



## Original Article

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## Assessment of antioxidant status of women with polycystic ovarian syndrome

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## ABSTRACT

**Objective:** To determine the antioxidant status of females with polycystic ovarian syndrome.

**Methods:** Blood samples of 85 females (45 newly diagnosed polycystic ovarian syndrome patients and 40 apparently healthy subjects) between 25 and 45 years of age were obtained from Ekiti State University Teaching Hospital, Ado Ekiti, Ekiti State, Nigeria. Spectrophotometry was used to evaluate the plasma concentrations of malondialdehyde, vitamin A, C and E, reduced glutathione and activities of glutathione peroxidase, catalase and superoxide dismutase.

**Results:** The concentrations of malondialdehyde and glutathione peroxidase activity significantly increased ( $P < 0.05$ ) in patients with polycystic ovarian syndrome compared with the healthy controls, while concentrations of reduced glutathione, vitamins A, C, E and activities of catalase and superoxide dismutase of patients with polycystic ovarian syndrome showed significant decrease ( $P < 0.05$ ) compared to the healthy controls. This study showed that oxidative stress may assume a role in the pathogenesis of polycystic ovarian syndrome. There were significant negative correlations between malondialdehyde and superoxide dismutase, catalase ( $P < 0.01$ ) and vitamin A ( $P < 0.05$ ), while there was a significant positive correlation ( $P < 0.01$ ) between malondialdehyde and glutathione peroxidase. In addition, vitamins A ( $P < 0.05$ ), C ( $P < 0.01$ ) and E ( $P < 0.01$ ) showed significant positive correlations with catalase antioxidant enzyme. However, vitamins C and E showed significant positive correlation ( $P < 0.05$ ) among each other.

**Conclusions:** The involvement of antioxidants in the management of polycystic ovarian syndrome may be helpful as secondary therapy to prevent oxidative damage and may be used as a potential approach to overcome metabolic as well as reproductive disorders associated with infertility in polycystic ovarian syndrome.

**KEYWORDS:** Malondialdehyde; Reduced glutathione; Vitamin A, C, and E; Glutathione peroxidase; Catalase; Superoxide dismutase; Polycystic ovary syndrome

## 1. Introduction

Polycystic ovary syndrome (PCOS) is a major commonly occurring endocrine disorder with prevalence of 5%-21% among reproductive-aged women[1]. PCOS is associated with a wide spectrum of complications in various parts of health, including reproductive, metabolic and psychological features. Several mechanisms suggested to contribute to the pathogenesis of PCOS include hormonal imbalance, resistance to insulin and genetic inheritance[1]. The primary cause of PCOS is multifactorial in origin, though hyperandrogenism, polycystic ovaries and oligoanovulation are common features in women with PCOS[2].

Hyperandrogenism favors excess luteinizing hormone secretion in fetal life in hypothalamic-pituitary unit programming, bringing about the development of insulin resistance and abdominal obesity[3]. Also, disruption of ovulatory function (anovulation) in PCOS may result from both altered steroid negative feedback regulation of luteinizing hormone and the compensatory hyperinsulinemia caused by insulin resistance[4]. The Rotterdam Consensus Criteria established by the European Society for Human Reproduction and Embryology and American Society for Reproductive Medicine in 2003 dependent on the broad studies during the most recent decades, have been used as diagnosing criteria for PCOS[5]. However, lifestyle modification, meditation, weight loss, herbal medicines have been used in the management of PCOS[1].

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Oxidative stress is an imbalance in the systemic expression of reactive oxygen species (ROS) and the inability of the biological system to readily detoxify or repair the reactive intermediates, thus producing peroxides and free radicals that might cause damages to cell components like lipids, DNA and proteins. An increase in oxidative stress, secondary to the free-radical mechanism, contributes to long term complications or development of metabolic diseases[6]. The utilization of antioxidants in the management of women with PCOS has generated great interests, though antioxidant supplementation has been reported to improve insulin sensitivity and other wellbeing undermining conditions[7]. A few features of PCOS, like increased androgen hormones, insulin resistance, abdominal adiposity and obesity, can induce oxidative stress in women with PCOS[8]. However, increased risk of cardiovascular disease, hypertension, central obesity and dyslipidemia, and insulin resistance may be caused by increased oxidative stress and decreased antioxidant ability[9,10].

Despite being one of the most well-known endocrine disorders in females, there is a dearth of information on the pathophysiology of PCOS. However, this research work estimates non-enzymatic antioxidants concentrations and enzymatic antioxidants activities in PCOS patients.

## 2. Materials and methods

### 2.1. Participants

A total of 45 women with PCOS, diagnosed for the first time and confirmed according to the 2004 Rotterdam Consensus Criteria, who attended Ekiti State University Teaching Hospital, Ado Ekiti, Ekiti State within the months of January and February 2019, were enrolled as patients group[11]. Forty apparently healthy women were enrolled as the control group. However, haphazard sampling was employed for sampling in this study.

Participants' exclusion criteria included PCOS patients not willing to be included in the study; Participants that were currently on medication and relatively health without PCOS; Unhealthy participants with diabetes, coronary artery disease, and other metabolic-related diseases. While participants' inclusion criterion included: Clinically diagnosed PCOS participants who were willing to participate or those who were relatively healthy without PCOS. The age range was 25–45 years old.

Some of the clinical manifestations of the PCOS in participants involved this study included menstrual dysfunction (34, 85%), amenorrhea (6, 15%), infertility (24, 60%), hirsutism/acne (32, 80%), ultrasound evidence of cysts within the ovary (36, 90%) and

high androgen concentration (26, 65%).

### 2.2. Ethical considerations

This study was approved by Ekiti State Teaching Hospital Ado Ekiti and Ekiti State University Ethics Committee (Ethical approval number: EKSUTH/A67/2019/04/008). Participants were provided with a proper understanding of the research work and written informed consent was provided by participants before collecting samples or any data. Relevant information about the participants' socio-demographic, anthropometric data and health history were collected by using questionnaires.

### 2.3. Collection of sample

Intravenous blood samples (10 mL) were collected into plain tubes at 10 days after the regular menstrual cycle of subjects by using a standard venipuncture technique. The serum of the collected blood samples was obtained by centrifugation at 3 000 rpm for 10 min using tabletop centrifuge (Scantriscan 60DV). The obtained serum was stored at -20 °C for biochemical analysis.

### 2.4. Biochemical analysis

#### 2.4.1. Malondialdehyde (MDA) evaluation

MDA, the by-product of the lipid peroxidation process was determined as confirmed by the formation of thiobarbituric acid reaction substances according to the method described by Matsunami *et al*[12].

The prepared reaction mixture containing 0.5 mL serum sample, 0.5 mL phosphate buffer (0.1 M, pH 8.0) and 0.5 mL 24% trichloroacetic acid was incubated for 10 min at room temperature. The incubated mixture was centrifuged at 2 000 rpm for 20 min by using tabletop centrifuge. Thiobarbituric acid (0.25 mL) of 0.33% in 20% acetic acid was added to 1.0 mL of the obtained supernatant and boiled for 1 h at 95 °C. The resulting product (pink color) was cooled and the absorbance was measured by using spectrometer at 532 nm. The concentration of MDA was determined by using extinction coefficient of MDA,  $\epsilon_{532} = 1.53 \times 10^5 \text{ M}^{-1} \text{ cm}^{-1}$ .

#### 2.4.2. Determination of superoxide dismutase (SOD) activity

The activity of SOD was determined spectrophotometrically by using the method described by Nur *et al*[13]. Into a 10 mL test tube, 200  $\mu\text{L}$  of the serum sample was pipetted and 2.5 mL of 75 mM of Tris-HCl buffer (pH 8.2), 30 mM ethylenediaminetetraacetic acid

and 300  $\mu\text{L}$  of 2 mM of pyrogallol were also added. Absorbance was read at 420 nm for 3 min by using spectrophotometer. However, 50% inhibition of the rate of autooxidation of pyrogallol which was determined by change in absorbance/min at 420 nm was one unit of the enzyme activity. The SOD activity was expressed as units/mg protein.

#### 2.4.3. Estimation of serum reduced glutathione (GSH) concentration

Nonenzymatic antioxidant, GSH concentration was estimated according to the method described by Abdel-Tawab *et al.*[14]. Mixture of 0.5 mL Ellman's reagent (10 mM) and 2.0 mL phosphate buffer (0.2 M, pH 8.0) were added to 1.0 mL of supernatant. The developed color (yellow) was read at 412 nm. The blank used contained 3.5 mL of phosphate buffer. Similarly, a series of standards were also treated. The amount of GSH was expressed in mmol/L.

#### 2.4.4. Estimation glutathione peroxidase (GPx) activity

The activity of an enzymatic antioxidant, GPx, was assayed for using the method described by Viswanadha *et al.*[15]. The prepared mixture used for the assay contained 250  $\mu\text{L}$  sodium phosphate buffer (0.2 M, pH 8.0), 50  $\mu\text{L}$  10 mM sodium azide, 100  $\mu\text{L}$  4 mM GSH and 50  $\mu\text{L}$  2.5 mM  $\text{H}_2\text{O}_2$ . Serum (50  $\mu\text{L}$ ) was added to the assay mixture, and the total volume was made up to 1.0 mL with distilled water. This was incubated at 37 °C for 3 min. To terminate the reaction, 250  $\mu\text{L}$  of 10 % trichloroacetic acid was added, and then centrifuged at 3 000 rpm for 10 min to obtain the supernatant. Finally, 2.0 mL of disodium hydrogen phosphate (0.3 M) solution and 0.5 mL of 5,5'-dithiobis-(2-nitrobenzoic acid) were added. The absorbance of the resulting product was read at 412 nm spectrophotometrically. The expression of the enzyme's activity was in Units/mg protein.

#### 2.4.5. Estimation of catalase activity

The activity of catalase, an enzymatic antioxidant was evaluated spectrophotometrically as the rate of disintegration of hydrogen peroxide according to the methods described by Kaya *et al.*[16]. Into a cuvette containing 450  $\mu\text{L}$  of phosphate buffer (0.1M, pH 7.4) and 500  $\mu\text{L}$  of 20 mM  $\text{H}_2\text{O}_2$ , 50  $\mu\text{L}$  of the sample was added. The activity of the enzyme was measured by using spectrophotometer at 240 nm for 1 min. The activity of the catalase enzyme was determined by using the molar extinction coefficient of  $\text{H}_2\text{O}_2$  ( $\epsilon_{240}=43.6 \text{ M cm}^{-1}$ ). Catalase activity was expressed as units per milligram of protein (one unit of activity equaled to 1 mmol of  $\text{H}_2\text{O}_2$  degraded per minute).

#### 2.4.6. Determination of vitamin C (ascorbic acid) concentrations

The concentration of vitamin C was determined by using the method described by Subash-Babu *et al.*[17]. Precipitation of 100  $\mu\text{L}$  of the samples was done with 250  $\mu\text{L}$  of 5% ice-cold tricarboxylic acid. The precipitated sample was centrifuged at 6 500 rpm for 20 min by using tabletop centrifuge. The mixture of one-tenth of 1.0 mL of supernatant with 0.2 mL of 2, 4 dinitrophenylhydrazine: thiourea: copper sulfate was done. The mixture was incubated for at 37 °C for 3 h. Finally, 1.5 mL of ice-cold 65%  $\text{H}_2\text{SO}_4$  was added and thoroughly mixed. For another 30 min, the solution was kept at room temperature and the absorbance was read at 530 nm by using spectrophotometer. The values of vitamin C were expressed as  $\mu\text{g}/\text{mg}$  protein.

#### 2.4.7. Estimation of vitamin-E (alpha-tocopherol) concentrations

The concentration of vitamin E (alpha-tocopherol) was determined spectrophotometrically by using the method of Okoduwa *et al.*[18]. Saponification of the sample was done by the addition of 0.3 mL of 60% (w/v in water) potassium hydroxide and 2.0 mL of 1% (w/v in ethanol) ascorbic acid to 0.2 mL of the serum sample. The resulting mixture was boiled at 70 °C for 30 min and cooled on ice. The *n*-hexane extract was prepared by adding 1.0 mL *n*-hexane and 2.0 mL water to the resulting sample solution and mixed. The mixture was allowed to rest for 10 min for phase separation. Drying of the hexane extract was done under nitrogen at 40 °C and 0.2 mL methanol was used to re-dissolve the extract. Similar treatment done for the test samples were repeated with the tubes containing  $\alpha$ -tocopherol standard. Also, the addition of 0.2 mL of 0.2% bathophenanthroline reagent to all the tubes including a reagent blank was done and the solution was thoroughly mixed. Exposure to direct sunlight was avoided from this point because the assay proceeded very fast. An addition of two-tenths of 1.0 mL of ferric chloride reagent was also done and test tubes were properly mixed by vortexing. Finally, 0.2 mL of orthophosphoric acid was added after 1 min and thorough mixing was done again. The absorbance of the resulting mixture was measured at 536 nm spectrophotometrically. The value of vitamin E was expressed as mg/gm tissue.

#### 2.4.8. Determination of vitamin A (beta-carotene bleaching assay) concentrations

The concentration of vitamin A was estimated by measuring the coupled autoxidation of  $\beta$ -carotene and linoleic acid using the method described by Hiroshi *et al.*[19]. The emulsion was prepared

by dissolving 5 mg  $\beta$ -carotene in 50 mL chloroform; 3 mL of the resulting mixture was taken and evaporated under reduced pressure at a temperature not exceeding 40 °C in a rotary evaporator. To the evaporated solution, 40  $\mu$ L linoleic acid, 40  $\mu$ L Tween 80 or Tween 20 and 100 mL/50 mL distilled water were added. The emulsion was shaken vigorously to form. The positive control used was quercetin; the negative control included 20  $\mu$ L methanol and 150  $\mu$ L emulsion, and test sample contained 20  $\mu$ L of the sample and 150  $\mu$ L of emulsion. Absorbance was read at 470 nm immediately [Time ( $T_0$ )]. The solution was also incubated at 50 °C for 60 min ( $T_{60}$ ) and the absorbance was read at 470 nm at  $T_{60}$ . The antioxidant activity was calculated as the follow formula:

Antioxidant activity (% inhibition) = 100 [Degradation rate of control (DRc) – Degradation rate of sample (DRs)]/DRc.

Whereby, DRc =ln [Initial absorbance (a) / Final absorbance after 60 min (b)]/60

$$DRs = \ln (a/b)/60$$

### 2.5. Statistical methods

Statistical Package for the Social Sciences (SPSS) version 22.0. was used in the study. One way analysis of variance and *t*-test were used for statistical analysis. Also, Bivariate Pearson Correlation analysis was used to determine the correlation between the antioxidant parameters. The obtained results were grouped and expressed as mean $\pm$ standard deviation (mean $\pm$ SD).

## 3. Results

### 3.1. Levels of MDA, vitamins A, E, C and activities of catalase, GSH, GPx, SOD in PCOS patients

Table 1 showed significant increases ( $P<0.05$ ) in MDA concentration and GPx activity in PCOS patients, while there were significant decreases ( $P<0.05$ ) in the level of GSH, vitamins A, E, C and in activities of catalase and SOD in PCOS patients compared to the healthy controls. However, the age of the PCOS patients showed no significant difference ( $P>0.05$ ) when compared with the healthy controls.

### 3.2. Correlation between antioxidant parameters of PCOS patients

Table 2 showed the correlation between MDA level, enzymatic and nonenzymatic antioxidants. This study showed a significant negative correlation between MDA and SOD ( $r=-0.61$ ,  $P<0.01$ ), catalase ( $r=-0.57$ ,  $P<0.01$ ) and vitamin A ( $r=-0.49$ ,  $P<0.05$ ), while there was a significant positive correlation between MDA and GPx ( $r=0.73$ ,  $P<0.01$ ). Also, vitamins A ( $r=0.48$ ,  $P<0.05$ ), vitamin C ( $r=0.65$ ,  $P<0.01$ ) and vitamin E ( $r=0.59$ ,  $P<0.01$ ) showed significant positive correlations with catalase antioxidant enzyme. However, vitamins C and E showed a significant positive correlation ( $r=0.51$ ,  $P<0.05$ ) among each other.

**Table 1.** Levels of MDA, vitamins A, E, C and activities of catalase, GSH, GPx, SOD in PCOS patients.

Parameters	Control	PCOS
Superoxide dismutase (SOD) (U/L)	65.99 $\pm$ 28.36 <sup>a</sup>	43.47 $\pm$ 30.73 <sup>b</sup>
Catalase (U/L)	1.48 $\pm$ 0.32 <sup>a</sup>	1.18 $\pm$ 2.01 <sup>b</sup>
Reduced glutathione (GSH) (mmol/L)	3.31 $\pm$ 2.60 <sup>a</sup>	2.69 $\pm$ 0.67 <sup>b</sup>
Glutathione peroxidase (GPX) (U/L)	0.18 $\pm$ 0.03 <sup>a</sup>	0.24 $\pm$ 0.19 <sup>b</sup>
Malondialdehyde (MDA) (mmol/L)	0.11 $\pm$ 0.06 <sup>a</sup>	0.18 $\pm$ 0.13 <sup>b</sup>
Vitamin C (mmol/L)	1.25 $\pm$ 0.19 <sup>a</sup>	1.13 $\pm$ 0.13 <sup>b</sup>
Vitamin E (mmol/L)	40.26 $\pm$ 3.35 <sup>a</sup>	37.43 $\pm$ 4.83 <sup>b</sup>
Vitamin A (mmol/L)	52.65 $\pm$ 38.74 <sup>a</sup>	39.11 $\pm$ 13.89 <sup>b</sup>
Age (year)	35.63 $\pm$ 12.03 <sup>a</sup>	34.90 $\pm$ 11.07 <sup>a</sup>

Values are presented as mean $\pm$ SD. Values with superscripts a and b are significantly different at  $P<0.05$  when compared with non PCOS control group. PCOS=polycystic ovarian syndrome.

**Table 2.** Correlation between antioxidant parameters of patients with polycystic ovarian syndrome.

Parameters	MDA	SOD	Catalase	GPX	GSH	Vitamin C	Vitamin E	Vitamin A
MDA	1	-0.61**	-0.57**	0.73**	-0.04	-0.38	-0.36	-0.47*
SOD	-0.61**	1	0.54*	-0.29	0.24	0.51*	0.20	0.15
Catalase	-0.57**	0.54*	1	-0.39	0.10	0.65**	0.59**	0.48*
GPX	0.73**	-0.29	-0.39	1	-0.12	0.24	-0.05	0.15
GSH	-0.04	-0.24	0.10	-0.12	1	-0.37	-0.29	-0.37
Vitamin C	-0.38	0.51*	0.65**	-0.37	0.24	1	0.51*	0.17
Vitamin E	-0.36	0.20	0.59**	-0.29	-0.05	0.51*	1	0.40
Vitamin A	-0.49*	0.15	0.48*	-0.37	0.15	0.17	0.40	1

Values are presented as Pearson correlation. The values represented with \* and \*\* represent 2-tailed correlation significant at 0.05 and 0.01 levels, respectively. MDA=malondialdehyde; SOD=superoxide dismutase; GPX=glutathione peroxidase; GSH=reduced glutathione.

#### 4. Discussion

MDA is one of several by-products of lipid peroxidation process, a biomarker that provides an indication of the level of lipid peroxidation. Products generated from lipid peroxidation reactions have been widely employed as biomarkers for oxidative stress[20]. This study shows that serum MDA concentration significantly increased in PCOS patients in comparison to the healthy controls. This is in line with the finding of Zhang *et al* who showed that the concentration of serum MDA in patients with PCOS was increased significantly when compared with the controls[21]. Also, alike result was gotten by Kuscu *et al* who ascribed increment in MDA to hyperglycemia and insulin resistance in PCOS[22]. Therefore, increased concentrations of MDA might be as a result of increased generation of ROS due to generation of inordinate oxidative damage in the participants. These oxygen species thusly can oxidize numerous other significant bio-molecules including membrane lipids.

Catalase is an intracellular antioxidant enzyme that catalyzes the decrease of hydrogen peroxide to water and molecular oxygen and is essentially situated in cell peroxisomes and somewhat in the cytosol. If there should be an occurrence of constrained glutathione content or decreased GPx activity, this enzyme assumes a significant role in the improvement of tolerance to oxidative stress. This study shows a significant decrease in the activity of catalase in serum samples of the PCOS patients compared with the controls. A significant decrease in the catalase activity was similarly reported by Al-Azzawie *et al* and Kandasamy *et al* in PCOS patients compared to the control group[23,24]. Therefore, the decrease in catalase activity might be due to accumulation of ROS in which catalase activity was decreased by the state of oxidative stress in PCOS patients.

SOD is a significant antioxidant enzyme having a neutralizing impact against superoxide anion. It is a noteworthy cellular defense system against superoxide. It is involved in the conversion of superoxide to hydrogen peroxide, which is then converted to water by GPx. This study shows a significant decrease in SOD activity in the serum samples of PCOS patients compared with the controls. This outcome is in concurrence with the findings of Zhang *et al* who expressed that the serum SOD levels in PCOS patients were significantly lower than those of controls[21]. The decrease in SOD activity in this study may be connected to increase utilization of SOD to scavenge produced ROS brought about by both hyperglycemia and excess free fatty acids.

GPx is an enzymatic antioxidant containing selenium. It effectively reduces hydrogen peroxide and lipid peroxides to water and lipid alcohols respectively and thus oxidizes glutathione to glutathione disulfide. However, when GPx activity or glutathione level is inadequate, hydrogen peroxide and lipid peroxides are not detoxified and might be changed over to hydroxyl radicals and lipid peroxyl radicals, respectively. This study shows a significant increase in the activity of GPx in serum sample PCOS patients compared to the

controls. Elevated GPx activity was to be expected with increased hydrogen peroxide, therefore decreased activity of GPx in women with PCOS might be due to the low level the substrate (GSH) of GPx[25]. However, Maha *et al* reported an increased GPx activity in patients with PCOS in comparison with the controls[26]. Meanwhile, Baskol *et al* found an increase but not significant difference in GPx activity in PCOS patients than those of controls[27]. The apparent increase in GPx activity might indicate that GPx has higher proclivity towards peroxides and scavenges most radicals produced.

GSH, a tripeptide, is a significant antioxidant present in millimolar concentrations in living cells and assumes the role of the intracellular radical scavenger. This study shows that GSH level in serum sample of PCOS patients showed a significant decrease in comparison with the controls. These results are similar to the findings of Sabuncu *et al* and Dincer *et al* who discovered that GSH level was significantly decreased in patients with PCOS than in the control group and proposed that the lower GSH levels may be indirectly related to insulin resistance[25,28]. The lower levels of GSH might be a result of increased utilization of glucose under hyperglycemic conditions in polyol pathway that utilizes nicotinamide adenine dinucleotide phosphate needed by GSH-reductase enzyme for regeneration of GSH. Therefore, hyperglycemia associated with insulin resistance reported found in PCOS patients might indirectly cause GSH depletion, and thus cause increased oxidative stress.

Vitamin A, a non-enzymatic antioxidant and fat-soluble vitamin, got from various compounds such as all-trans-retinol,  $\beta$ -carotene, and retinyl esters. The ROS scavenging properties that are usually involved in the prevention of lipid peroxidation were exerted by long-chain arrangement of conjugated double bonds in retinoids structures[29]. There was a significant decrease in serum vitamin A level in the group with PCOS when compared to the control group. A lower level of this nonenzymatic antioxidant parameter with increased MDA levels may be because of the higher turnover, for forestalling oxidative damage in these patients with PCOS, suggesting an increased safeguard against oxidative damage.

Also, vitamin E, a non-enzymatic and lipid-soluble antioxidant, reacts with lipid peroxyl radicals that lead to ending the peroxidation chain reaction and in this manner lessening oxidative damage. The serum concentration of vitamin E in the present study was significantly decreased in PCOS patients when compared to the controls. Similar reports of decreased vitamin E concentrations in PCOS patients were reported by different studies[28]. The possibility of rapid reaction of vitamin E with molecular oxygen and free radicals might be the cause of significantly decreased concentration of vitamin E in this study. Therefore, it is suggested that vitamin E protects polyunsaturated fatty acid from peroxidation reaction by acting as a scavenger.

Vitamin C is the major nonenzymatic water-soluble antioxidant in the biological system and furthermore acts as a cofactor in several metabolic reactions. It is the first antioxidant to be utilized during lipid peroxidation brought about by free radicals. Serum vitamin

C levels in the present study were significantly lower in the group with PCOS in comparison to the control group. This corresponds to the studies of Zhang *et al* and Surapaneni *et al*[21,30]. Also, Polak *et al* found a significant decrease in levels of vitamin C both in the peritoneal fluid and endometrial tissue in women with PCOS[31]. The significant decrease might be because of the exhaustion of vitamin C in free radicals neutralization.

In the correlation between MDA, non-enzymatic and enzymatic antioxidants, this study shows a negative correlation between MDA, SOD, CAT and vitamin A in the group with PCOS. However, the mechanism of oxidative stress brought about by the imbalance in the formation of ROS and attenuation of anti-oxidative defense supports the negative correlation between the antioxidants parameters and MDA in PCOS patients. Therefore, this study suggests an increased lipid peroxidation and depletion of antioxidants leading to oxidative stress in patients with PCOS. This study also shows a significant positive correlation between MDA concentration and GPX activity, which implies that the increase in MDA concentration prompts an increment in GPX activity and this positive correlation may be to scavenge the effect of increased oxidative stress in PCOS patients.

However, vitamin C and E had a positive correlation which shows vitamin C and vitamin E interrelationship for scavenging free radicals. Vitamin C was reported to regenerate vitamin E by decreasing formed vitamin E radicals when oxygen radicals are scavenged by vitamin E[32]. However, vitamins C and E were reportedly found differently outside and inside the membranes respectively, in both liposomal membrane systems and inhomogeneous solutions, where the collaboration between vitamin C and vitamin E radicals was shown to occur and vitamin C plays a role of synergist[32]. Hence, maintenance of normal concentration of vitamin C is required to prevent oxidative stress. Finally, this study showed no statistically significant difference in both groups regarding age. Therefore, age was not affected in PCOS in this study. In conclusion, both non-enzymatic and enzymatic antioxidants were implicated in this present study in patients with PCOS in comparison to the controls. However, according to this study, oxidative stress might play a significant role in the pathogenesis of PCOS. The increased antioxidant enzyme activities may be because of biological response to elevated oxidative stress. Therefore, increased risk of long term complications (*i.e.* cardiovascular diseases, cancer, diabetes) reported associated with women with PCOS might be attributed to decreased antioxidant capacity. Therefore, treatment on rectifying menstrual irregularities, decreasing insulin resistance, reducing androgen excesses and treatment with antioxidants in the underlying phases of the ailment might be helpful in the management of PCOS. The limitation of this study includes small number of sample. This limitation affected the sampling method used for the study.

## Conflict of interest statement

No conflict of interest was declared by the authors regarding the publication of this research study.

## Authors' contributions

This research study was carried out in collaboration with the authors. Authors Olufisayo Grace Oyeibanji, Modupe Fisayo Asaolu conceived and designed the research study. Both authors performed the experiment and data collection. Author Olufisayo Grace Oyeibanji carried out data analysis and interpretation with support from author Modupe Fisayo Asaolu. Author Olufisayo Grace Oyeibanji wrote the manuscript with support from author Modupe Fisayo Asaolu. Authors Olufisayo Grace Oyeibanji and Modupe Fisayo Asaolu revised the manuscript. Both authors made the final approval of the manuscript for publication.

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