

## **Inter-relationship of circulating biochemical markers of oxidative stress and comorbid condition in polycystic ovary syndrome.**

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### **Abstract**

**Introduction:** Polycystic Ovarian Syndrome (PCOS), commonly found in 5% of all women of reproductive age is a heterogeneous endocrine disorder in which woman's levels of estrogen and progesterone are imbalance leading to the growth of benign masses in the ovaries (ovarian cysts). Rotterdam criteria used to diagnose PCOS include two of three clinical features: 1) oligo- or anovulation, 2) hyperandrogenism e.g., hirsutism, acne etc. and 3) sonographic morphologic evidence of polycystic ovaries.

**Aim:** To investigate the relationship between circulating biomarkers and their heterogeneity in PCOS.

**Material and methods:** A prospective case control study was conducted including 70 females of age ranging from 20 to 35 y out of which 20 served as healthy controls and 50 were patients of PCOS. Lipid levels, renal profile and antioxidative status of Malondialdehyde (MDA), Catalase (CAT), Superoxide dismutase (SOD) and Reduced glutathione (GSH) was analysed quantitatively. Serum electrolytes (Na<sup>+</sup>, K<sup>+</sup>, Mg<sup>2+</sup>, PO<sub>4</sub><sup>3-</sup>), anti-oxidants vitamins C and E and steroidal hormones like Follicle Stimulating Hormone (FSH), Sex Hormone Binding Globulin (SHBG) were measured by the commercially available kits.

**Results:** A total of 70 patients were enrolled to the study. Serum electrolytes level like potassium (K) and Phosphorus (P) were significantly increased while antioxidants were decreased in the study group as compared to the control group. Oxidative stress was also increased with higher levels of MDA. Follicle Stimulating Hormone (FSH) and Sex Hormone Binding Globulin (SHBG) were decreased in the study group. The PCOS patients showed statistically significant correlation among the circulating biomarkers like FSH vs. prolactin ( $r=0.400^{**}$ ).

**Conclusion:** PCOS is found strongly associated with increased lipid peroxidation, oxidative stress, electrolyte imbalance as well as variation in lipid profile in biological system. As the lipid peroxidation increases it increases MDA (end product of lipid peroxidation), significantly it characterizes PCOS by quantifying lipid hydroperoxides and therefore it may serve as a good biomarker of oxidative stress of PCOS.

**Keywords:** Polycystic ovarian syndrome (PCOS), Rotterdam criteria, Endocrine, Metabolic disorder, Electrolytes, Oxidative stress, MDA, FSH, SHBG, Prolactin.

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## Introduction

About 5%-10% of all the women in their reproductive age suffer from Polycystic Ovarian Syndrome (PCOS) which is a heterogeneous endocrine and metabolic disorder [1-3]. PCOS may involve biological systems other than the reproductive one. It is a well-established cardiovascular disease risk factor as well as a risk factor for various metabolic abnormalities [4-6]. Moreover, pancreatic  $\beta$ -cell dysfunction and Insulin Resistance (IR) are considered to be major biological aspects that lead to the development of type-2 diabetes. Hence, women suffering from PCOS were reported to have higher rates for type-2 diabetes in the long term [7-11].

Oxidative stress is considered to be a major role player in the pathogenesis of Cardiovascular Disease (CVD) related to PCOS [12-14]. IR has also been documented in patients with PCOS due to oxidative stress [15-17]. Moreover, oxidative stress not only affects the cardiovascular system but also impairs female reproductive physiology, mainly ovulation [18-20]. Hyperglycemia and IR are important factors responsible for the elevated oxidative stress in PCOS patients [21-23]. Lean women with PCOS were also reported to have an elevated oxidative stress without IR [24-26]. The present study was designed to investigate the relationship between circulating biomarkers of stress either enzymatic or non-enzymatic their heterogeneity of PCOS.

## Materials and Methods

Fifty females in the age group of 20-35 y constituted the study group and 20 age-matched females without PCOS constituted the control group. Patients were diagnosed according to the Rotterdam criteria [4]. The experimental protocol was approved by the Research Ethical Committee of The Institute of molecular biology and biotechnology, The University of Lahore. Five milliliter (5 ml) of venous blood sample was taken from each participant. The samples were centrifuged within one hour of collection, after which the sera were separated and stored at  $-20^{\circ}\text{C}$  until assayed.

### Evaluated parameters

Lipid and renal profile as well as vitamins C and E were estimated by the commercially available kits (Human Diagnostics<sup>®</sup>). Spectrophotometric method was used to measure SOD [27]. Methodology of Ohkawa was employed to

measure MDA [28]. Measurement of CAT was performed by Aebi spectrophotometric method [29]. Moron Method been used to measure GSH [30]. Electrolytes ( $\text{Na}^+$ ,  $\text{K}^+$ ,  $\text{Mg}^{2+}$ ,  $\text{PO}_4^{-3}$ ) were estimated by the flame photometer. Hormones like Luteinizing Hormone (LH), FSH, and prolactin were estimated by the commercially available ELISA (Enzyme-Linked Immunosorbent Assay) Kit.

## Results

The study included 70 patients. All the women were from low socioeconomic status as they were at high risk to attain PCOS due to risk for engaging adverse health behaviours, including smoking and poor nutritional diet. Informed written consent was obtained from each patient according to Helsinki declaration. Table 1 represents circulating biochemical markers and various hormones in 2 study groups. Elevated levels of BUN, uric acid as well as BMI in patients suffering from PCOS as compared to controls were observed. Hb level was alleviated in patients ( $9.48 \pm 0.21$ ) as compared to controls ( $14.14 \pm 0.21$ ,  $p=0.043$ ). All the studied markers were significantly altered between the groups ( $P<0.05$ ). Oxidative stress was increased with the increase of MDA and Nitric Oxide (NO) in the study as compared to controls. On the other hand the anti-oxidative stress was decreased with reference to SOD, GSH and CAT as compared to controls.

Elevated levels of total cholesterol (TCh), triglycerides (TG), Low Density Lipoproteins (LDL) and Fasting Blood Sugar (FBS) were observed in PCOS group. Inversely, study group's High Density Lipoprotein (HDL) level ( $1.39 \pm 0.01$ ) was decreased as compared to controls ( $1.73 \pm 0.03$ ,  $p \leq 0.05$ ). Electrolytes levels were also increased in PCOS patients, i.e., potassium (K) and phosphate (P) ( $6.04 \pm 0.13$  and  $5.81 \pm 0.08$  respectively,  $p \leq 0.05$ ). The levels of calcium (Ca), magnesium (Mg). In the hormonal profile, increased levels of LH, progesterone and prolactin were noticed in the PCOS group compared to controls. Conversely, FSH and Sex Hormone Binding Globulin (SHBG) showed decreased levels in the study group as compared to the control group. The levels of vitamin E and C were decreased. As presented in Table 2, statistically significant correlation between FSH and prolactin ( $r=0.400^{**}$ ) was detected. Similarly strong negative correlation

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could be seen in between GSH and progesterone ( $r=-0.402^{**}$ ), which is a significant finding.

**Table 1.** Circulating biochemical markers and various hormones in the 2 study groups.

Variables	Control (n=20)	PCOS (n=50)	P<0.05
BUN (mg/dL)	13.75 ± 0.51	22.44 ± 0.37	0.022
Uric acid (mg/dL)	4.01 ± 0.17	10.17 ± 0.24	0.041
Hb (g/dL)	14.14 ± 0.21	9.48 ± 0.21	0.043
BMI (Kg/m <sup>2</sup> )	21.55 ± 0.35	36.76 ± 1.09	0.003
MDA (nM/ml)	1.34 ± 0.06	3.69 ± 0.12	0.002
SOD (ng/ml)	0.47 ± 0.03	0.15 ± 0.01	0.042
GSH (nM/ml)	9.88 ± 0.26	2.25 ± 0.10	0.013
GPx (µM/ml)	0.63 ± 0.07	0.22 ± 0.01	0.004
CAT (µM/mol of protein)	4.13 ± 0.18	0.92 ± 0.08	0.025
NO (µM/L)	11.28 ± 0.30	15.50 ± 0.23	0.030
TCh (mM/L)	4.44 ± 0.08	5.48 ± 0.13	0.001
Tg (mM/L)	1.24 ± 0.03	2.59 ± 0.07	0.022
LDL (mM/L)	2.31 ± 0.03	2.47 ± 0.03	0.008
HDL (mM/L)	1.73 ± 0.03	1.39 ± 0.01	0.033
Fasting BS (mg/dL)	90.30 ± 1.82	130.18 ± 1.67	0.035
K (mmol/L)	3.88 ± 0.12	6.04 ± 0.13	0.001
Ca (mmol/L)	8.81 ± 0.19	6.01 ± 0.21	0.025
Mg (mmol/L)	1.77 ± 0.04	1.41 ± 0.02	0.039
Phosphate (mmol/L)	3.87 ± 0.13	5.81 ± 0.08	0.032
Vitamin C (µg/ml)	0.57 ± 0.01	0.33 ± 0.01	0.010
Vitamin E (µg/ml)	0.29 ± 0.01	0.21 ± 0.01	0.000
LH (IU/L)	5.58 ± 0.12	13.29 ± 0.49	0.037
FSH (IU/L)	5.84 ± 0.09	5.19 ± 0.15	0.001
Progesterone (ng/dL)	0.49 ± 0.01	0.69 ± 0.00	0.001
SHBG (nmol/L)	66.48 ± 0.89	53.59 ± 1.07	0.020
PC (µM/ml)	2.98 ± 0.11	5.42 ± 0.15	0.029
Prolidase activity (µM/L)	60.90 ± 32.62	69.77 ± 9.69	0.001
Prolactin (ng/ml)	15.15 ± 0.72	21.02 ± 0.40	0.019
TAS (µmol/ml)	13.33 ± 1.27	84.40 ± 1.40	0.023

BUN: Blood Urea Nitrogen; TCh: Total Cholesterol; Tg: Triglycerides; LDL: Low Density Lipoprotein; HDL: High Density Lipoprotein; MDA: Malondialdehyde; SOD: Superoxide Dismutase; GSH: Reduced Glutathione; CAT: Catalase; GPx: Glutathione Peroxidase; LH: Luteinizing Hormone; FSH: Follicle Stimulating Hormone; SHBG: Sex Hormone Binding Globulin; PC: Protein Carbonyl; TAS: Total Antioxidant Status. Significant P<0.05.

**Table 2.** Pearson's correlation showing relationship between antioxidants, electrolytes and hormones in PCOS patients.

Correlation	R value	P value
Mg vs. MDA	-0.324*	0.022
SOD vs. Ca	-0.450**	0.001
SOD vs. vitamin C	0.343*	0.015
SOD vs. PPC	0.305*	0.031
GSH vs. Ca	0.326*	0.021
GSH vs. Phos	-0.315*	0.026
GSH vs. progesterone	-0.402**	0.004
CAT vs. Mg	-0.455**	0.001
K vs. prolidase	0.365**	0.009
Mg vs. Hb	0.371**	0.008
NO vs. Tg	0.363**	0.010
Vitamin C vs. Tg	-0.334*	0.018
Vitamin E vs. LDL	0.339*	0.016
FSH vs. prolactin	0.400**	0.004

\*Significant p<0.05, \*\*Highly significant p<0.01.

## Discussion

The major findings of the study depict that reduced antioxidants resulted in the increased oxidative stress certainly in the PCOS patients as well as dyslipidemia is a contributing risk factor for future coronary artery diseases and even diabetes. The important components of antioxidative system are GSH, SOD, and CAT which play crucial role into various cellular function. The present study shows the elevated levels of MDA in patients suffering from PCOS as compared to controls and alleviated levels of enzymatic as well as non-enzymatic antioxidants in PCOS. These findings are in complete agreement with Victor's observation [31], who evaluated 20 healthy individuals and 20 women with PCOS for oxidative stress. The authors observed lower levels of GSH. The reduced levels were referred to the association between abnormal ROS production in mitochondria and IR among the PCOS patients. The findings of the current study are also consistent with other studies from Sabuncu, Victor, Kuscu and Var [8,12,31]. Unlike our results, some previous studies did not observe any statistical significant difference in MDA levels in case of women with PCOS as compared to control [32].

LH and FSH levels were considered to be important factors for the diagnosis of PCOS in the form of LH/FSH ratio. Accordingly, in the current study, LH showed elevated levels in PCOS patients while FSH showed decreased levels as compared to control. Although our results correlate with previous studies addressing the LH/FSH ratio, this ratio shows low sensitivity for the diagnosis of PCOS [33,34] and therefore it is not used as a diagnosis parameter. However, this study reinforces the use of the LH/FSH ratio as a complementary parameter in PCOS patients.

Significantly elevated prolactin levels were observed in PCOS patients as compared to the control group. Prolactin is a known role parameter in normal ovulation and elevated levels interfere ovulation. As shown in this study prolactin levels are increased. Previous studies [35] observed no significant prolactin elevation even while were being treated with metformin which is one of the first line treatments of PCOS. Moreover results of current study are in agreement with Filho's reports [36]. Regarding hyperprolactinemia, but according to Filho et al. raised levels of prolactin are not clinical manifestations of PCOS. Significant correlation between FSH and prolactin presents that these are the good biomarkers for PCOS.

## Conclusion

The present study concluded that the women suffering from PCOS have increased lipid peroxidation and oxidative stress as well as they reflected electrolyte imbalance hence increased MDA serves as a significant marker for the identification of disease. Moreover, it emphasizes on the hormonal imbalance so, investigations at hormonal level should be done in the future.

## Conflict of Interest

Declared none

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