AJAB

Asian J Agric & Biol. xxxx(x).
DOI: 10.35495/ajab.2023.212

Original Article

Oxidative stress modulates endocrine profiling in polycystic ovarian syndrome patients

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Received:
September 23, 2023
Accepted:
October 15, 2023
Published Online:
November 15, 2023

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Abstract

Oxidative stress accelerates the pathophysiological development of various anomalies like polycystic ovarian syndrome (PCOS). The current study aimed to assess the role of oxidative stress in polycystic ovarian syndrome (PCOS). Adult women (n=100) aged ~20–40 years which were diagnosed with polycystic ovarian syndrome on ultrasound following the Rotterdam criteria, were randomly selected from various hospitals within the city of Faisalabad, Pakistan. Fifty healthy women (n=50) with same age and regular menstrual cycles, biochemical and metabolic markers were designated as a control group. Blood samples were collected from both the groups on 2nd day of menstrual cycle. Serum was separated from the blood samples and evaluated for oxidative stress markers, lipid and hormonal profile in both groups. Stress scale-21 (DASS-21) questionnaire was employed to assess the depression and anxiety scale. Results exhibit a strong correlation of FSH levels with oxidative stress parameters. The questionnaire revealed severe depression, anxiety, and stress in patients of PCOS. Apart from elevated oxidative stress markers, dyslipidemia was also prevalent in the PCOS patients. In conclusion the current study highlights the permissive role of oxidative stress in PCOS which results in hormonal disruption.

Keywords: Polycystic ovarian syndrome, Oxidative stress, Lipid profile, Thyroid stimulating hormone, Dyslipidemia

How to cite this:

Mughal IA, Hussain G, Mukhtar I, Irfan S and Haseeb Anwar H. Oxidative stress modulates endocrine profiling in polycystic ovarian syndrome patients. Asian J. Agric. Biol. xxxx(x): 2023212. DOI: https://doi.org/10.35495/ajab.2023.212

Introduction

The polycystic ovarian syndrome (PCOS) affects 5 to 15% women of conceptive age with multiple secondary complication (Bhushan and Sinha, 2022; Kanbour and Dobs, 2022).

The most important clinical presentations in PCOS is obesity (Sangouni et al., 2022; Li et al., 2022; Kim and Lee, 2022). PCOS is characterized by ovarian dysfunction resulting in oligo-ovulation/anovulation, hyperandrogenism, and presence of ovarian cysts during gynecological ultrasound (Louwers and Laven, 2020). High levels of total cholesterol, triglycerides, LDL, FBG, insulin and its resistance and low levels of HDL, sex hormone-binding globulin (SHBG), dehydroepiandrosterone sulfate (DHEA-S) are directly linked with obesity. Despite of changing overall metabolism, obesity changes hormonal

metabolism leading to PCOS, infertility and endometrial carcinomas in obese women (Sayın et al., 2020).

Most of the PCOS cases co-exist with insulin resistance, hyperinsulinemia, obesity, hypertension, nonalcoholic steatohepatitis and finally fully developed metabolic disorder. To diagnose polycystic ovarian syndrome, Rotterdam criteria from 2003 must be followed. According to which any two out of three criteria must be presented, without different elements that may cause oligo-ovulation and/or anovulation, signs of raised androgen levels (clinically or biochemically) and ovaries with multiple cysts (one or two) on gynecological ultrasound (Eshre and Group, 2004).

In obesity, increased level of oxidative stress results in generation of high levels of free radicals (super oxide, hydrogen peroxide and hydroxyl ions) as compared to antioxidant (superoxide dismutase and glutathione peroxidase). Elevated levels of reactive oxygen species (ROS) causes cellular damage due to oxidation of LDL. Furthermore, many oxidative enzymes (myeloperoxidase and lipoxygenases) involved in LDL oxidation are associated with the development of obesity and insulin resistance (Wang et al., 2014; Neels, 2013).

Oxidative stress impacts cellular biology and might impact steroid producing cells of the ovary and promote excessive androgens by minimizing androgen conversion into estrogens, disrupting developing follicles resulting in infertility (Sadeghi et al., 2022; Ji et al., 2022). High oxidative stress depicted by increased level of plasma/serum oxidative stress markers results in a severe redox condition which favor's the progression of the PCOS (Wang and Zhang, 2022). PCOS may contribute in developing risk of hypercholesterolemia and atherosclerosis (Panda et al., 2022). The current study was designed to explore the association of oxidative stress, lipid profile, and endocrine in women with PCOS in the district of Faisalabad, Pakistan.

Material and Methods

One hundred women aged (20 – 40 years) diagnosed as polycystic ovarian syndrome on ultrasound (According to Rotterdam criteria) were randomly selected from outpatient departments of DHQ hospital Faisalabad, Aziz Fatima Trust hospital Faisalabad and two private clinics in Faisalabad, Pakistan. Fifty healthy women having same age and body mass index with regular menstrual cycles lasting for 25 – 35 days with normal ovulation, biochemical and metabolic markers were recruited as control group in the current study. Women in control group had shown normal ovarian morphology on ultrasonography and reported no hirsutism. All the participants of the study were informed for their consent. Patient history regarding name, age, address, marital status, parity, and symptoms of the disease (fertility, menstrual disturbance, obesity and hirsutism, duration, and treatment in part) was obtained at the time of selection of the subjects. Initially, women were also examined for body height, weight, body mass index, hirsutism, and galactorrhea.

Inclusion criteria

Women of child bearing age (18-45 years) with presence of polycystic ovarian morphology (PCOM) on ultrasound and clinical findings were included in the study.

Exclusion criteria

Women with pregnancy, thyroid dysfunction, blood pressure, abdominal and pelvic surgery, chronic alcoholics, cigarette smoking, administering CVS, carbamazepine, methotrexate therapy, phenytoin, contraceptive pills and anti-obesity drugs. Females on antidepressants and strenuous physical activity were all excluded from the study.

DASS-21 survey

The DASS survey form designed by Lovibond and Lovibond, was used to evaluate the patients for symptoms of stress, anxiety and depression along with patient's response to given therapy. The survey demonstrates psychometric qualitative parameters appropriately which can be compared to any available standardized instrument. Furthermore, Performa is a summarized edition of the 42-points previous DASS, the total score is calculated by multiplying each subscale's score by two. According to the Performa, the results are rated and classified as: "normal, mild, moderate, severe, or extremely severe" (Lovibond and Lovibond, 1995). All the participants of the study were interviewed to fill the DASS-21 questionnaire for overall stress evaluation.

Ethical board approval

Approval of the current project was provided by the Ethical Review Committee, Government College University Faisalabad, Pakistan, under reference

number GCUF/ERC/40 which is regulated by the guideline of National Biosafety committee and Punjab Biosafety rules, 2014.

Blood sampling

Blood samples of almost 10 mL were collected from overnight fasted women at 2nd day of menstrual cycle. Serum was separated by centrifugation at 2000 rpm for ten minutes and stored in freezer for further lab investigations.

Biochemical analysis

Hormonal analysis

Serum hormonal profile of the patients was assessed by ELISA kits by Elabscience, USA. Thyroid Stimulating Hormone (TSH; Catalogue # E_EL-R0976; Measurement range: 1.25-80 ng/mL), Follicle stimulating hormone (Human FSH; mIU/mL; Catalogue # E_EL-H1143; Measurement range: 1.56-100 mIU/mL), Luteinizing hormone (Human LH; mIU/mL; Catalogue # E_EL-H6019; Measurement range: 0.16-1 mIU/mL), Testosterone (Human LEP; pg/mL; Catalogue # E_EL-0165; Measurement range: 31.25-2000 pg/ml), and Prolactin (Human PRL; pg/mL; Catalogue # E_EL-H0141; Measurement range: 1.56-100 ng/mL). All kits have CV of less than 10%. Assays were performed following instructions provided in the kits.

Oxidative stress evaluation

Physiological and stressful conditions lead to the excessive generation of free radicals in the form of oxidative reacting species in the body and ultimately cause oxidative stress due to imbalance between oxidants and antioxidants. In oxidative stress assay as previously described (Nisar et al., 2017) ferrous ion is oxidized to ferric ion due to presence of oxidants in the serum sample. Ferric ion forms a color complex with xyenol orange in acidic medium which is measured spectrophotometrically as change in color intensity by colorimetric method. The calibration curve was constructed by using different dilutions of H_2O_2 and results were expressed µmol of H_2O_2 $_{equivalent}$/L. Sensitivity was measured as 1.13 H_2O_2 $_{equivalent}$/L, while precision was found less than 3% with linearity of 200 µmol H_2O_2 $_{equivalent}$/L. Antioxidant provides greater protection against oxidant as free radicals. Plasma antioxidant status also called as total antioxidant capacity (TAC) collectively represents various compounds and metabolic interaction. TAC is a valuable parameter to assess antioxidant status of the body as compared to estimate the antioxidant property of an individual molecule. Antioxidant status was measured by colorimetric method (Nisar et al., 2017). Calibration curve was constructed by standardizing Trolox (vitamin E analogue) in different dilutions and expressed in mM Trolox $_{equivalent}$/L. Assay was linear up to 6mmol Torolx $_{eqivalent}$/L with precision less than 3%. Paraoxonase 1 (PON1) is capable to hydrolyze various organophosphates and aromatic esters and lipid peroxidation products ultimately reduces their accumulation. For the evaluation of PON1 enzymatic activity was estimated by paraoxon hydrolysis into *p*-nitrophenol while change in color intensity was measured spectrophotometrically in in Unit/min/L. Arylesterase is an aromatic esterase an isoform of paraoxonase hydrolyses lipid peroxidation compounds and measured in KU/min/L. PON1 and Arylesterase enzymes activities were measured following method as described previously by Anwar et al. (2019).

Serum lipid profile

The serum Total Cholesterol (Cat# CS0005; Measurement range: 1–5 g), Triglycerides (Cat# MAK266; Measurement range: 2–9999 mM), and HDL-Cholesterol (Cat# MAK045). The instructions supplied in the kits were followed to process samples. The Friedrick equation was used to determine serum LDL cholesterol levels.

Statistical analysis

Descriptive statistics was employed to examine the mean values of the derived data with standard deviation of all parameters. Software SPSS-23 was used to analyze and compare the values of each of the measurements as well as some parameter among the groups, an independent t-test was used. The correlation between different factors was investigated using Pearson's correlation coefficients. The correlation's significance and the proportionate influence of each variable were calculated. It was determined that a P value ≤ 0.05 was considered as significant.

Results

Hormonal status

Serum levels of FSH, LH, Testosterone hormone, Prolactin hormone, and TSH in the study and control group are represented by Figure 1 (A, B, C, D, E). The mean levels of FSH (4.54±1.45 mIU/mL) and LH (11.73±0.94 mIU/mL) were significantly ($P \leq 0.05$)

reduced in the PCOS patients in comparison to healthy women of control group (8.88±2.56 mIU/mL and 12.88±2.76 mIU/mL respectively). Whereas the mean serum levels of prolactin hormone, testosterone hormone, and TSH were significantly ($P \leq 0.05$) raised in women of study group in contrast to women of control group.

Oxidative stress

Oxidative stress parameters measured in both groups are shown in Figure 2 (A, B, C, D). The TAC level was reduced significantly ($P \leq 0.01$) in the PCOS patients (0.68 ± 0.22 mM/L) as compared to the healthy women in control group (2.0 ± 0.08 mM/L). Mean paraoxonase activity and mean arylesterase activity were also decreased significantly ($P \leq 0.01$) in the PCOS patients as compared to the healthy women of control group as shown in Figure 1 (C and D). However, mean TOS levels were found significantly ($P \leq 0.01$) higher in the PCOS patients (33.22 ± 4.44 µM/L) when compared with healthy women of control group (18.59 ± 3.17 µM/L).

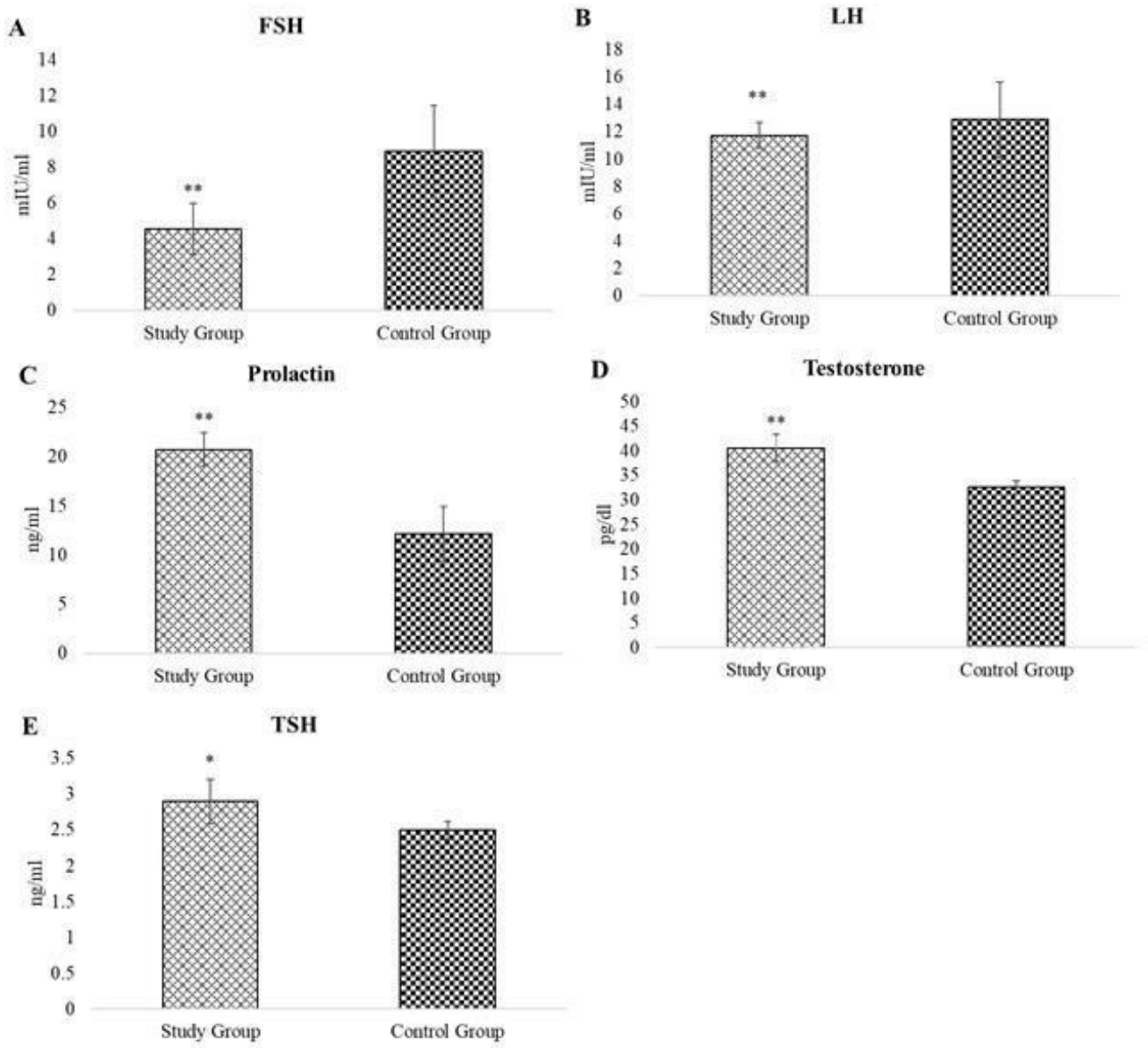

Figure-1: Serum levels of different hormones in Study group and control group. Data are expressed as means ± standard deviations. ** Shows significance at the 0.01 level. * Shows significance at the 0.05 level.

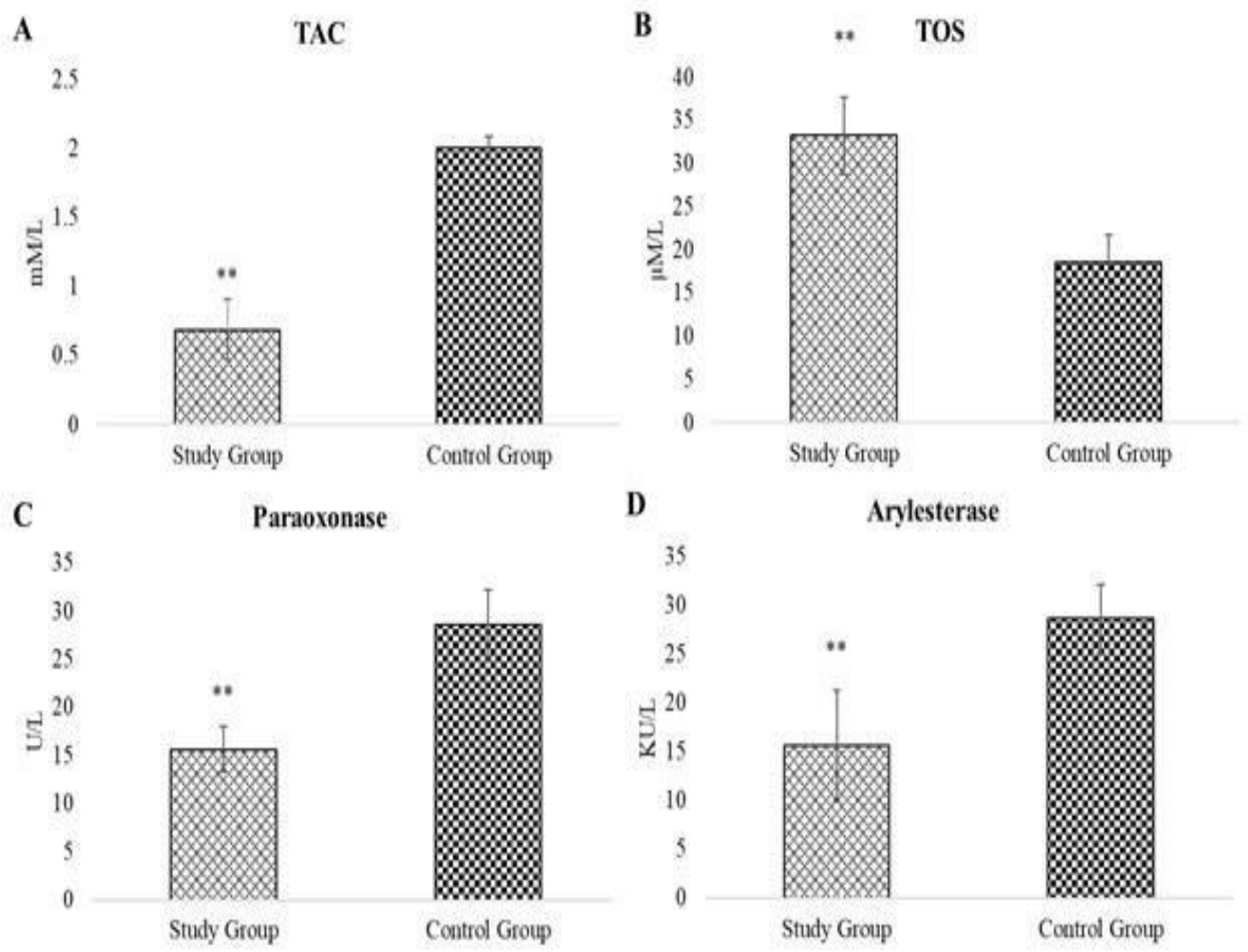

Figure-2: Different parameters of oxidative stress in Study group and control group. Data are expressed as means ± standard deviations. ** shows significance at the 0.01 level. * Shows significance at the 0.05 level.

Depression can be ruled out if the score lies between 0 to 9, anxiety is indicated if it is between 0 to 7, and 0 to 14 stands for stress on each of the three subscales of the DASS-21 were judged normal. Anxiety, stress and moderate depression were defined as summation of 14 to 20 to label depression, 10 to 14 for anxiety, and stress is indicated if it lies between 19 to 25. Finally, depression total scores of 21–27, anxiety sum scores of 15–19, and stress sum scores of 26–33 were considered severe. Any score higher than this was deemed highly serious. Results of the study show the percentage of participants affected with PCOS are more depressed, anxious and stressed as presented in Table 1.

Table-1: Percentage of study participants with mild, moderate and severe Depression, Anxiety and Stress according to DASS-21.

Variable	Parameter	Control Group		Study Group		Results
		N	%	N	%	
Depression	Mild	3	6	20	20	P < 05
	Moderate	0	0	46	46	P < 05
	Severe	0	0	9	9	P < 05
Anxiety	Mild	9	18	8	8	P < 05
	Moderate	19	38	12	12	P < 05
	Severe	0	0	71	71	P < 05
Stress	Mild	7	14	8	8	P < 05
	Moderate	15	30	32	32	P < 05
	Severe	0	0	52	52	P < 05

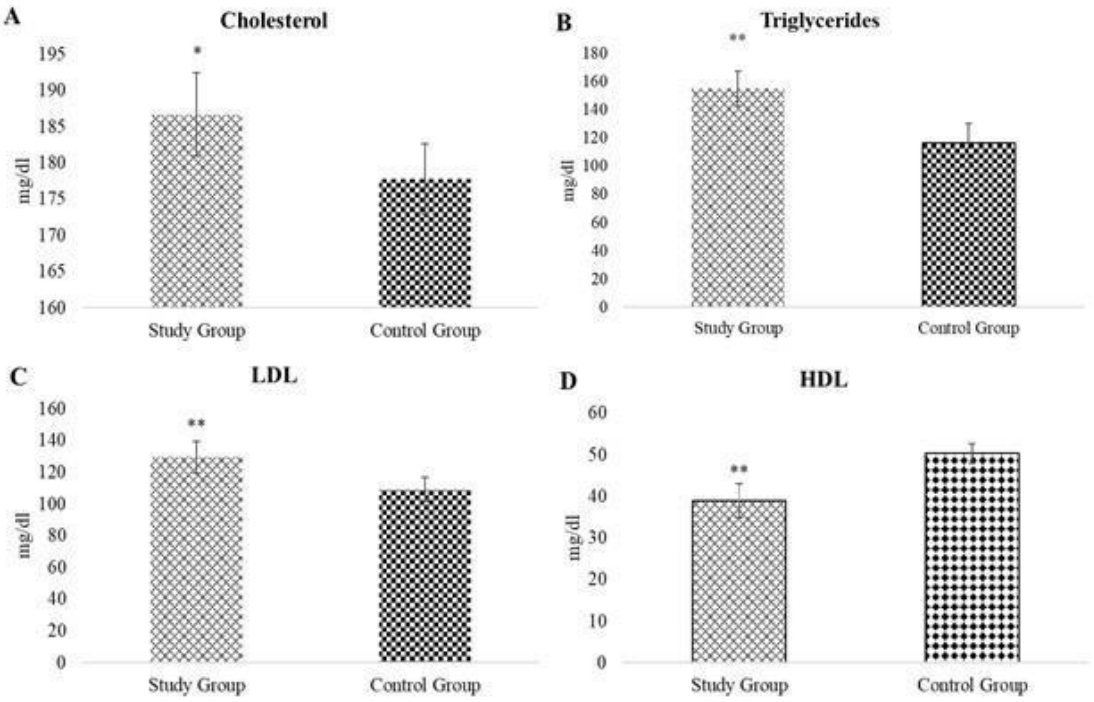

Figure-3: Different parameters of lipid profile in Study group and control group. Data are expressed as means ± standard deviations. ** Shows significance at the 0.01 level. * indicates significance at the 0.05 level.

Lipid profile

Results of different parameters of serum lipid profile of PCOS patients and healthy women of control group are presented in Figure 3 (A, B, C, D). The mean total cholesterol level was significantly ($P \leq 0.05$) raised in women of study group (186.67 ± 5.74 mg/dL) when compared with the healthy women of control group (177.82 ± 4.85 mg/dL). Triglycerides and LDL levels were found significantly ($P \leq 0.05$) higher in the PCOS patients in comparison to healthy women of control group. Whereas the mean HDL levels were significantly ($P \leq 0.01$) raised in healthy women of control group (50.3 ± 2.4 mg/dL) as compared with the PCOS patients (38.93 ± 4.2 mg/dL).

Correlation among oxidative stress, lipid profile and hormonal status

Relationship of lipid profile parameters with the TAC (total antioxidant capacity) is formulated in Table 2. There is significant negative correlation of TAC with cholesterol (r^2 = -0.19; $P \leq 0.05$), triglycerides (r^2 = -0.270; $P \leq 0.01$), and LDL (r^2 = -0.229; $p \leq 0.01$). Whereas the positive correlation of TAC with HDL (r^2 = 0.559; $P \leq 0.01$) shows that by increasing the TAC also increases the HDL levels. The HDL shows negative correlation with TOS (r^2 = -0.46; $P \leq 0.01$). Paraoxonase and arylesterase enzyme activities are also significantly ($P \leq 0.01$) negatively correlated with triglycerides and LDL levels and significantly ($P \leq 0.01$) positively correlated with HDL levels. There is a significant ($P \leq 0.01$) positive correlation of FSH with TAC (r^2 = 0.615), PON (r^2 = 0.591), and ARY (r^2 = 0.520), while significant ($P \leq 0.01$) negative correlation with that of TOS (r^2 = -0.47). In case of prolactin, a significantly ($P \leq 0.01$) negative correlation has been observed with TAC (r^2 = -0.333), PON (r^2 = -0.34), and ARY (r^2 = -0.303), while significant ($P \leq 0.01$) positive correlation with TOS (r^2 = 0.139).

Depression can be ruled out if the score lies between 0 to 9, anxiety is indicated if it is between 0 to 7, and 0 to 14 stands for stress on each of the three subscales of the DASS-21 were judged normal. Stress, anxiety and moderate depression were estimated as summation of 14 to 20 to label depression, 10 to 14 for anxiety, and stress is indicated if it lies between 19 to 25. Conclusively, depression total scores of 21–27, sum scores of 15–19 in anxiety, and sum scores of 26–33 in stress were considered severe. If score achieved higher than such values was considered highly serious. Results of the study shows the percentage of participants affected with PCOS are more depressed, anxious and stressed as presented in Table 1.

Table-2: Pearson's correlation coefficients of different parameters of oxidative stress with lipid profile and hormones.

	Cholesterol	Triglyceride	HDL	LDL	TSH	LH	FSH	Prolactin	Testosterone	TAC	TOS	PON	ARY
Cholesterol	1	.413**	.045	.422**	.000	.041	-.142	.049	-.084	-.190*	.070	-.146	-.138
Triglyceride	.413**	1	-.132	.308**	.062	-.074	-.24**	.061	.149	-.270**	.126	-.26**	-.261**
HDL	.045	-.132	1	-.25**	-.065	.028	.379**	-.236**	-.100	.559**	-.46**	.676**	.535**
LDL	.422**	.308**	-.249**	1	.083	-.063	-.287**	.258**	.098	-.229**	.079	-.33**	-.295**
TSH	.000	.062	-.065	.083	1	.002	-.078	-.010	-.064	-.058	.079	-.100	-.032
LH	.041	-.074	.028	-.063	.002	1	.293**	-.144	-.073	.085	-.067	.122	.104
FSH	-.142	-.239**	.379**	-.29**	-.078	.293**	1	-.347**	-.151	.615**	-.47**	.591**	.520**
Prolactin	.049	.061	-.236**	.258**	-.010	-.144	-.347**	1	.073	-.333**	.139	-.34**	-.303**
Testosterone	-.084	.149	-.100	.098	-.064	-.073	-.151	.073	1	-.088	-.050	-.080	-.123
TAC	-.190*	-.270**	.559**	-.23**	-.058	.085	.615**	-.333**	-.088	1	-.62**	.763**	.665**
TOS	.070	.126	-.455**	.079	.079	-.067	-.472**	.139	-.050	-.619**	1	-.78**	-.523**
PON	-.146	-.255**	.676**	-.33**	-.100	.122	.591**	-.338**	-.080	.763**	-.71**	1	.722**
ARY	-.138	-.261**	.535**	-.3**	-.032	.104	.520**	-.303**	-.123	.665**	-.52**	.722**	1

**Correlation is significant at the 0.01 level (2-tailed).

*Correlation is significant at the 0.05 level (2-tailed).

Discussion

Oxidative stress markers are considerably enhanced in PCOS patients and are thought to be a potential contributory factor of PCOS pathogenesis (Mohammadi, 2019). Current study reveals the close association of oxidative stress markers and dyslipidemia in PCOS patients from district Faisalabad, Pakistan. We observed an increase in the oxidative stress markers is also associated with increased mental and psychological stress in the PCOS patients (Figure 1; A, B, C, D). Previously, Fenkci et al. (2003) examined TAC concentration in PCOS women and compared with the control group with similar age, BMI, and smoking history. They revealed a significantly lower TAC concentration in PCOS women.

Literature archives demonstrated the increased oxidative stress in terms of raised ROS production, high MDA levels (Murri et al., 2013), and glycation end products (Garg and Merhi, 2016) in the women with PCOS. Obesity induces gender specific issues in females that occurs with onset of early puberty. dysovulation, amenorrhea and proceed furthermore with infertility, PCOS and breast and endometrial cancers (Damiati, 2018).

Mental stress is generally defined as an emotional condition characterized by various symptoms of depression; loss of interest, hopelessness, melancholy, and worry indicating tenseness and restlessness. Women having PCOS have lesser self-esteem, a higher bad self-image, depression, and mental stress (Scaruffi et al., 2014) due to the physical presence of hyperandrogenism signs, like hirsutism, obesity, seborrhea, cystic acne, and hair loss, probably due to compromised feminine personality (Dixon et al., 2003). Current study revealed that the percentage of depressed, anxious, and stressed participants with PCOS are more as compared to the control group participants.

Current study depicts that stress level might have an exaggerative role in the development of PCOS. So, it is important to treat the oxidative and mental stress in parallel while treating the hormonal imbalance. In previous research, Jedel et al. (2010) stated that anxiety levels, mental stress, depression, and social fears are markedly higher in women with PCOS.

Increased LH, testosterone, TSH and prolactin while decreased FSH levels are characteristic features of PCOS however such hormonal alteration may be

affected via oxidative stress parameters. The hormonal levels of FSH and LH were found lower in our study group women as compared to the healthy women of control group. Whereas serum levels of testosterone, prolactin and TSH were found higher PCOS study group as compared to the healthy control group (Figure 3; A, B, C, D, E). There are various studies which build a direct link of oxidative stress with the imbalance of FSH, LH, Testosterone, TSH, and Prolactin leading to the development of PCOS (Mizgier et al., 2021; Liu et al., 2021).

Hyperlipidemia among various metabolic abnormalities usually co-exist in PCOS women (Kim and Choi, 2013; Pergialiotis et al., 2018). The current research project also explained correlation of oxidative stress with the hyperlipidemia as oxidative stress parameters were increased along with hyperlipidemia. Several studies support our results and explain a close association of dyslipidemia and oxidative stress in the patients of PCOS (Sulaiman et al., 2018).

Oxidative stress enhances lipid peroxidation thereby impairing insulin signaling mechanism with the cell, aggravating insulin resistance. Such impairment initiates a vicious cycle where insulin resistance lead to increased persistent insulin levels which further worsen oxidative stress. Higher levels of sex steroid hormones also contribute to insulin resistance, make more challenging situation for glucose to enter the cell to meet the energy requirements of the cell (Li et al., 2022). While oocyte need energy produced from citric acid cycle for its maturation (Turathum et al., 2021). However, increased levels of androgens in PCOS, suppress the citric acid cycle to produce less energy having negative impact on maturation of oocyte leading to impaired pregnancy in females PCOS and hyperandrogenism (Jimenez et al., 2013). Antioxidants have potential benefit in managing PCOS by decreasing levels of oxidative stress and symptoms (Li et al., 2022). Antioxidants vitamin C, E and polyphenols from plant source and mushroom supplementation may support to neutralize ROS thereby defending cells against oxidative stress (Austria et al., 2021; Arguelles, 2022; Heidari et al., 2022).

Conclusion

In conclusion a strong association between incident of PCOS and the psychological distress levels was observed in the current study as compared to healthy women. In routine clinical practice the treatment of PCOS mainly focusses on the hormonal therapy to correct endocrine imbalance. Our study revealed the significant association between oxidative and psychological distress with the hormonal imbalance in patients with PCOS. Possibly, oxidative and psychological distress may be the cause of the hormonal imbalance. Thus, it might be recommended to treat the oxidative stress along with treating the hormonal imbalance in the patients with PCOS.

Disclaimer: None.
Conflict of Interest: None.
Source of Funding: None.

Contribution of Authors

Mughal IA: Performed the laboratory experiments, collected data and drafted initial manuscript
Hussain G & Irfan S: Literature review, proof read and edited the final manuscript
Mukhtar I: Analyzed and interpreted data
Anwar H: Conceived the research idea and developed research methodology

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