Original Research
ISSN (Print): 0971-913X
J Endocrinol Reprod. *Vol 27(1), 29-39, March 2023, DOI: 10.18311/jer/2023/33508* ISSN (Online): 2455-1244

Relationship between Serum Levels of Oxidative Stress Markers and Metabolic Syndrome Components in PCOS Women

K. Lakshmi[1] and Suttur S. Malini[2]*

[1]Department of Studies in Zoology, University of Mysore, Manasagangotri, Mysuru – 570005, Karnataka, India
[2]Department of Studies in Genetics and Genomics, University of Mysore, Manasagangothri, Mysuru, Karnataka, India; sutturmalini@yahoo.com

Abstract

Background: Polycystic Ovarian Syndrome (PCOS) is a common endocrinological problem that leads to infertility in reproductive age. It is strongly associated with oxidative stress, which increases the risk of Metabolic Syndrome (Met-S) in women. This study aimed to evaluate the relationship between oxidative stress markers and metabolic syndrome parameters in PCOS women. **Methods:** In this cross-sectional study, we included age-matched 100 control and 150 PCOS (according to Rotterdam criteria). Anthropometric measurements were obtained from each subject. Lipid profile, Fasting Plasma Glucose (FPG), and insulin were determined. Serum Malondialdehyde (MDA), Nitric Oxide (NO), and Reactive Oxygen Species (ROS) levels are pro-oxidant indicators, while for antioxidant activities, Superoxide Dismutase (SOD), Catalase (CAT), Glutathione (GSH), Vitamin-C (Vit C), and Total Antioxidant Capacity (TAC) activity were measured by spectrophotometry. **Results:** In the PCOS group the SOD, CAT, GSH, Vit C, and TAC activity were significantly low, whereas NO, ROS, and MDA were significantly high ($p < 0.05$). In the PCOS group, the pro-oxidant MDA showed a negative correlation with HDL and a positive correlation with DBP. The antioxidants SOD and CAT showed a negative correlation with fasting blood glucose and triglycerides. **Conclusion:** The metabolic syndrome components of PCOS can induce oxidative stress, which is evidenced by a decrease in antioxidant defence mechanisms. It is probably because oxidative stress itself is the consequence of PCOS, more so with Met-S which increases the pro-oxidant state and decreases the anti-oxidant capacity in women.

Keywords: Antioxidants, Metabolic Syndrome, Oxidative Stress, PCOS

1. Introduction

Polycystic Ovarian Syndrome (PCOS) is the most frequent endocrinological abnormality in women of reproductive age, with a prevalence range of 6% to 21% worldwide[1]. It is described by clinical and/or biochemical Hyperandrogenism (HA), as well as Ovulatory Dysfunction (OA) and Polycystic Ovaries (PCO)[2]. Furthermore, PCOS is a heterogeneous condition that has a significant reproductive (a leading cause of anovulatory infertility), psychologic (anxiety and depression), and metabolic impact on women (increased type 2 diabetes mellitus and Cardiovascular Disease (CVD))[3–5].

Metabolic Syndrome (Met-S) is a combination of metabolic conditions, such as dyslipidemia, abdominal obesity, hypertension, and impaired fasting glucose[6]. These metabolic consequences might be seen in PCOS patients, increasing the risk of developing Met-S[7]. Numerous studies found an increased incidence of Met-S among women with PCOS[8,9].

Oxidative Stress (OS), defined as an imbalance between pro-oxidant molecules (reactive oxygen and nitrogen species) and antioxidant defence, has been linked to the pathophysiology of infertility in females[10]. PCOS is associated with abnormal circulating oxidative

*Author for correspondence

Article Received: 19.12.22 Revised on: 23.03.23 Accepted on: 07.04.23

stress indicators[11]. Obesity and Insulin Resistance (IR), both of which are frequent in PCOS patients, are likely to contribute to increased Oxidative Stress[12]. Meanwhile, OS level is strongly associated with obesity, Insulin Resistance, hyperandrogenism, and chronic inflammation[13]. The previous studies reported a correlation between circulating markers of oxidative stress and PCOS, indicating the significance of oxidative stress in the pathogenesis of PCOS[11,14]. However, the specific mechanisms of action are yet to be well defined.

Studies on PCOS patients have suggested that IR may be crucial to the pathogenesis of developing Met-S and that this may lead to leukocytes producing more Reactive Oxygen Species (ROS)[13,15]. However, investigations on oxidative stress in PCOS with Met-S, as well as the link between these two disorders, are few. As a result, more research into the association between oxidative stress and metabolic syndrome in PCOS is needed. The present study aimed to evaluate the activity of oxidative stress markers and their association with Met-S components in PCOS with and without Met-S.

2. Materials and Methods

For the present study, 150 PCOS and 100 control subjects were recruited from various gynaecological clinics and infertility centres in the Southern part of Karnataka, India. The study duration was from 2017 to 2018 and was approved by the Institutional Ethical Committee (University of Mysore- IHEC-UOM No.154/Ph.D/2017-18). Each one of the women who were part of the study provided informed consent.

PCOS was diagnosed based on the Rotterdam PCOS (ESHRE/ASRM) criteria[16]. PCOS was diagnosed when two or more of the following criteria are fulfilled:

- Oligo/anovulation (O).
- Clinical and/or biochemical Hyperandrogenemia (H).
- Ultrasound-detected Polycystic Ovaries (P).

The presence of androgen-secreting neoplasms, congenital adrenal hyperplasia, Cushing's syndrome, hyperprolactinemia, and thyroid problems in women was ruled out. The control group included women of age ranging from 18 to 39 years, with regular menstrual cycles (21-35 days), no hirsutism and normal ovaries on ultrasound.

A physical examination was done on each participant. The smallest circumference between the iliac crest and the rib cage was used as the measurement point for Waist Circumference (WC). At the level of the femoral trochanters, the Hip Circumference (HC) was measured at its largest measurement. Body Mass Index (BMI) was computed using the body weight (kg) and height (cm) measurements. BMI was classified as ≤ 25 (normal weight) and ≥ 25 (obese/overweight) (WHO, 2000). Waist to Hip Ratio (WHR), Waist to Height Ratio (WHtR), Body Adiposity Index (BAI) [BAI = HC (cm)/Height (m) 1.5-18] and blood pressure were measured for all the subjects.

After a 10-12 h overnight fasting, blood samples were collected from all individuals in their early follicular phase (2^{nd}-4^{th} day) and were used for the assessment of endocrine, biochemical and oxidative stress markers. The glucose oxidase-peroxidase test was used to assess plasma glucose (ARKRAY Kit, Mumbai, Maharashtra, India). The enzymatic test was used to estimate the lipid profile, which included cholesterol, triglycerides, and High-Density Lipoprotein (HDL) (Meril Kit, Vapi, Gujarat, India) and the Friedewald equation was used to compute Low-Density Lipoprotein (LDL) and Very Low-Density Lipoprotein (VLDL)[17,18]. The fasting insulin level was measured using ELISA (Prime Biomed, Bengaluru, Karnataka, India).

For Met-S diagnosis, we used revised NCEP ATP III, 2005[5]. According to this definition, a person is considered to have Met-S if they meet three out of the following five criteria:

Central obesity (in this study, defined as WC ≥ 80 cm for South Asian females).

- High triglycerides (TG ≥ 150 mg/dL).
- Low HDL (female HDL < 50 mg/dL).
- High Blood Pressure (SBP/ DBP ≥ 130/85 mmHg).
- Fasting Blood Glucose (FBG ≥ 100 mg/dL).
- Malondialdehyde (MDA), a byproduct of the lipid peroxidation process, was detected by the thiobarbituric acid reaction[19]. Reactive Oxygen Species (ROS) were detected fluorometrically using the Dichlorofluorescindiacetyl (DCFDA) oxidation technique[20]. Serum Nitric Oxide (NO) was measured using the Griess reaction using a modified method by Grisham[21].

The enzymatic antioxidants such as Super Oxide Dismutase (SOD)[22], Catalase (CAT)[23], and non-enzymatic antioxidants reduced Glutathione (GSH)[24], Vitamin C

(Vit-C)[25] activity were assessed spectrophotometrically. Total Antioxidant Capacity (TAC) was measured spectrophotometrically through the formation of a phosphomolybdenum complex[26].

3. Statistical Analysis

The collected data were aggregated and represented as mean ± standard deviation. Student's t-test and one-way ANOVA followed by Duncan's multiple range test were used to determine the significance of the difference between the groups. The correlation between the oxidative stress markers and Met-S parameters was ascertained using bivariate Pearson Correlation analysis. A p-value < 0.05 was considered significant for this study. All statistical analyses were performed using SPSS, version 23.0; IBM (IBM Corp. Armonk, NY, USA). Graphs were made using Graph Pad Prism version 8.0.2 (La Jolla, USA).

4. Results

In the present study, we examined the clinical and anthropometric parameters of 150 PCOS and 100 control women. The mean BMI of the PCOS and control groups was 27.02 ± 6.03 and 24.31 ± 4.93 years, respectively (Table 1). It shows that women in the PCOS group were

Table 1. Clinical, endocrine and metabolic features of PCOS and control group

Variables	PCOS (n = 150)	Control (n = 100)	*p*-Value
Age (in year)	24.87 ± 4.73	24.38 ± 5.27	0.08
BMI (kg/m²)	27.02 ± 6.03	24.31 ± 4.93	**< 0.0001**
WC (in cm)	92.87 ± 11.92	87.64 ± 9.56	**< 0.0001**
WHR	0.926 ± 0.09	0.91 ± 0.07	**< 0.0001**
WHtR	58.84 ± 8.20	56.64 ± 6.18	0.3636
BAI	22.39 ± 7.07	19.54 ± 4.55	**< 0.0001**
SBP (mmHg)	124.11 ± 8.28	124.55 ± 8.38	0.09
DBP (mmHg)	82.66 ± 2.28	82.7 ± 2.42	**0.004**
TC(mg/dL)	212.83 ± 51.12	184.84 ± 61.76	**0.0001**
TG(mg/dL)	155.39 ± 76.62	140.19 ± 32.43	**0.04**
HDL (mg/dL)	39.42 ± 13.56	44.36 ± 9.31	**0.002**
LDL (mg/dL)	142.58 ± 44.67	115.74 ± 59.22	**< 0.0001**
VLDL (mg/dL)	31.13 ± 15.26	28.03 ± 6.48	**0.047**
TG/HDL	4.50 ± 3.83	1.45 ± 0.64	**< 0.0001**
FBG (mg/dL)	97.12 ± 3.62	89.43 ± 6.43	**<0.001**
Fasting Insulin (mU/L)	15.31 ± 2.13	9.40 ± 2.15	**<0.005**

Data are expressed as mean ± SD and analysed by independent t-test. *BMI*- Body Mass Index; *WC*- Waist Circumference; *WHR*- Waist Hip Ratio; *WHtR*- Weight Height Ratio; *BAI*- Basal Adipose index; *SBP*- Systolic Blood Pressure; *DBP*- Diastolic Blood Pressure; *TC*- Total Cholesterol; *TG*- Triglyceride; *HDL*- High Density Lipoprotein; *LDL*- Low Density Lipoprotein; *VLDL*- Very Low Density Lipoprotein; *FBG*-Fasting Blood Glucose. The comparison between the groups was considered significant if $p < 0.05$.

Table 2. Oxidative stress and antioxidant parameters in PCOS and control

	PCOS (n=150)	Control (n=100)	*p*-Value
SOD [units/mg protein]	0.04 ± 0.01	0.09 ± 0.01	**< 0.0001**
CAT [nmol H_2O_2 oxidised/min/mL]	0.03 ± 0.01	0.08 ± 0.01	**< 0.0001**
GSH [nmol/mL]	36.65 ± 10.73	52.16 ± 13.96	**< 0.0001**
VIT C [µmol/L]	34.32 ± 9.46	39.89 ± 12.05	**< 0.0001**
TAC [µmol a-tocopherol/L]	126.39 ± 13.72	144.49 ± 18.47	**< 0.0001**
MDA [nmol/ml]	4.8 ± 1.18	2.26 ± 1.14	**< 0.0001**
ROS [mmol DCF formed/min/mg protein]	0.538 ± 0.14	0.324 ± 0.07	**< 0.0001**
Serum NO [µmoles/L]	60.26 ± 14.22	37.39 ± 12.05	**< 0.0001**

Data are expressed on Mean ± SD and analyzed by independent *t*-test. *SOD*- Superoxide Dismutase; *CAT*- Catalase; *TAC*- Total Antioxidant Capacity; *GSH*- Glutathione; *Vit C*- Vitamin C; *MDA*- Malondialdehyde; ROS- Reactive Oxygen Species; *NO*- serum Nitric Oxide; The comparison between the groups was considered significant if $p < 0.05$.

Table 3. Anthropometric, endocrine and metabolic features of control and PCOS sub-groups of metabolic syndrome

Variables	PCOS with Met-S (n = 85)	PCOS without Met-S (n = 65)	Control (n = 100)	p-Value
Age (in year)	24.76 ± 4.9	24.21 ± 4.53	24.38 ± 5.27	0.775
BMI (kg/m²)	27.12 ± 5.8[a]	26.18 ± 6.7[b]	24.31 ± 4.93[c]	**<0.0001**
WC (in cm)	97.71 ± 12.1[a]	87.82 ± 13.40[b]	87.64 ± 9.56[b]	**<0.0001**
WHR	0.925 ± 0.1	0.915 ± 0.095	0.91 ± 0.07	0.920
WHtR	61.56 ± 8.3[a]	55.63 ± 9.19[b]	56.64 ± 6.18[b]	**<0.0001**
BAI	23.10 ± 7.3[a]	21.10 ± 9.19[b]	19.54 ± 4.55[b]	**<0.0001**
SBP (mmHg)	125.48 ± 8.0	122.94 ± 7.12	124.55 ± 8.38	0.183
DBP (mmHg)	82.98 ± 2.4	82.39 ± 2.05	82.7± 2.42	0.597
TC(mg/dL)	218.69 ± 55.4[a]	206.23 ± 44.03[a]	184.84 ± 61.76[b]	**0.0002**
TG (mg/dL)	169.18 ± 85.7[a]	141.72 ± 53.32[b]	140.19 ± 32.43[b]	**<0.0001**
HDL (mg/dL)	36.50 ± 12.2[b]	42.50 ± 14.22[a]	44.36 ± 9.31[a]	**<0.0001**
LDL (mg/dL)	147.25 ± 46.7[a]	138.05 ± 38.46[a]	115.74 ± 59.22[b]	**<0.0001**
VLDL (mg/dL)	33.89 ± 17.1[a]	28.25 ± 10.66[b]	28.03 ± 6.48[b]	**<0.0001**
TG/HDL	5.40 ± 4.5[a]	3.68 ± 1.88[b]	1.45 ± 0.64[c]	**<0.0001**
FBG (mg/dL)	98.60 ± 5.4[a]	96.17 ± 2.72[b]	89.43 ± 6.43[c]	**<0.001**
Fasting Insulin (mU/L)	16.07 ± 6.8[a]	14.65 ± 6.59[a]	9.40 ± 2.15[b]	**<0.005**

Data are expressed as mean ± SD and analysed one way ANOVA followed by Duncan's multiple range test. The same superscript letters in the given column are not significantly different whereas those with different superscript letters are significantly ($p < 0.05$). *BMI*- Body Mass Index; *WC*- Waist Circumference; *WHR*- Waist Hip Ratio; *WHtR*- Weight Height Ratio; *BAI*- Basal Adipose Index; *SBP*- Systolic Blood Pressure; *DBP*-Diastolic Blood Pressure; *TC*- Total Cholesterol; *TG*- Triglyceride; *HDL*- High Density Lipoprotein; *LDL* - Low Density Lipoprotein; *VLDL*- Very Low Density Lipoprotein; *FBG*- Fasting Blood Glucose.

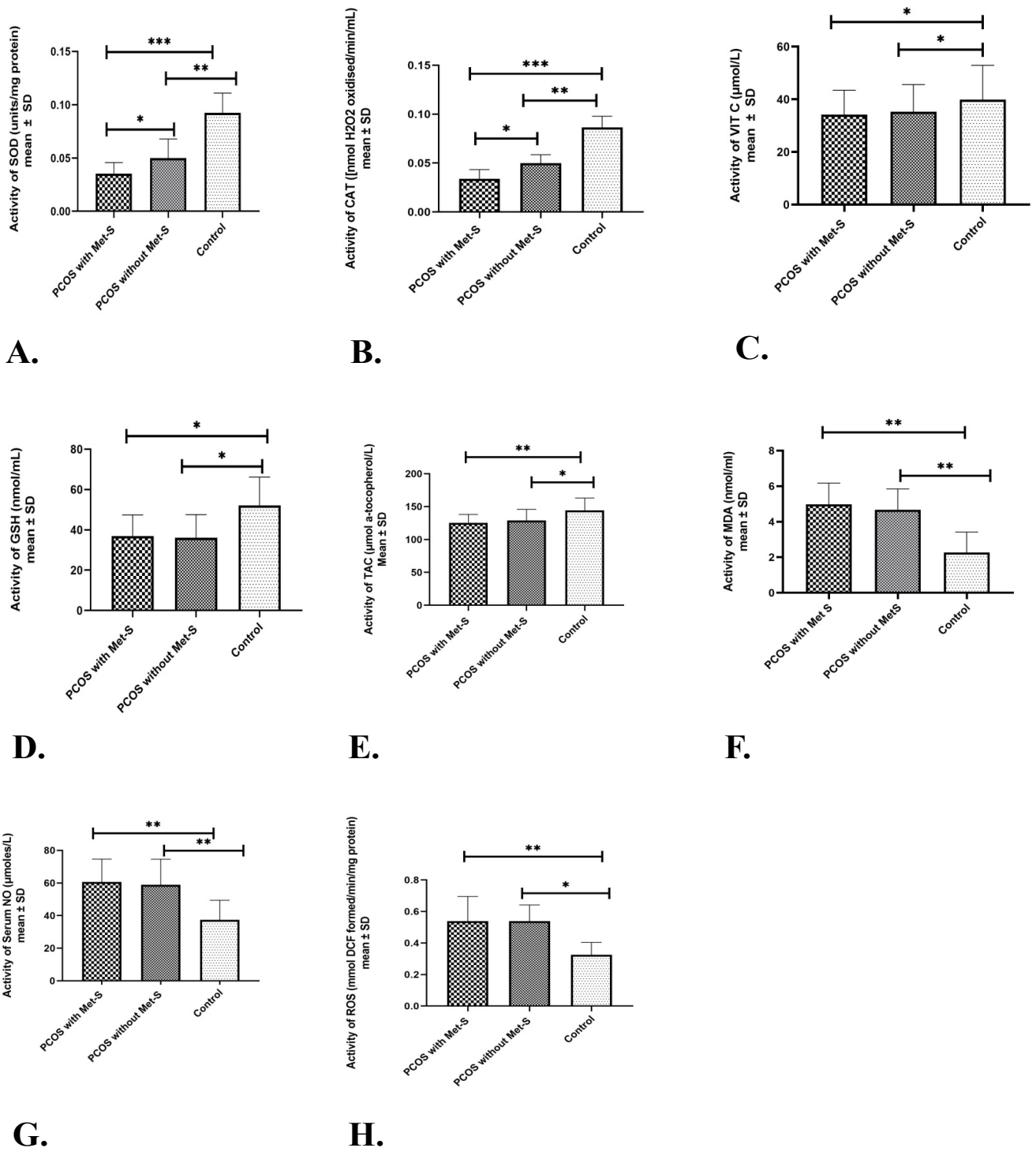

Figure 1. Oxidative and antioxidant markers between group analysis. MDA- Malondialdehyde; SOD- Superoxide Dismutase; CAT- Catalase; TAC- Total Antioxidant Capacity; GSH- Glutathione; Vit C- Vitamin C; ROS- Reactive Oxygen Species; NO- Serum Nitric Oxide.

obese when compared to the control. No statistically significant difference was found between PCOS and control women in terms of age, SBP and WHtR. The metabolic parameters- WC, WHR TC, TG, LDL, VLDL, TG/HDL ratio, FBG and insulin level were significantly higher and HDL was significantly lower in PCOS women compared to those in the control group.

The activities of various pro-oxidant and antioxidant markers of control and PCOS women are shown in Table 2. In PCOS women, enzymatic and non-enzymatic antioxidants such as SOD ($p < 0.0001$), CAT ($p < 0.0001$), GSH ($p < 0.0001$), total antioxidant capacity ($p < 0.0001$), and vitamin C ($p < 0.0001$) were significantly lower than in the control. Pro-oxidant stress indicators NO ($p < 0.0001$), ROS ($p < 0.0001$), and MDA ($p < 0.0001$) were insignificantly higher in PCOS subjects.

According to NCEP ATP III criteria (2005) in our study PCOS women were sub-grouped into PCOS with Met-S and PCOS without Met-S. Table 3 displays the results of our analysis of the anthropometric, endocrine, and metabolic characteristics of the PCOS with Met-S (n = 85), PCOS without Met-S (n = 65), and control (n = 100) groups. Except for Age, WHR, SBP and DBP there was a statistically significant difference between the three groups in other parameters (Table 3).

Figure 1 depicts a comparison of PCOS with Met-S and PCOS without Met-S groups with the control group. The majority of the oxidative stress indicators were statistically significant ($p < 0.05$) between the three groups. MDA, ROS and NO levels increased in the PCOS women with Met-S and PCOS women without Met-S compared to the control, while SOD, CAT, GSH, Vit-C and TAC levels decreased ($p < 0.05$). Within PCOS subgroups not that many differences were found, except SOD and CAT activities which greatly decreased in PCOS with the Met-S group compared to the PCOS without Met-S group.

Table 4 shows the bivariate analysis of the correlation between MDA, TAC, SOD, CAT and various features of Met-S (WC, SBP, DBP, HDL, LDL, TG, and FBG) in the three different groups. In PCOS with the Met-S group, HDL showed a negative correlation with MDA (r = -0.323) and a positive correlation with TAC (r = 0.253) with statistical significance ($p = 0.001$). There was a significant negative correlation between fasting blood glucose and CAT (r = -0.428). Both SOD and CAT were significantly negatively correlated with TG in the PCOS with Met-S group ($p < 0.05$), especially SOD (r = -0.520). In PCOS without Met-S group MDA showed a positive correlation with DBP (r = 0.428) and a negative correlation with HDL

Table 4. Correlation between oxidant-antioxidant parameters and features of Met-S

Variables	MDA			TAC			SOD			CAT		
	Control	**PCOS without Met-S**	**PCOS with Met-S**	**Control**	**PCOS without Met-S**	**PCOS with Met-S**	**Control**	**PCOS without Met-S**	**PCOS with Met-S**	**Control**	**PCOS without Met-S**	**PCOS with Met-S**
WC	0.098	-0.145	0.119	0.066	-0.178	-0.221	-0.067	0.024	0.09	0.261	0.056	-0.124
SBP	0.147	-0.025	0.002	-0.072	-0.045	0.060	-0.048	-0.048	-0.02	-0.146	-0.123	0.078
DBP	0.028	**0.428***	-0.08	0.256	0.014	-0.171	-0.148	-0.148	0.015	-0.041	-0.024	-0.210
HDL	0.017	**-0.541***	**-0.323***	-0.186	0.001	**0.253***	-0.248	-0.248	0.036	-0.007	-0.145	0.036
TG	0.219	-0.074	0.135	0.075	-0.041	-0.243	-0.073	-0.073	**-0.520***	-0.072	-0.078	**-0.328***
FBG	0.041	-0.046	0.014	0.023	0.186	-0.453	-0.143	-0.143	-0.225	0.211	0.169	**-0.428***

MDA- Malondialdehyde; SOD- Superoxide Dismutase; CAT- Catalase; TAC-Total Antioxidant Capacity; WC- Waist Circumference; SBP- Systolic Blood Pressure; DBP- Diastolic Blood Pressure; TG- Triglyceride; HDL- High-Density Lipoprotein; FBG- Fasting Blood Glucose.

* $p<0.05$

(r = -0.541) ($p<0.05$). In the control group, markers of OS did not correlate with Met-S parameters.

5. Discussion

In this study, PCOS women were sub-grouped into PCOS with Met-S and PCOS without Met-S, according to NCEP ATP III criteria (2005). The prevalence of Met-S, according to this criterion in PCOS women, was 56.66% (n = 85).

The relationship between metabolic syndrome and oxidative stress is undeniable and unquestionable. We investigated pro-oxidant markers MDA, ROS, and NO in control and PCOS women. It is generally known that lipid abnormalities are frequently detected in PCOS women[27,28]. Furthermore, dysfunctional lipid metabolism and elevated levels of oxidative stress indicators are seen frequently in PCOS women[29].

MDA is a typical measure of lipid peroxidation, which is regarded to be one of the most representative pro-oxidant markers of oxidative stress in PCOS[30]. We found high MDA levels in PCOS subgroups (with and without Met-S) compared to the control group but higher in PCOS with the Met-S group. Like our study, the study by Wang, *et al*., observed increased MDA levels in the Met-S PCOS group[31]. This is consistent with the conclusion of Zhang, *et* al., who reported that women with PCOS had considerably higher MDA concentrations than controls[32]. Similar findings were made by Kuscu, *et al*., who linked PCOS-related hyperglycemia and insulin resistance to an increase in MDA[33]. In our study, MDA levels in PCOS with Met-S and PCOS without Met-S groups did not show any significant difference.

Elevated ROS production as a result of excessive oxidative damage caused by the participants may thus be the cause of increased MDA concentration. Thus, these oxygen species can oxidise a variety of other important biomolecules, such as membrane lipids[34]. In our study both the PCOS subgroups (PCOS with and without Met-S) had ROS levels that were considerably greater than controls. Similarly, Gonzalez, *et al*., also observed a considerable increase in ROS in the PCOS group[13].

Nitric Oxide (NO) promotes vessel homeostasis by limiting vascular smooth muscle contraction and growth, platelet aggregation, and leukocyte adherence to the endothelium. Diabetes, atherosclerosis, and hypertension are all associated with altered NO pathways[35]. Insulin has been shown to stimulate NO release from cultured endothelial cells through a phosphatidylinositol-3 (PI-3) kinase pathway[36]. We found a significant difference in NO levels between women in the control and PCOS groups. PCOS women showed increased insulin levels. Many studies showed that there is no difference in insulin levels between PCOS and control groups[37–39].

We found that women with PCOS had significantly lower levels of both enzymatic and non-enzymatic antioxidant parameters such as SOD, CAT, GSH, vitamin C and TAC. Because of higher turnover for preventing oxidative damage, the levels of these antioxidant parameters might have decreased.

The activity of CAT in PCOS women was significantly low. Other studies also showed a substantial drop in catalse activity in PCOS women[40,41]. The decline in catalase activity may be caused by the build-up of ROS, whereupon the oxidative stress in PCOS women would have reduced catalase function.

A study conducted by Seleem, *et al.,* reveals that PCOS women had significantly lower SOD activity than in the control[42]. Compared to our control group, SOD was lower in PCOS women with or without Met-S. These findings are consistent with the study by Wang, *et al.,* and Zhang *et al*[31,32]. There is one study which shows results similar to our data and explains that a decrease in SOD activity may be connected with increased utilization of SOD to scavenge ROS produced due both to hyperglycemia and excess free fatty acids[34].

GSH is an essential antioxidant that is found in all cells. It protects cells against free radicals, peroxides, and other hazardous substances[43]. GSH levels were decreased in PCOS women with or without Met-S. This finding is similar to that of many other studies and it was hypothesized that the lower GSH levels may be indirectly associated with insulin resistance[39,44,45].

Vitamin C in the present study was significantly lower in PCOS women with or without Met-S. This is consistent with the finding of Fathima, *et al*[39]. Furthermore, Polak and his colleagues found a substantial drop in vitamin C levels in both peritoneal fluid and endometrial tissue in women with PCOS[47].

TAC has been described as an antioxidant marker representing the potency to destroy free radicals. There have been a few studies that describe the overall antioxidant level in PCOS. According to the findings of our investigation, PCOS women have a lower TAC

level than controls. Another research reveals that TAC levels were lower in PCOS women[48]. These findings are consistent with those of Wang, *et al.,* which showed significantly lower TAC levels in PCOS women with or without Met-S[31].

Our study also shows a significant positive correlation between HDL with TAC and a negative correlation between HDL with MDA. In PCOS women in the Met-S group showed that CAT significantly negatively correlated with TG and FBG indicating that there is a decrease in anti-oxidative capacity as TG and FBG increase. Also, another antioxidant, SOD showed a negative correlation with TG. In addition, a recent study in PCOS women found that oxidative stress can contribute to PCOS pathogenesis due to lipid metabolism dysfunction and relative antioxidant deficiency[27]. In PCOS without Met-S group there is an inverse relationship between MDA and HDL and a positive relationship between MDA and DBP. High blood pressure, independent of Met-S, is correlated with MDA highlighting its role in oxidative stress-induced pathogenesis in PCOS.

Obesity is one of the risk factors for cardiovascular disorders. Met-S components can be more prevalent in overweight/obese individuals and often are associated with indicators of oxidative stress[49]. Similarly, we found that Met-S components are essential features of oxidative stress indicators in PCOS patients. PCOS women were overweighed compared to the control group which may be one of the reasons we did not find a single case of Met-S in the control group. Our study found an increase in oxidative capacity and a decrease in antioxidant capacity especially in PCOS with Met-S, further indicating that Met-S can accelerate oxidative stress in PCOS patients. As a result, PCOS in Met-S women may have an elevated risk of developing cardiovascular diseases. There may be additional factors influencing the antioxidant abilities of an individual based on the different results of studies assessing OS parameters in PCOS subjects. Larger population-based case-control studies are needed to establish the relationship between the components of Met-S and oxidative stress in PCOS.

In conclusion, the present study evidences the coexistence of increased oxidative stress and metabolic syndrome components in both PCOS with Met-S and PCOS without Met-S subjects. The metabolic syndrome components of PCOS can induce oxidative stress, which is evidenced by a decrease in antioxidant defence mechanisms. It is probably because oxidative stress itself is the consequence of PCOS, additionally with Met-S which increases the pro-oxidant state and decreases anti-oxidant capacity in women. Furthermore, oxidative stress may participate in the future development of cardiovascular diseases in women, in addition to dyslipidemia, central obesity, and hyperglycemia. Also, in the future, more oxidative stress studies should be conducted on PCOS patients with and without Met-S. We suggest the implementation of antioxidants in PCOS therapy, in addition to maintenance of a healthy lifestyle, physical activity and proper diet pattern which should all have beneficial therapeutic effects and also avoid oxidative stress-induced complications in the future in PCOS women.

6. Acknowledgments

We thank all volunteers who took part in our study. We are especially grateful to Dr Zeinab Nagsband for their support during the research. We thank Dr Sonia Mandappa, gynaecologist and obstetrician, Ashwini and Mediwave Hospital lab staff for their assistance with sample collection. We thank the Department of Studies in Zoology, University of Mysore, Manasagangothri, Mysuru, India.

7. References

1. Lizneva D, Suturina L, Walker W, *et al*. Criteria, prevalence, and phenotypes of polycystic ovary syndrome. Fertil Steril. 2016; 106:6-15. https://doi.org/10.1016/j.fertnstert.2016.05.003 PMid:27233760
2. Norman RJ, Dewailly D, Legro RS, Hickey TE. Polycystic ovary syndrome. Lancet. 2007; 370(9588):685-97. https://doi.org/10.1016/S0140-6736(07)61345-2 PMid:17720020
3. Deeks AA, Gibson-Helm ME, Teede HJ. Anxiety and depression in polycystic ovary syndrome: A comprehensive investigation. Fertil Steril. 2010; 93:2421-3. https://doi.org/10.1016/j.fertnstert.2009.09.018 PMid:20117778
4. Moran LJ, Misso ML, Wild RA, Norman RJ. Impaired glucose tolerance, type 2 diabetes and metabolic syndrome in polycystic ovary syndrome: A systematic review and meta-analysis. Hum Reprod Update. 2010; 16:347-63. https://doi.org/10.1093/humupd/dmq001 PMid:20159883

5. Chen L, Xu WM, Zhang D. Association of abdominal obesity, insulin resistance, and oxidative stress in adipose tissue in women with Polycystic Ovary Syndrome. Fertil Steril. 2014; 102(4):1167-74. https://doi.org/10.1016/j.fertnstert.2014.06.027 PMid:25064406
6. Grundy SM, Cleeman JI, Daniels SR, *et al*. Diagnosis and management of the metabolic syndrome: an American Heart Association/National Heart, Lung, and Blood Institute Scientific Statement. Circulation. 2005; 112:2735-52. https://doi.org/10.1161/CIRCULATIONAHA. 105.169404 PMid:16157765
7. Caserta D, Adducchio G, Picchia S, *et al*. Metabolic syndrome and polycystic ovary syndrome: an intriguing overlapping. Gynecol Endocrinol. 2014; 30:397-402. https://doi.org/10.3109/09513590.2014.887673 PMid: 24552422
8. Lim SS, Kakoly NS, Tan JWJ, *et al*. Metabolic syndrome in polycystic ovary syndrome: A systematic review, meta-analysis and meta-regression. Obes Rev. 2019; 20(2):339-52. https://doi.org/10.1111/obr.12762 PMid:30339316
9. Otaghi M, Azami M, Khorshidi A, *et al*. The association between metabolic syndrome and polycystic ovary syndrome: A systematic review and meta-analysis. Diab Metab Syndr. 2019; 13(2):1481-9. https://doi.org/10.1016/j.dsx.2019.01.002 PMid:31336510
10. Agarwal A, Aponte-Mellado A, Premkumar BJ, *et al*. The effects of oxidative stress on female reproduction: a review. Reprod Biol Endocrinol. 2012; 10:49. https://doi.org/10.1186/1477-7827-10-49 PMid:22748101 PMCid: PMC3527168
11. Murri M, Luque-Ramírez M, Insenser M, *et al*. Circulating markers of oxidative stress and Polycystic Ovary Syndrome (PCOS): A systematic review and meta-analysis. Hum Reprod Update. 2013; 19(3):268-88. https://doi.org/10.1093/humupd/dms059 PMid:23303572
12. Desai V, Prasad NR, Manohar SM, *et al*. Oxidative stress in non-obese women with polycystic ovarian syndrome. J Clin Diagn Res. 2014; 8:CC01-03.
13. Gonzalez F, Rote NS, Minium J, Kirwan JP. Reactive oxygen species-induced oxidative stress in the development of insulin resistance and hyperandrogenism in polycystic ovary syndrome. J Clin Endocrinol Metab. 2006; 91:336-40. https://doi.org/10.1210/jc.2005-1696 PMid:16249279
14. Papalou O, Victor VM, Diamanti-Kandarakis E. Oxidative stress in polycystic ovary syndrome. Curr Pharm Des. 2016; 22:2709-22. https://doi.org/10.2174/1381612822666160216151852 PMid:26881435
15. Victor VM, Rocha M, Bañuls C, *et al*. Mitochondrial complex I impairment in leukocytes from polycystic ovary syndrome patients with insulin resistance. J Clin Endocrinol Metab. 2009; 94:3505-12. https://doi.org/10.1210/jc.2009-0466 PMid:19567514
16. The Rotterdam ESHRE/ASRM-Sponsored PCOS Consensus Workshop Group. Revised 2003 consensus on diagnostic criteria and long-term health risks related to polycystic ovary syndrome. Fertil Steril. 2004; 81:19-25. https://doi.org/10.1016/j.fertnstert.2003.10.004
17. Friedewald WT, Levy RI, Fredrickson DS. Estimation of the concentration of low-density lipoprotein cholesterol in plasma, without use of the preparative ultracentrifuge. Clin Chem. 1972; 18:499-502. https://doi.org/10.1093/clinchem/18.6.499 PMid:4337382
18. Cleeman JI, Grundy SM, Becker D, *et al*. Cholesterol Educ program, executive summary of the third report of the National Cholesterol Education Program (NCEP) expert panel on detection, evaluation, and treatment of high blood cholesterol in adults (adult treatment panel III). JAMA 2001; 285:2486-97. https://doi.org/10.1001/jama.285.19.2486 PMid:11368702
19. Bernheim F, Bernheim MLC, Wilbur KM. The reaction between thiobarbituric acid and the oxidation products of certain lipids. J Biol Chem. 1948; 174(1):257-64. https://doi.org/10.1016/S0021-9258(18)57394-4 PMid: 18914082
20. Black MJ, Brandt RB. Spectrofluorometric analysis of hydrogen peroxide. Anal Biochem. 1974; 58(1):246-54 https://doi.org/10.1016/0003-2697(74)90464-3 PMid: 4825377
21. Grisham MB, Johnson GG, Lancaster JR. Quantitation of nitrate and nitrite in extracellular fluids. Methods Enzymol. 1996; 268:237-46, https://doi.org/10.1016/S0076-6879(96)68026-4 PMid:8782590
22. Kazari D. A modified spectrophotometric assay of superoxide dismutase using nitrate formation by superoxide radical. Ind J Biochem Biophys. 2000; 57:201-4.
23. Aebi H. Catalase in vitro methods. Enzymology 1984; 105:121-6. https://doi.org/10.1016/S0076-6879(84)05016-3 PMid: 6727660
24. Moron M, Depierre J, Mannervik B. Levels of glutathione, glutathione reductase and glutathione s-transferase activities in rat lung and liver. Biochim Biophys Acta (BBA)- General Subjects. 1979; 582(1):67-78 https://doi.org/10.1016/0304-4165(79)90289-7
25. Subash-Babu P, Alshatwi AA, Ignacimuthu S. Beneficial antioxidative and antiperoxidative effect of cinnamaldehyde protect streptozotocin induced pancreatic β-cells

damage in Wistar rats. Biomol Therapeut. (Seoul). 2014; 22(1):47-54. https://doi.org/10.4062/biomolther.2013.100 PMid: 24596621 PMCid:PMC3936432

26. Prieto P, Pineda M, Aguilar M. Spectrophotometric quantitation of antioxidant capacity through the formation of a phosphomolybdenum complex: Specific application to the determination of vitamin E. Anal Biochem. 1999; 269(2):337-41. https://doi.org/10.1006/abio.1999.4019 PMid:10222007
27. Rocha MP, Marcondes JAM, Barcellos CRG, *et al*. Dyslipidemia in women with polycystic ovary syndrome: incidence, pattern and predictors. Gynecol Endocrinol. 2011; 27:814-19. https://doi.org/10.3109/09513590.2010.508852 PMid:20807166
28. Ruan X, Li M, Mueck AO. Why does Polycystic Ovary Syndrome (PCOS) need long-term management? Curr Pharm Des. 2018; 24:4685-92. https://doi.org/10.2174/1381612825666190130104922 PMid:30706800
29. Mujica LS, Bridi A, Méa RD, *et al*. Oxidative stress and metabolic markers in pre- and postnatal polycystic ovary syndrome rat protocols. J Infamm Res. 2018; 11:193-202. https://doi.org/10.2147/JIR.S160264 PMid:29805266 PMCid:PMC5960249
30. Abuja PM, Albertini R. Methods for monitoring oxidative stress, lipid peroxidation and oxidation resistance of lipoproteins. Clin Chim Acta. 2001; 306(1-2):1-17. https://doi.org/10.1016/S0009-8981(01)00393-X PMid: 11282089
31. Wang H, Ruan X, Li Y, *et al*. Oxidative stress indicators in Chinese women with PCOS and correlation with features of metabolic syndrome and dependency on lipid patterns. Arch Gynecol Obstet. 2019; 300(5):1413-21. https://doi.org/10.1007/s00404-019-05305-7 PMid: 31549221
32. Zhang D, Luo WY, Liao H, *et al*. The effects of oxidative stress to PCOS. J Sichuan Univ Med Sci Ed. 2008; 39:421-3.
33. Kuşçu NK, Var A. Oxidative stress but not endothelial dysfunction exists in non-obese, young group of patients with polycystic ovary syndrome. Acta Obstet Gynecol Scand. 2009; 88:612-7. https://doi.org/10.1080/00016340902859315 PMid:19308750
34. Oyebanji O, Asaolu, M. Assessment of antioxidant status of women with polycystic ovarian syndrome. Asian Pac J Reprod. 2020; 9(1):9. https://doi.org/10.4103/2305-0500.275523
35. Taylor BS, Kim YM, Wang Q, *et al*. Nitric oxide downregulates hepatocyte-inducible nitric oxide synthase gene expression. Arch Surg. 1997; 132:1177-83. https://doi.org/10.1001/archsurg.1997.01430350027005 PMid:9366709
36. Zeng G, Quon MJ. Insulin-stimulated production of nitric oxide is inhibited by wortmannin. Direct measurement in vascular endothelial cells. J Clin Investig. 1996; 98:894-8. https://doi.org/10.1172/JCI118871 PMid: 8770859 PMCid:PMC507502
37. Nacul AP, Andrade CD, Schwarz P, *et al*. Nitric oxide and fibrinogen in polycystic ovary syndrome: Associations with insulin resistance and obesity. Eur J Obstet Gynecol Reprod Biol. 2007; 133:191-6. https://doi.org/10.1016/j.ejogrb.2006.09.009 PMid:17049715
38. Baskol G, Aygen E, Erdem F, *et al*. Assessment of paraoxonase 1, xanthine oxidase and glutathione peroxidase activities, nitric oxide and thiol levels in women with polycystic ovary syndrome. Acta Obstet Gynecol Scand. 2012; 91:326-30. https://doi.org/10.1111/j.1600-0412.2011.01337.x PMid:22168506
39. Fatima Q, Amin S, Kawa IA, *et al*. Evaluation of antioxidant defense markers in relation to hormonal and insulin parameters in women with polycystic ovary syndrome (PCOS): A case-control study. Diabetes Metab Syndr. 2019; 13(3);1957-61. https://doi.org/10.1016/j.dsx.2019.04.032 PMid:31235121
40. Al-Azzawie HF, Humadi EH. Oxidative stress and antioxidant mechanisms in a sample of Iraqi patients with polycystic ovary syndrome. Iraqi J Comm Med. 2010; 3:196-200.
41. Kandasamy S, Sivagamasundari RI, Bupathy A, *et al*. Evaluation of insulin resistance and oxidative stress in obese patients with polycystic ovary syndrome. Int Jappl Biol Pharm. 2010; 2:391-98.
42. Seleem AK, El Refaeey AA, Shaalan D, *et al*. Superoxide dismutase in polycystic ovary syndrome patients undergoing intracytoplasmic sperm injection. J Assist Reprod Genet. 2014; 31:499-504. https://doi.org/10.1007/s10815-014-0190-7 PMid:24526356 PMCid: PMC3969459
43. Sies H. Glutathione and its role in cellular functions. Free Radic Biol Med. 1999; 27:916-21. https://doi.org/10.1016/S0891-5849(99)00177-X PMid:10569624
44. Sabuncu T, Vural H, Harma M, Harma M. Oxidative stress in polycystic ovary syndrome and its contribution to the risk of cardiovascular disease. Clin Biochem. 2001; 34(5):407-13. https://doi.org/10.1016/S0009-9120(01)00245-4 PMid:11522279
45. Dincer Y, Akcay T, Erdem T, *et al*. DNA damage, DNA susceptibility to oxidation and glutathione level in women with polycystic ovary syndrome. Scand J Clin Lab Invest. 2005; 65(8):721-28. https://doi.org/10.1080/00365510500375263 PMid:16509054

46. Surapaneni KM, Vishnu PV. Lipid peroxidation, glutathion, ascorbic acid, vit E, antioxidant enzyme and serum homocysteine status in patients with polycystic ovary syndrome. Biol Med. 2009; 1(3):44-9.
47. Polak G, Kozioł-Montewka M, Gogacz M, *et al*. Total antioxidant status in peritoneal fluid in infertile women. Eur J Obstet Gynecol Reprod Biol. 2001; 94:261-63. https://doi.org/10.1016/S0301-2115(00)00352-3 PMid: 11165736
48. Moti M, Amini L, MirhoseiniArdakani SS, *et al*. Oxidative stress and antioxidant defense system in Iranian women with polycystic ovary syndrome. Iran J Reprod Med. 2015; 13:373-78.
49. Morelli NR, Scavuzzi BM, Miglioranza LHDS, *et al*. Metabolic syndrome components are associated with oxidative stress in overweight and obese patients. Arch Endocrinol Metab. 2018; 62:309-18. https://doi.org/10.20945/2359-3997000000036 PMid:29791650