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Insulin resistance and Oxidant-Antioxidant Markers in Young Women with Polycystic Ovarian Syndrome

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ABSTRACT:
Introduction: Oxidative stress and insulin resistance (IR) may contribute to the pathogenesis of polycystic ovarian syndrome (PCOS). IR promotes hyperglycaemia, which may increase the production of reactive oxygen species and decrease in antioxidant levels in PCOS.
Aim: To investigate the oxidant-antioxidant status and its association with insulin resistance in young women with PCOS.
Materials and methods: The present study involved two groups, PCOS (n = 55) and age and BMI matched controls (n = 55). Serum levels of various oxidative stress markers malondialdehyde (MDA), antioxidants- superoxide dismutase (SOD), catalase, and total antioxidant capacity (TAC) were measured using spectrophotometric assays. HOMA-IR method was used to assess the insulin resistance. Results were analysed to compare and correlate insulin resistance with these oxidative stress markers.
Results: PCOS group had significantly higher MDA and lower TAC activity, SOD, catalase levels than the control group ($p < 0.001$). PCOS patients with IR had significantly higher MDA, while SOD, catalase, TAC levels lower than the PCOS patients without IR ($p< 0.001$). Infertile PCOS patients had significantly higher MDA, and lower SOD, catalase, TAC level than the fertile PCOS patients ($p<0.001$). There was a statistically significant and positive correlation between HOMA-IR and MDA levels, whereas HOMA-IR showed statistically significant negative correlation with SOD, catalase and TAC levels.
Conclusion: Our findings suggest that young PCOS women may experience oxidative stress due to insulin resistance. As a result, the existence of insulin resistance, hyperinsulinemia, and oxidative damage in PCOS is likely to hasten the gradual progression of cardiovascular disease.

Keywords: Infertility; Insulin resistance; Oxidative stress; Polycystic ovary syndrome; Antioxidants

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INTRODUCTION

Polycystic ovarian syndrome (PCOS) is the most common endocrinological disorder in women of reproductive age, with a prevalence of 6% to 21% depending on the different diagnostic criteria and ethnicity (Zhao et al., 2023). PCOS is

associated with both reproductive and metabolic complications. Reproductive complications include hyperandrogenism, ovarian dysfunction, pregnancy complications and infertility (Boomsma et al., 2006). Furthermore, metabolic problems include insulin resistance, dyslipidemia, decreased glucose tolerance, and diabetes mellitus (Talbott et al., 2000). The mechanism underlying polycystic ovarian syndrome (PCOS) is not well understood.

Insulin resistance has been found in 50-70% of PCOS women; it appears to interfere with both ovarian steriodogenic abnormalities and anovulatory mechanisms (Poretsky et al., 1999; Legro et al., 2004). Insulin resistance leads to metabolic syndrome and raises the risk of cardiovascular problems independently (OvalleandAzziz, 2002). Insulin resistance leads to oxidative stress due to the production of reactive oxygen species (ROS) by hyperglycemia and increased free fatty acid levels (YeonLee et al., 2010; Bloch-Damti & Bashan, 2005).

Oxidative stress (OS) is defined as an imbalance between oxidants and antioxidants that results in an abnormal redox state of cells (Sulaiman et al., 2018). Reactive oxygen species (ROS) such as superoxide, hydrogen peroxide, and hydroxyl radical ions are the agents of the OS and are mostly generated at low physiological levels in the mitochondria and peroxisomes. When ROS are present in high concentrations, they become toxic and damage body tissues, despite their role in the control of various vital physiological processes (Schrieber and Chandel, 2014). Antioxidants are a type of protective physiological mechanism that living systems have evolved to deal with OS. They can neutralise the effects of oxidants and have ability to dispose, scavenge, and suppress the free radical formation (Fang, 2002). Antioxidants are either enzymatic, such as superoxide dismutase, catalase, glutathione peroxidase (GPx), and glutathione reductase (GR), or non-enzymatic, such as glutathione (GSH), α-tocopherol (vitamin E), ascorbate (vitamin C), and β-carotene. It has been observed that these antioxidants have a significant function in the female reproductive system and in the pathophysiology of female infertility (Agarwal, 2005).

OS is thought to play role in endometriosis, unexplained infertility, male factor infertility, ovulatory regulation and impairment of oocyte quality in the human reproductive system (Agarwal, 2012). When excessive ROS generation overwhelms the body's natural antioxidant defence system, it can create an unstable environment for normal female physiological reactions (Al-Gubory, 2010). As a result, it is essential to assess the oxidant/antioxidant state of PCOS and infertile women.

In this study we intend to estimate oxidant-antioxidant status and to assess the association of oxidant-antioxidant markers with insulin resistance in young PCOS patients.

MATERIALS AND METHODS

This case-control study was approved by the Institutional Human Ethical Clearance Committee of University of Mysore (IHEC-UOM No. 154/PhD/2017-18) which was conducted between January 2017 and December 2018. A total of 110 women, including 55 PCOS subjects and 55controls were enrolled in the present study age range between 18- 30 years. The subjects received written consent before the study, and all the subjects were fully informed about the study protocol.

The diagnosis of PCOS was based on the Rotterdam criteria that require the presence of two of the three following criteria (ESHRE and ASRM-Sponsored PCOS Consensus Workshop Group, 2004): (1) the presence of oligomenorrhea or amenorrhea, (2) clinical and/or biochemical hyperandrogenism, (3) polycystic ovaries on ultrasonography. Other related diseases, such as Cushing syndrome, adrenal congenital hyperplasia, and androgen-secreting tumors, hyper-prolactinemia and patients with other medical diseases were excluded. The women in the control group were healthy volunteers with regular menstrual cycles of 4-5 days in length and 25-30 days in frequency, with no evidence of hyperandrogenism and normal sonographic

appearance of the ovaries. General information (age, marital status), reproductive information (menstruation, fertility), clinical features of PCOS, drug history were collected from each patient. All women were subjected to anthropometric assessment like waist circumferences (WC), hip circumferences (HC), waist-hip ratio (WHR), and body mass index (BMI) (kg/m^2).

Blood samples were collected from each subject after 10-12 hours of overnight fasting between 08:00 and 09:00 in the morning during their early follicular phase (2nd - 4th day) of the spontaneous or induced menstrual cycles or any day in amenorrheic patients. After blood samples were centrifuged at 3,000 rpm for 10 minutes, serum further used for endocrine and biochemical analysis.

Insulin resistance was calculated using the homeostasis model assessment (HOMA-IR) index (fasting glucose x fasting insulin/constant). Since the fasting glucose value was measured in mg/dL, the constant was 405 (Legro et al., 2004). The HOMA-IR level > 2.5 is considered insulin resistant. All PCOS subjects were divided into the following groups: PCOS women with insulin resistance (IR+) (n=22) and PCOS women without insulin resistance (IR-) (n=38). Subgroup analysis was also done based on fertility status. Two subgroups were created: patients with PCOS who did not become pregnant despite 1-year of unprotected intercourse (n=18) and PCOS patients who became pregnant without treatment (n=22). Plasma glucose levels were measured by enzymatic glucose oxidase-peroxidase assay (ARKRAY kit, Mumbai, Maharashtra, India). The serum levels of follicle stimulating hormone (FSH), luteinizing hormone (LH) and insulin were measured by enzyme-linked immune sorbent assay (Prime Biomed, Bangaluru, Karnataka, India).

Super oxide dismutase (SOD) analysis was conducted according to the method specified by Kazari Das (Kazari, 2000). In this method, the superoxide radicals produced by the reduction of riboflavin are scavenged by SOD. The leftover superoxide radicals react with hydroxylamine hydrochloride to produce nitrites which in turn react with Griess reagent to produce a pink azo compound whose absorbance was determined at 543 nm. The SOD level was expressed as units/mg protein.

Aebi method (Aebi, 1984) was used for the determination of catalase activity where the decomposition of hydrogen peroxide by catalase was noted by observing the decrease in absorbance read at 240nm. The variation in absorbance per unit time was used as a measure catalase activity. The catalase level was expressed as nmol H_2O_2 oxidised/min/mL. The malondialdehyde (MDA) was measured by thiobarbituric acid- trichloroacetic acid – hydrochloric acid assay (TBA-TCA-HCl assay) (Bernheim et al., 1948). The MDA level was expressed as (nmol/ml). The total antioxidant capacity (TAC) is measured by the modified method of the phosphomolybdic method (Prieto et al., 1999). The TAC level was expressed as μmol a-tocopherol/L.

Statistical Analysis:

Data are expressed as mean ± standard deviation (SD. Student t-test and one-way analysis of variance (ANOVA) were used for the comparisons. A *post hoc* analysis was carried out to determine the two variables between which there is a statistically significant difference. p values less than 0.05 were accepted to be statistically significant. Pearson's correlation coefficient was used to determine the relationship between two variables. Statistical analysis was performed using IBM SPSS version 23.0 (SPSS, Chicago, IL, USA) and GraphPad Prism version 8.0 (GraphPad Software, San Diego, CA, USA).

RESULTS

This study compares the demographic and clinical characteristics of 55 women with PCOS and 55 healthy controls. The demographic features of the PCOS and control group are summarized in Table 1. When compared with the healthy controls, the women with PCOS had significantly lower SOD, TAC levels and catalase activity ($p = 0.016$ and $p = 0.025$ respectively) as well as significantly higher MDA levels ($p < 0.001$ and $p = 0.043$ respectively).

Table 1: Comparison of anthropometric, endocrine parameters and oxidant-antioxidant status between PCOS and controls

Variables	PCOS(n=55)	Control(n=55)	p-value
Age (in years)	23.74 ± 0.64	23.43 ± 1.13	0.082
BMI(kg/m²)	21.95 ± 2.08	22.11± 3.36	0.760
WC (in cm)	91.45 ± 10.94	81.40 ± 5.45	**<0.001**
FSH(mIU/ml)	4.24 ± 1.59	5.84 ± 2.50	**0.001**
LH(mIU/ml)	12.01 ± 6.36	7.14 ± 1.85	**<0.001**
Testosterone(nmol/L)	1.78 ± 0.61	1.13 ± 0.54	**<0.001**
Fasting glucose (mg/dl)	91.96 ± 5.91	87.82 ± 4.9	**<0.001**
Fasting insulin(mIU)	12.1 ± 2.21	11.33 ± 1.20	**<0.001**
Homa-IR	2.78 ± 0.62	2.45 ± 0.44	**<0.001**
SOD(units/mg protein)	0.031 ± 0.005	0.094 ± 0.01	**<0.001**
Catalase (nmol H_2O_2 oxidised/min/mL)	0.031 ± 0.007	0.085 ± 0.01	**<0.001**
MDA (nmol/ml)	4.97 ± 1.22	2.67 ± 1.28	**<0.001**
TAC (μmol a-tocopherol/L)	122.01 ± 9.17	151.72 ± 18.26	**<0.001**

Data are expressed as mean ± SD and analyzed by student t test. BMI- Body Mass Index; WC-Waist Circumference; FSH- Follicle Stimulating Hormone; LH- Leiuntinizing hormone; HOMA-IR: Homeostasis Model for Insulin Resistance; SOD- Superoxide Dismutase; MDA- Malondialdehyde; TAC-Total Antioxidant Capacity. $p<0.05$ significant.

There were 22 PCOS women with insulin resistance (IR+) and 33 PCOS women without insulin resistance (IR-). The mean ages of the PCOS women without insulin resistance (IR-), PCOS women with insulin resistance (IR+), and controls were 23.75 ± 0.57 years, 23.75 ± 0.99 years and 23.43± 1.3 years, respectively, and the mean BMIs were 22.06 ± 2.23 kg/m² , 22.39 ± 3.97 kg/m² and 22.1± 3.36 kg/m², respectively. There were no significant differences among the groups in age and BMI (p=0.123; p=0.075).

When compared with the healthy controls, IR +PCOS had significantly lower SOD, TAC levels and catalase activity ($p < 0.001$) and significantly higher MDA levels ($p< 0.001$) (Figure1).When compared to IR- PCOS, the IR + PCOS had significantly higher MDA levels, significantly lower SOD, catalase and TAC activity ($p = 0.011$, $p = 0.003$, $p <0.001$ and $p< 0.001$ respectively). (Table 2)

Table 2: Comparison of anthropometric and endocrine characteristics of control and PCOS after sub-dividing into IR + PCOS and IR – PCOS groups

Variables	IR + PCOS(n=22)	IR – PCOS(n=33)	Control(n=55)	p-value
Age (in years)	23.86 ± 0.63	23. 66 ± 0.64	23.43 ± 1.13	0.164
BMI(kg/m²)	22.3 ± 1.34	21.72 ± 2.45	22.11± 3.36	0.719
WC (in cm)	91.45 ± 12.14^{b}	91.45 ± 10.26^{c}	81.40 ± 5.45	**<0.001**
FSH (mIU/ml)	4.18± 1.95^{b}	4.28 ± 1.32^{c}	5.84 ± 2.50	**0.001**
LH (mIU/ml)	9.23 ± 6.33^{b}	11.33 ± 5.06^{c}	7.14 ± 1.85	**<0.001**
Testosterone(nmol/L)	2.03 ± 0.30^{ab}	1.61 ± 0.71^{c}	1.13 ± 0.54	**<0.001**
Fasting glucose (mg/dl)	98.09 ± 4.11^{ab}	87.88 ± 3.9	87.82 ± 4.9	**<0.001**
Fasting insulin(mIU)	14.14 ± 0.85^{ab}	10.87 ± 1.61	11.33 ± 1.20	**<0.001**
Homa-IR	3.42 ± 0.21^{ab}	2.35 ± 0.27	2.45 ± 0.44	**<0.001**
SOD(units/mg protein)	0.025 ± 0.002^{ab}	0.035 ± 0.002^{c}	0.094 ± 0.01	**<0.001**

Catalase (nmol H_2O_2 oxidised/min/mL)	0.022± 0.002^{ab}	0.036 ± 0.004^{c}	0.085 ± 0.01	**<0.001**
MDA (nmol/ml)	5.53 ± 1.30^{ab}	4.6 ± 1.03^{c}	2.67 ± 1.28	**<0.001**
TAC (µmol a-tocopherol/L)	111.97 ± 1.13^{ab}	128.70 ± 5.08^{c}	151.72 ± 18.26	**<0.001**

Data are expressed as mean ± SD and analyzed by one way anova followed by LSD *post hoc* test. BMI-Body Mass index; WC-Waist Circumference; FSH- follicle stimulating hormone; LH- leiuntinizing hormone; HOMA-IR: homeostasis model for insulin resistance; SOD- superoxide dismutase; MDA-malondialdehyde ; TAC-total antioxidant capacity.

(a) Comparison of IR (+) PCOS patients with IR(-) PCOS patients; $p < 0.05$

(b) Comparison of IR (+) PCOS patients with healthy controls; $p < 0 .05$

(c) Comparison of IR (-) PCOS patients with healthy controls; $p < 0.05$

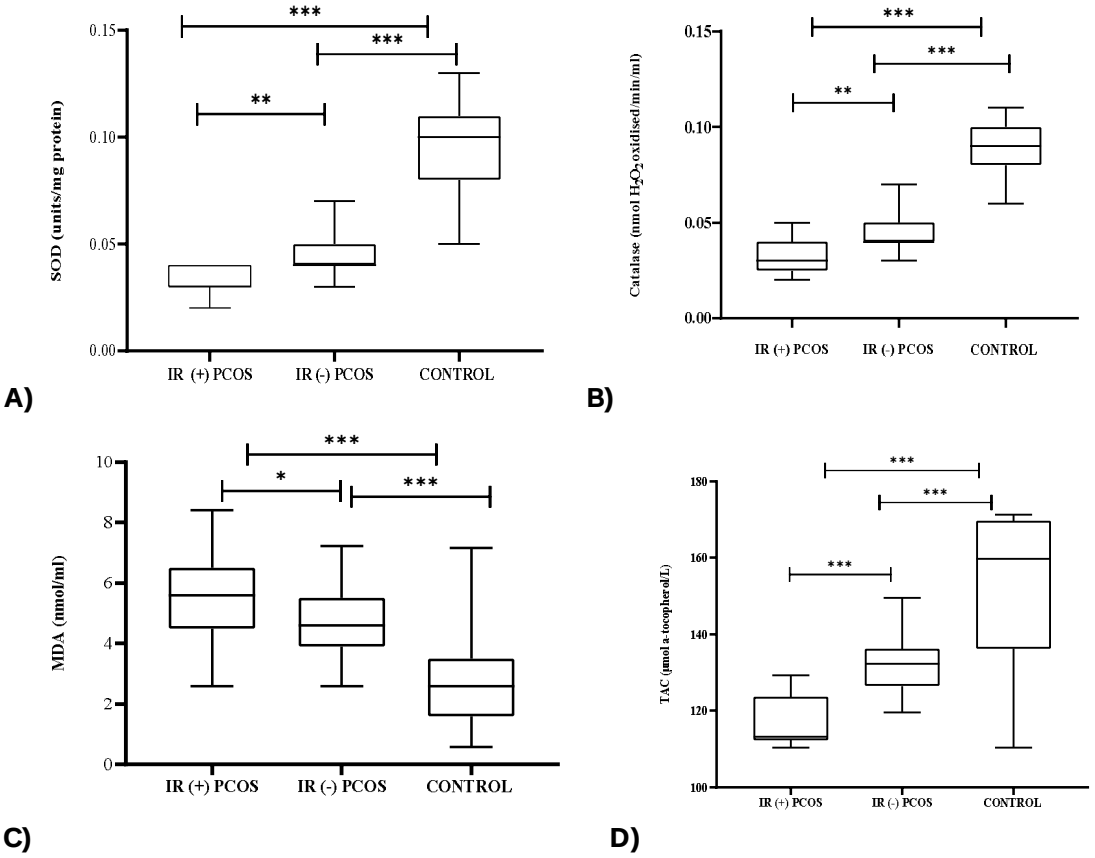

Figure 1: Oxidative and antioxidant markers between group analysis.

MDA- Malondialdehyde; SOD- Superoxide dismutase; CAT- Catalase; TAC-Total antioxidant capacity

IR (+) PCOS-PCOS women with insulin resistance

IR (-) PCOS-PCOS women without insulin resistance

* denotes significance of $p < 0.05$

Among the 40 married PCOS women, 18 were infertile, and 22 were fertile in our study group. When compared with the healthy controls, the infertile PCOS patients had significantly lower concentrations of SOD, catalase and TAC activity (p<0.001) as well as significantly higher MDA levels (p < 0.001). When compared to fertile PCOS patients, infertile PCOS patients had significantly higher MDA levels and lower SOD, catalase and TAC activity (p<0.001) (Table 3).

Table 3: Comparison of anthropometric and endocrine characteristics of control and PCOS after sub-dividing into infertile and fertile PCOS groups

Variables	Infertile PCOS (n=18)	Fertile PCOS (n=22)	Control (n=55)	p-value
Age (in years)	23.72 ± 0.46	24.0 ± 1.2	23.43 ± 1.13	0.164
BMI(kg/m²)	23.96 ± 2.34	22.95 ± 1.39	22.11± 3.36	<0.001
WC (in cm)	95.94 ± 8.48	93.59 ± 10.5	81.40 ± 5.45	0.001
FSH(mIU/ml)	4.57 ± 1.42	4.58 ± 1.79	5.84 ± 2.50	<0.001
LH(mIU/ml)	11.34 ± 3.31	10.10 ± 5.38	7.14 ± 1.85	<0.001
Testosterone(nmol/L)	1.83 ± 0.78	1.25 ± 0.21	1.13 ± 0.54	<0.001
Fasting glucose (mg/dl)	98.39 ± 2.56	96.57 ± 4.10	87.82 ± 4.9	<0.001
Fasting insulin(mIU)	15.71 ± 2.13	14.9 ± 1.65	11.33 ± 1.20	<0.001
Homa-IR	3.86 ± 0.52	3.57 ± 0.41	2.45 ± 0.44	<0.001
SOD(units/mg protein)	0.031 ± 0.006	0.043 ± 0.011	0.094 ± 0.01	<0.001
Catalase (nmol H_2O_2 oxidised/min/mL)	0.030 ± 0.009	0.040 ± 0.008	0.085 ± 0.01	<0.001
MDA (nmol/ml)	5.72 ± 1.34	4.40 ± 1.09	2.67 ± 1.28	<0.001
TAC (μmol a-tocopherol/L)	128.19 ± 12.53	131.00 ± 11.86	151.72 ± 18.26	<0.001

Data are expressed as mean ± SD and analyzed by one way anova followed by LSD *post hoc* test. BMI-Body Mass index; WC-Waist Circumference; FSH- Follicle stimulating hormone; LH- Leiuntinizing hormone; HOMA-IR: Homeostasis Model for Insulin Resistance SOD- Superoxide ismutase; MDA-Malondialdehyde ; TAC-Total antioxidant capacity.

Pearson (r) correlation coefficient analysis was carried out for HOMA-IR, glucose levels with oxidant-antioxidant markers levels. When exploring possible correlations between HOMA-IR and oxidant-antioxidant markers we found that the HOMA-IR was positively correlated with MDA (p<0.001, r = 0.304) and negatively correlated with SOD level (r = -0.675, p < 0.001), catalase activity (r = -0.667, p< 0.001), and TAC (r = -0.542, p < 0.001) (Figure 2).

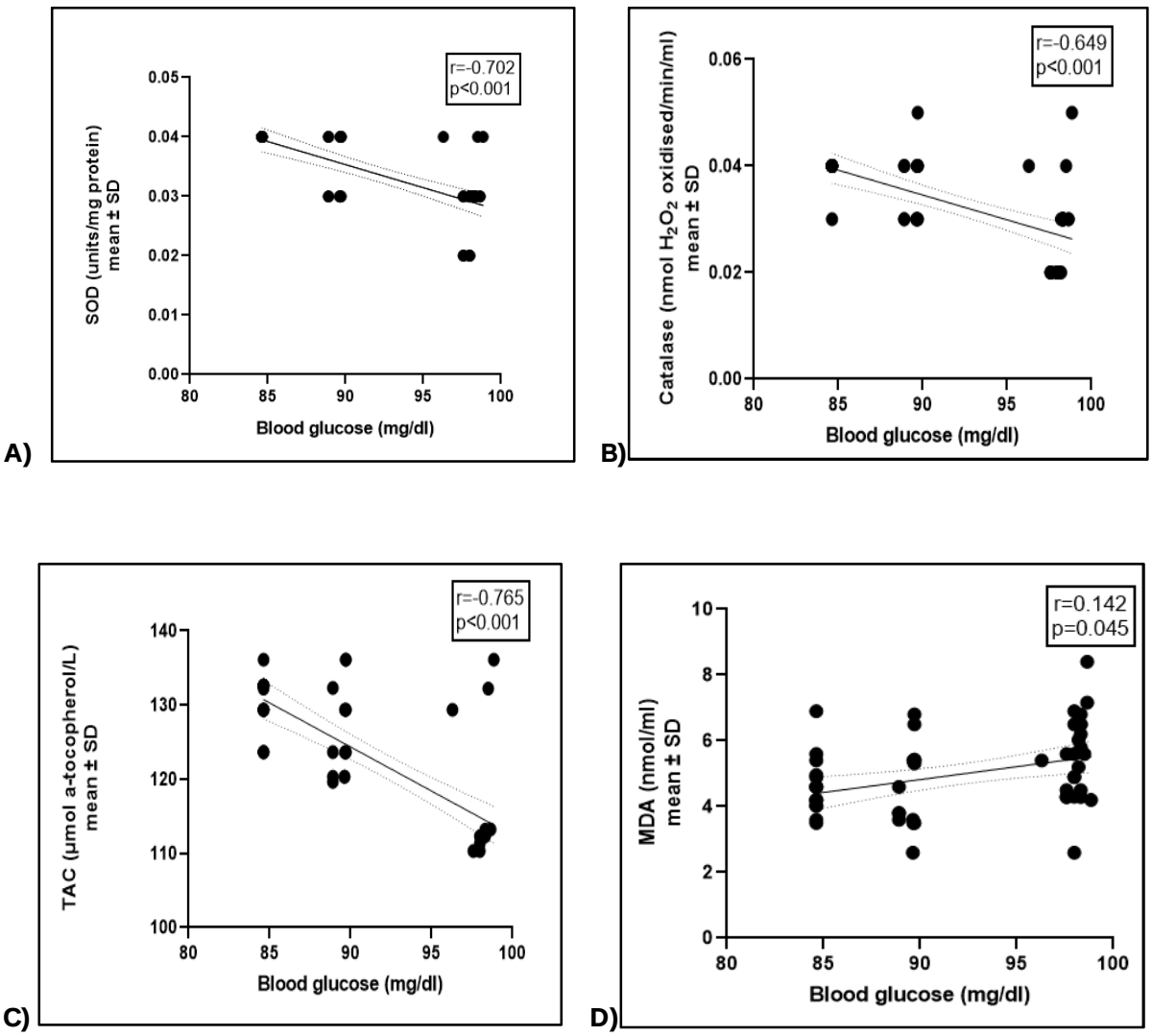

Figure 2: Relationship between serum glucose and oxidant-antioxidant markes in PCOS women

There was a statistically significant negative correlation between blood glucose and SOD level ($r = -0.675$, $p < 0.001$), catalase activity ($r = -0.667$, $p< 0.001$) and TAC ($r = -0.542$, $p < 0.001$). There was a statistically significant positive correlation showed between blood glucose and MDA ($r = 0.513$, $p< 0.001$) (Fig.3).

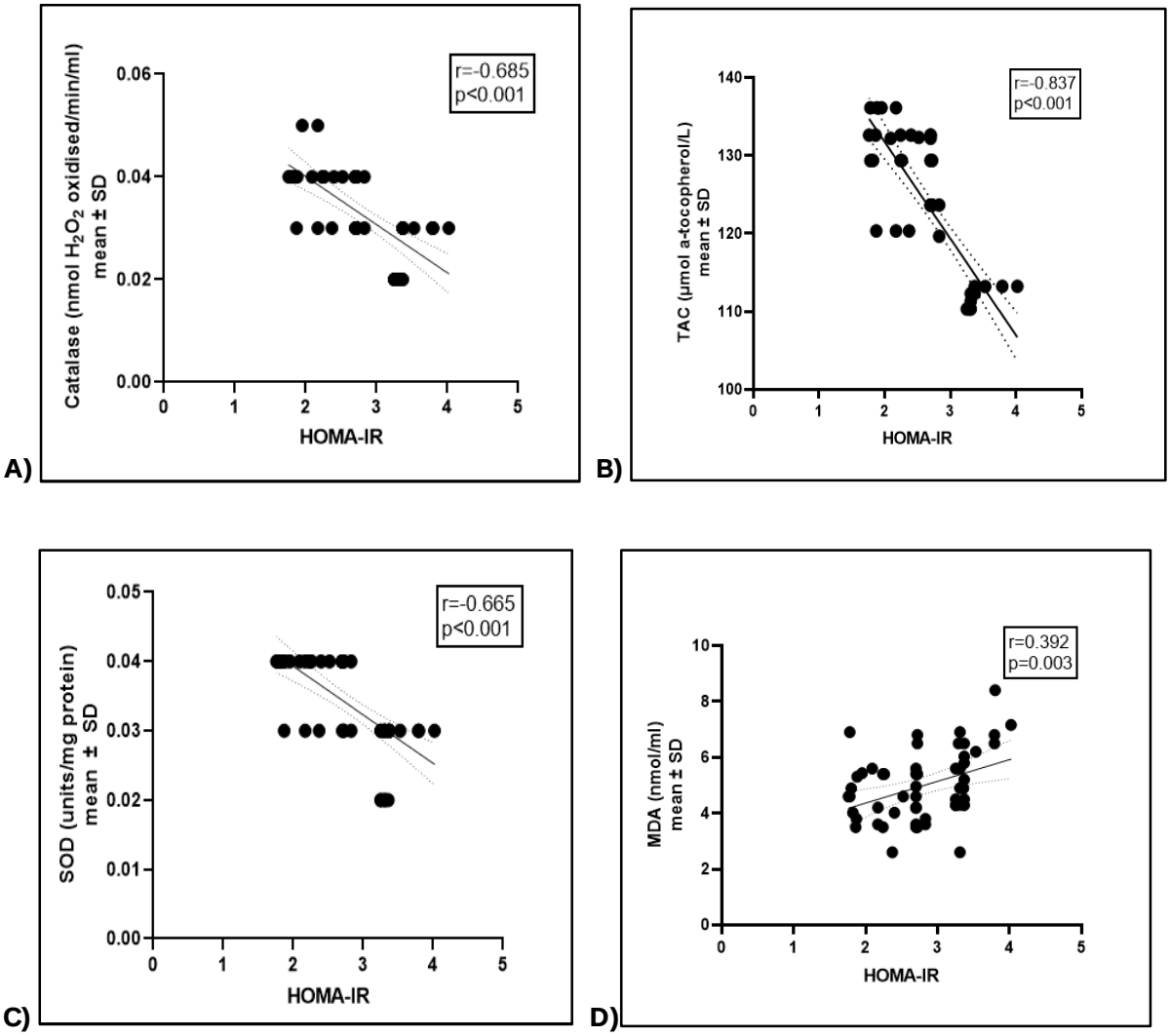

Figure 3: Relationship between HOMA-IR and oxidant-antioxidant markes in PCOS women

DISCUSSION

PCOS has been defined as a chronic systemic disease rather than a simple local disease, and it is frequently associated with insulin resistance (IR), hyperandrogenemia, chronic inflammation, and OS (OS), though the pathogenesis is unknown (Zuo et al., 2016). In the current study, young PCOS participants had higher mean levels of fasting glucose, serum insulin, and HOMA-IR when compared to age-matched controls. Our study results shows consistent with the previous findings. In their investigation, in PCOS women HOMA-IR was considerably higher than that of age-matched healthy women, indicating that insulin resistance played a crucial role in the pathogenesis of PCOS (Xiang et al, 2012).

We assessed the levels ofoxidant-antioxidant indicators in patients with and without insulin resistance (IR), as well as infertile and fertile PCOS, and also investigated significant associations between insulin resistance, hyperglycemia, and oxidant-antioxidant parameters.

Many studies that demonstrated an impaired oxidant-antioxidant balance in patients with PCOS, the antioxidant response varied despite increased OS (Sabuncu et al., 2001; Kusçu and Var, 2009). The most common cause of this

variation may be the genetic difference in oxidative defense mechanisms and varying BMI indices and age ranges of the patients (Sabuncu et al., 2001).

We found PCOS patients with infertility had significantly higher MDA levels, significantly lower SOD, catalase and TAC levels than those of the fertile PCOS patients, similar to previous studies (Turan et al., 2015; Ozer et al., 2016).

PCOS is associated with a decline in antioxidant levels. It is one of the states with elevated OS, which disrupts the follicular and luteal phases of the ovarian cycle (Agarwal et al. 2012). In women with PCOS, the follicular fluid contained elevated levels of ROS and MDA. It decreased TAC, which was directly linked to decreased oocyte maturation and fertilisation rates, poor embryo quality, and decreased pregnancy rates (Das et al. 2006, Singh et al. 2013, Nuez-Calonge et al. 2016).

Several prior Turkish studies reported that, PCOS patients with insulin resistance were found to have significantly higher MDA levels, significantly lower thiol levels, significantly lower SOD and lower catalase activity than the PCOS patients without insulin resistance (Turan et al., 2015; Ozer et al, 2016). Our findings also report that PCOS patients with insulin resistance had significantly higher MDA levels. In contrast, reduced SOD and catalase levels may indicate antioxidant enzyme suppression in PCOS individuals with insulin resistance.

OS lowers insulin production from pancreatic cells while impairing glucose absorption in muscle and adipose tissue (Takeda et al., 2005). Hyperglycemia which triggers the release of reactive oxygen species from the mononuclear cells which leads to cellular damage and activates the transcription of pro-inflammatory cytokines such as tumor necrosis factor-alpha which is a known mediator of insulin resistance. This pro-inflammatory state may contribute to the development of both insulin resistance and hyperandrogenism (Desai et al., 2014). Recent research has shown that activation of stress-sensitive intracellular signalling pathways leads to insulin resistance and reduced insulin production, both in vitro (Gao et al., 2010) and in vivo (Masharani et al., 2011).

The results of the present study showed that HOMA-IR was positively correlated with MDA and negatively correlated with SOD, catalase and TAC activity. Insulin resistance in polycystic ovary syndrome is caused by both receptor and post receptor abnormalities, including phosphatidylinositol 3-kinase and GLUT-4 glucose transporter deficiencies (Seow et al., 2004).

The limitation of the present study was the relatively small sample size. Also, the studied PCOS women were confined to a certain geographical area. Therefore, future studies should replicate the investigation in a larger sample size and various populations to confirm our results.

CONCLUSION

In conclusion, subjects with PCOS are under the OS and it is high in infertile and insulin resistance, Furthermore, our study revealed an association between OS markers and insulin resistance which are accepted risk factors for diabetes and future CVD. In the future, clinicians should pay special attention to these patients in long term follow-up to monitor chronic diseases that may occur as a result of OS-induced DNA damage. Further randomised controlled trials are necessary to examine the beneficial effects of antioxidants in the treatment of infertility or chronic disease in selected patients.

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Conflict of Interest

There are no conflicts of interest

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