	0.161±0.097 <sup>***</sup>	1397.68±517.45 <sup>***</sup>
Second (25 – 35)	Control (no.=27)	95.267±7.929	1.145±0.207	2673.95±330.26
	PCOS (no.=36)	38.175±10.490 <sup>***</sup>	0.184±0.081 <sup>***</sup>	1484.84±482.72 <sup>***</sup>
Third (36 – 45)	Control (no.=25)	90.403±3.694	1.004±0.130	2555.33±216.09
	PCOS (no.=30)	40.342±7.301 <sup>***</sup>	0.142±0.075 <sup>***</sup>	1509.44±590.08 <sup>***</sup>

Data are represented as Mean ± SD, <sup>\*\*\*</sup>P< 0.0001, <sup>\*\*</sup>P< 0.001, <sup>\*</sup>P< 0.05,

Assorted values of levels belonging to GPx, CAT, and SOD enzymes are represented by 41.682±8.304, 0.161±0.097 and 1397.68±517.45 respectively in PCOS women at the first age category whereas the same enzymatic antioxidants recorded levels equal to 38.175±10.490, 0.184±0.081 and 1484.84±482.72 respectively at the second age category for the same patients. In the third category of age, the levels of GPx, CAT, and SOD were 40.342±7.301, 0.142±0.075 and 1509.44±590.08 for the same PCOS women. The highest levels of GPx, CAT, and SOD enzymes were recorded in the 1<sup>st</sup>,

2<sup>nd</sup> and 3<sup>rd</sup> categories respectively while the lowest values were noticed in the second, third and first categories.

Marital status is an important variable to follow the alteration in the levels of enzymatic antioxidants. Table (2) shows the levels of GPx, CAT, and SOD in the blood sera of PCOS women depending on marital status factors.

**Table (2): Activity of GPx, CAT and SOD enzymes in blood sera of PCOS women according to marital status variable.**

marital status	Women Groups	GPx (pmol/ml)	CAT (k/ml)	SOD (pg/ml)
Married	Control (no.=40)	95.617±7.536	1.086±0.207	2568.55±338.21
	PCOS (no.=49)	39.857±9.839***	0.173±0.095***	1520.71±542.08***
Single	Control (no.=40)	93.476±7.045	1.088±0.213	2607.47±288.71
	PCOS (no.=51)	40.172±8.002***	0.154±0.075***	1407.56±505.45***

Data are represented as Mean ± SD, \*\*\*P< 0.0001, \*\*P< 0.001, \*P< 0.05,

GPX, CAT, and SOD enzymes showed various values of levels represented by 39.857±9.839, 0.173±0.095 and 1520.71±542.08 respectively in the blood sera of married PCOS females, whereas the same enzymatic antioxidants recorded levels equal to 40.172±8.002, 0.154±0.075 and 1407.56±505.45 respectively in the blood sera of single PCOS females. The greatest levels for GPX, CAT, and SOD were found in single, married and married women.

Copper and zinc are considered necessary biochemical parameters that are measured in different disorders including PCOS. So, the levels of these trace elements were estimated in women diagnosed with PCOS depending on age factor as shown in Table 3.

**Table (3): Activity of copper and zinc in the blood serum of PCOS women according to age variable.**

Age category (year)	Women Groups	Cu ( $\mu\text{g}/\text{dl}$ )	Zn ( $\mu\text{g}/\text{dl}$ )
First (14 -24)	Control (no.=28)	76.464 $\pm$ 13.908	85.680 $\pm$ 1.746
	PCOS (no.=34)	137.645 $\pm$ 32.621 <sup>***</sup>	56.330 $\pm$ 12.564 <sup>***</sup>
Second (25 – 35)	Control (no.=27)	84.444 $\pm$ 19.228	83.560 $\pm$ 0.783
	PCOS (no.=36)	136.917 $\pm$ 28.613 <sup>***</sup>	57.613 $\pm$ 12.055 <sup>***</sup>
Third (36 – 45)	Control (no.=25)	84.440 $\pm$ 18.681	82.712 $\pm$ 1.823
	PCOS (no.=30)	131.798 $\pm$ 32.854 <sup>***</sup>	58.945 $\pm$ 2.931 <sup>***</sup>

Data are represented as Mean  $\pm$  SD, <sup>\*\*\*</sup>P< 0.0001, <sup>\*\*</sup>P< 0.001, <sup>\*</sup>P< 0.05,

The level values of copper were recorded to be 137.645 $\pm$ 32.621, 136.917 $\pm$ 28.613 and 131.798 $\pm$ 32.854 in PCOS women at the 1<sup>st</sup>, 2<sup>nd</sup> and 3<sup>rd</sup> sections respectively whereas zinc concentration values were 56.330 $\pm$ 12.564, 57.613 $\pm$ 12.055 and 58.945 $\pm$  2.931 at the same categories for the same women with PCOS. The highest values of Cu and Zn were found in the first and third categories, but the lowest concentrations were recorded in the third and first categories.

Also, copper and zinc were assessed in the blood serum of PCOS women according to marital status variable as shown in Table (4) where different levels were recorded in PCOS and healthy women.

**Table (4): Activity of copper and zinc in blood serum of PCOS women according to marital status factor.**

Marital status	Women Groups	Cu ( $\mu\text{g}/\text{dl}$ )	Zn ( $\mu\text{g}/\text{dl}$ )
Married	Control (no.=40)	79.550 $\pm$ 17.373	85.303 $\pm$ 1.680
	PCOS (no.=49)	139.835 $\pm$ 33.308 <sup>***</sup>	57.245 $\pm$ 10.640 <sup>***</sup>
Single	Control (no.=40)	83.750 $\pm$ 17.719	82.771 $\pm$ 1.295
	PCOS (no.=51)	131.589 $\pm$ 28.541 <sup>***</sup>	57.894 $\pm$ 10.208 <sup>***</sup>

Data are represented as Mean  $\pm$  SD, \*\*\*P< 0.0001, \*\*P< 0.001, \*P< 0.05,

Two various values of concentrations belonging to copper were estimated represented by 46.612 $\pm$ 11.103 and 43.863 $\pm$ 9.514 in the blood serum of married and single PCOS women respectively whereas zinc concentrations also showed two assorted values equal to 57.245 $\pm$ 10.640 and 57.894 $\pm$ 10.208 at the same for married and single women with PCOS.

## **Discussion**

Polycystic ovary syndrome (PCOS) is a biochemical, clinical, and physiological syndrome that takes place in the female reproductive system leading to the occurrence of multiple health problems and varied complex disorders. This clinical status leads to alterations in the biochemical system represented by non-enzymatic and enzymatic antioxidants (17). Given the importance of PCOS and its complexity, therefore this research was performed for the assessment of the oxidative stress system represented by glutathione peroxidase, catalase, and superoxide dismutase enzymes and also copper and zinc levels in the blood sera of PCOS groups. The levels of GPx, CAT, and SOD decreased highly significantly (P\*\*\*<0.0001) in PCOS women in contrast with healthy individuals in accordance with the age variable. It is known that the age factor belonging to PCOS women is very necessary to follow the severity of PCOS, especially in married women. Therefore, the alteration in the levels of GPx, CAT, and SOD was noticed clearly. Pre-studies confirmed the role of age of PCOS women in the assessment of CAT, GPx and SOD where alteration of the age category of patients led to change in the levels of these enzymes. So, this biochemical statement indicated the presence of a highly significant difference (18,19). Marital status is considered one of the biochemical variables belonging to women with PCOS which leads to the alteration in biological, biochemical, and clinical rules in the female reproductive system. Therefore, the biochemical markers will differ in their levels, leading to a significant variation when they are compared in married and single healthy women (20,21). Enzymes of GPx, CAT, and SOD recorded a high significance (P\*\*\*< 0.0001) in the blood serum of married and single PCOS women when compared with healthy individuals. When comparing the levels of the enzymes above in married and single PCOS groups, there was a slight variation among the levels of these enzymes. It was noticed that CAT and SOD levels were higher in married women when compared with single women which may lead to reduced levels of oxidative stress in married women. Whereas, GPX recorded a slight decrease between the two groups mentioned above. The levels of copper and zinc

are important biochemical markers that follow the acuteness of polycystic ovary syndrome in various females of assorted ages because the levels of Cu and Zn are correlated directly with oxidative stress, especially with antioxidant enzymes (22,23). Marital status and quality of life are considered two of the variables that affect the considerations of trace elements including copper and zinc, (24,25). In this research, there was a high significance ( $P^{***}<0.0001$ ) in the levels of Zn and Cu of women diagnosed with PCOS for both single and married females. Copper indicated a significant increase ( $P^{***}< 0.0001$ ) in its values, specifically in married PCOS women, whereas Zn decreased significantly ( $P^{***}<0.0001$ ) in PCOS females in contrast with healthy individuals (26).

## **Conclusions**

Enzymatic antioxidants exemplified by glutathione peroxidase, catalase, and superoxide dismutase showed a clear clinical and biochemical role in the reproductive system of women with PCOS across various age groups, categorized into single and married females. Therefore, highly significant differences were recorded for GPx, CAT, and SOD activity depending on age and marital status variables. Also copper and zinc as trace elements had a biochemical role in the alteration of the clinical system of PCOS women leading to distinctions in physiological actions according to age and marital status among PCOS females.

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