

Original Investigation

Oxidative Stress Status, Metabolic Profile and Cardiovascular Risk Factors in Patients with Polycystic Ovary Syndrome

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Abstract

We aimed to investigate the relationships between Polycystic Ovary Syndrome (PCOS) and oxidative stress status, metabolic profile and cardiovascular risk factors in patients with polycystic ovary syndrome

Methods: *We measured Body Mass Index (BMI), glucose, cholesterol, triglyceride, HDL, LDL, high sensitive CRP (hsCRP), insulin, AST and ALT, Malondialdehyde (MDA), erythrocyte reduced glutathione (GSH), Nitric Oxide (NO) levels in PCOS patients and in normal individuals.*

We used spectrophotometrical method to determine glucose, cholesterol, triglyceride, HDL, LDL, AST and ALT levels. Nephelometrical method was used to measure hsCRP. Immunoassay method was used for insulin. Oxidant status was evaluated by using erythrocyte MDA and NO levels, while antioxidant status was evaluated by GSH levels.

Results: *Study population were consist of 32 women with PCOS (Study group) and 32 healthy volunteers (Control group). In study group, we found statistically higher levels of MDA, NO ($p<0.001$) and lower levels of GSH ($p<0.001$) compared to those of controls. hsCRP, insulin ($p<0.001$) and triglyceride ($p<0.05$) levels were statistically higher in study group. We also observed a statistically higher BMI in women with PCOS ($p<0.05$).*

Conclusion: *Our results revealed that PCOS is associated with imbalanced oxidative/antioxidative status, impaired metabolic profile and increased cardiovascular risk factors.*

Key words: PCOS, oxidants, antioxidants, MDA, NO, GSH

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Introduction

Polycystic ovary syndrome (PCOS) is one of the most common endocrinological disorders, affecting 5–10% of the population of women in reproductive age [1]. Nowadays, PCOS is accepted to be not only a gynecological disorder, but also include cardiovascular risk factors and several metabolic problems. Besides classical definition as accepted by the Rotterdam criteria [2], metabolic, endocrinological, and cardiovascular disorders may coexist. Insulin resistance and β -cell dysfunction are supposed to be one of the major pathogenic determinants for the development of type 2 diabetes. Therefore, women with PCOS had an increased risk for type 2 diabetes [3]. In addition to the insulin resistance (IR) and hyperinsulinemia, pancreatic β -cell dysfunction were observed in PCOS [4]. IR accompanies to a certain extent whether the patient is obese or lean [5]. The majority of studies evaluating the prevalence of glucose intolerance in PCOS, primarily include obese women, which aggravates their risk for glucose intolerance. Likewise, high prevalence of abnormal glucose intolerance has also been documented in women with PCOS.

Oxidative stress, which is well-known to participate in the pathogenesis of cardiovascular disease (CVD), was documented in PCOS [6]. And it is also reported to affect IR in these patients [7]. Oxidative stress may influence on not only cardiovascular system but also female reproductive system [8]. Insulin resistance and hyperglycemia are profound factors to increase oxidative stress, but non-obese PCOS patients without IR were also reported to have elevated oxidant status [9]. Although no difference at cardiovascular risk was reported in some studies [10], endothelial dysfunction, which may precede manifest CVD [11], was reported in even young women with PCOS [12].

High inflammatory and endothelial markers were accepted to cause endothelial dysfunction in these patients [13]. Subclinical CVD was reported in obese PCOS group [14]. Lipid peroxidation process revealing malonyldialdehyde (MDA) is accepted to reflect oxidative stress [15] while superoxide dismutase (SOD) is an antioxidant enzyme serving as a defensive mechanism of the body. Increased reactive oxygen species (ROS) may cause intracellular and cell wall damage causing an increase in MDA levels. SOD defends the body against free oxygen radicals and balances increased oxidative load. Nitric oxide (NO) is continuously released from endothelial cells to keep vessels dilated and shows endothelial integrity. Free oxygen radicals may also interact with NO release and decreased NO will also demonstrate endothelial dysfunction.

The aim of our study was to investigate the relationships between PCOS and oxidative stress status, metabolic profile and cardiovascular risk factors in patients with polycystic ovary syndrome.

Material and Methods

Thirty-two patients with PCOS who admitted to outpatient clinics of Isparta Gulkent State Hospital, Gynecology and Obstetrics Department, and 32 healthy volunteers were included in the study. PCOS diagnosed according to the Rotterdam criteria [2]. The patients having two or more of the following criteria were defined as PCOS:

- 1- History of oligo- and/or anovulation in reproductive age.
- 2- Clinical and/or biochemical signs of hyperandrogenism: hirsutism score of >6 and/or high total testosterone level.
- 3- Typical ovarian imaging of polycystic ovaries on ultrasound: multiple follicles in each ovary measuring 2–9 mm in diameter and/or increased ovarian volume (>10 ml).

All the human subjects were fully informed about the study protocol. Written and informed consent were obtained. None of the participants had a history of hypertension, alcohol consumption, disorders of glucose tolerance, hyperuricemia, cardiovascular events, and had received treatment with oral contraceptives or antiandrogens for the previous 6 months.

Blood samples were obtained during the midfollicular phase (days 3–7) of the menstrual cycle after overnight fasting.

NO is rapidly oxygenated to NO₂ and further to NO₃ and hence direct assessment of NO is almost impossible (*in vivo*). So the combined production of NO₂ and NO₃ can be used for NO synthesis *in vitro* and *in vivo*. Nitrate was assayed by a modification of the cadmium-reduction method [16]. The produced nitrite was determined by diazotization of sulfanilamide and coupling to naphthylethylene diamine (NDA). After samples were deproteinized with somogyl reagent, the nitrate was reduced by Cu-coated Cd in glycine buffer at pH 9.7. The reduction followed pseudo-first order reaction kinetics, a 90 min. interval. Mix, then read absorbances against the blank at 545 nm after 20 to 60 min. Results are expressed in µmol/mg ml.

MDA in tissues were determined by the method of Uchiyama and Mihara [17]. A 3 ml aliquot of 1 % phosphoric acid and 1 ml of 0.6 % thiobarbituric acid solution were added to 0.5 ml of 10 % tissue homogenate pipetted into a tube. The mixture was heated in boiling water for 45 min. After cooling, the color was extracted into 4 ml of n-butanol. The absorbance was measured in spectrophotometer (Ultraspec Plus, Pharmacia LKB Biochrom, UK) at 532 nm.

The amounts of lipid peroxides were calculated as thiobarbituric acid reactive substances of lipid peroxidation and are given as nmol/ml.

Determination of glutathione was performed by observation of absorbance of yellow colored product which forms as a result of reaction of Elman reactive with sulfhydryl groups in 410 nm, spectrophotometrically. The method developed by Fairbanks and Klee was used [18]. By multiplying sample absorbance obtained from standard graphic with the factor, GSH activity was calculated as $\mu\text{mol/l}$.

High Sensitive CRP (hsCRP): The hs-CRP test can more accurately detect lower concentrations of the protein (it is more sensitive), which makes it more useful than the CRP test in predicting a *healthy* person's risk for cardiovascular disease. hsCRP is promoted by some as a test for determining the potential risk level for cardiovascular disease, heart attacks and strokes. The current thought is that hsCRP can play a role in the evaluation process before one encounters one of these health problems. More clinical trials that involve measuring hsCRP levels are currently underway in an effort to better understand its role in cardiovascular events and may eventually lead to guidelines on its use in screening and treatment decisions. In our study we measured hsCRP with nephelometry (Beckmann Coulter immage). Results were reported in mg/L.

Glucose, cholesterol, triglyceride, ALT, AST, HDL and LDL levels were measured spectrophotometrically by a biochemistry autoanalyzer (Dade Behring RXL Dimension).

Insulin levels were measured by an automated chemiluminescence system (Immulite 2000 hormone autoanalyzer).

Statistical Analysis:

The statistical analysis were performed SPSS for windows version 13.00 program. All data were reported as mean $\pm$ standard deviation (SD). Normality for continuous variables in groups were determined by the Shapiro Wilk test. The variables showed normal distribution ($P > 0.05$). So, unpaired t test was used for comparison of variables between the groups. Statistical significance was defined as $p < 0.05$.

Results

In our study there were no statistically significant differences between groups in terms of age and height. The mean ages of the study and control groups were 25.0 ± 6.14 and 27.4 ± 6.9 years. However, there were significant differences between study and control groups in terms of weight and body mass index (BMI) (71.5 ± 5.72 vs. 55.09 ± 4.18 Kg) ($p < 0.001$) and (27.3 ± 5.15 vs. 21.5 ± 2.5 kg/m^2) respectively ($p < 0.005$).

The MDA levels were significantly higher in the study group compared to the those of controls (15.93 ± 2.1 vs. 9.41 ± 3.1 nmol/ml respectively) ($p < 0.001$). GSH levels were significantly higher in the control group 6.7 ± 0.6 nmol/ml vs. 7.7 ± 2.1 nmol/ml respectively ($p < 0.001$). There were significant differences between the groups in terms of NO levels. NO levels were significantly higher in control group compared to study group 27.65 ± 4.7 vs. 43.36 ± 3.54 $\mu\text{mol/mg}$ respectively ($p < 0.001$) (Table 1).

TABLE 1. Comparison of Age, Height , Weight, BMI, and MDA, GSH, NO levels in two groups.

	AGE	HEIGHT	WEIGHT	BMI	MDA	GSH	NO
Study (PCOS)	25.0 ± 6.14	160.90 ± 6.14	71.5 ± 5.72	27.3 ± 5.15	15.93 ± 2.1	6.7 ± 0.6	43.36 ± 3.54
Control	27.4 ± 6.9	159.28 ± 4.18	$55.09 \pm 4.18^*$	21.5 ± 2.5	$9.41 \pm 3.1^*$	$7.7 \pm 2.1^*$	$27.65 \pm 4.7^*$

* = Statistically significant differences between the groups $P < 0.001$

PCOS: Polycystic ovary syndrome

Glucose, cholesterol, ALT, AST, LDL and HDL there were similar in the groups ($p > 0.05$). However, triglyceride levels were significantly higher in the study group (127.13 ± 71.5 vs. 88.3 ± 41.2 mg/dl) respectively ($p < 0.005$). High Sensitive CRP (hsCRP) levels were significantly higher in the study group compared to the controls (0.5 ± 0.4 mg/L vs. 0.11 ± 0.1 mg/L) respectively ($p < 0.001$). Insulin levels were significantly higher in the study group compared to the control group (13.33 ± 4.8 $\mu\text{IU/ml}$ vs. 9.5 ± 1.44 $\mu\text{IU/ml}$) respectively ($p < 0.001$) (Table 2).

TABLE 2: Comparison of Glucose, Triglycerid, Tcholesterol, HDL, LDL, CRP, ALT, AST, Insulin levels in two groups.

	GLUCOSE	TRIGLICERID	T. CHOL	HDL	LDL	CRP	ALT	AST	INSULIN
Study(PCOS)	82.1 ± 8.12	127.13 ± 71.5	185.03 ± 33.9	43.36 ± 3.54	84.73 ± 24.18	0.5 ± 0.4	34.36 ± 12.05	17.06 ± 6.76	13.33 ± 4.8
Control	83.0 ± 6.11	$88.3 \pm 41.2^{**}$	175.68 ± 19.4	51.78 ± 7.05	89.9 ± 16.2	$0.11 \pm 0.1^*$	33.81 ± 8.72	17.34 ± 3.6	$9.5 \pm 1.44^*$

* = Statistically significant differences between the groups $P < 0.001$

**= Statistically significant differences between the groups $P < 0.05$

Discussion

Insulin resistance, hyperinsulinemia and oxidative stress are supposed to play important roles in the pathogenesis of polycystic ovary syndrome (PCOS). In addition, some women with PCOS have been shown to have insulin secretory defects and can be predicted to be at an increased risk for glucose intolerance [19].

It has been shown that lipid peroxidation and ROS formation may cause oxidative stress and damage. The detoxification of these products occurs through transformation of reduced form

of glutathion (GSH) to oxidized form (GSSG) by glutathion peroxidase enzyme. Oxidized glutathion again turns to reduced form by glutathion reductase enzyme.

When cells are exposed to excessive amounts of oxidants, due to formation of oxidized dimer form of glutathione (GSSG) exceeds metabolic limit, there will be oxidative stress. In this situation oxidants that cannot be detoxified affect membrane lipids, DNA, carbohydrates and enzymes in a negative way[20,21].

In our study, we observed lower GSH levels in PCOS group. Victor et al. [22] performed a study with 20 PCOS and 20 healthy volunteers for control group and they observed lower GSH levels in PCOS group. They suggest that this is due to the association between insulin resistance and an impaired mitochondrial oxidative metabolism. Our findings are also consistent with those of Victor et al. [23] and Sabuncu et al. [6].

It has been shown that increased production of ROS in PCOS may causes tissue damages [7]. Lipid peroxidation is supposed to one of the tissue damage mechanisms of oxidative stress and it has been shown as the damage of unsaturated fatty acids that is dependent on free radicals and oxygen [6]. In this study, we measured MDA levels, which were very harmful effects on the cells, in order to determine lipid peroxidation level. We found higher MDA levels in PCOS group. Our findings were similar to those of Sabuncu et al. [6], Yilmaz et al. [24] and Kuscü et al. [4]. On the other hand Erdogan et al. showed no statistically significant difference between PCOS patients and control group as regards MDA levels [25]. In the literature it has been known that MDA levels are higher in men compared to women [26]. Although exact mechanism has not been clearly understood, hyperandrogenemia in PCOS may be the reason for these higher MDA levels. There are conflicting studies in the literature concerning this hypothesis.

Nitric oxide (NO) contributes to vessel homeostasis by inhibiting vascular smooth muscle contraction and growth, platelet aggregation, and leukocyte adhesion to the endothelium. Humans with atherosclerosis, diabetes or hypertension often show impaired NO pathways [27]. Insulin regulates blood vessel tonus via its actions on endothelial NO synthesis [28,29]. Although insulin sensitivity and endothelial NO synthesis are positively related in healthy individuals, insulin resistant states such as obesity, gestational diabetes, hypertension, and type 2 diabetes are all associated with impaired insulin-mediated nitric oxide-dependent vasodilatation [30]. Human endothelial cells possess insulin receptors, and insulin has been demonstrated to increase NO release from cultured endothelial cells via a phosphatidylinositol-3 (PI-3) kinase pathway [31]. In our study, there was a significant difference in terms of NO levels. NO levels were higher in PCOS group when compared to

that of controls. On the other hand, Kuscü et al. [4] and Erdogan et al. [25] did not observe significant differences in terms of NO levels in their studies.

Our study revealed that there is an imbalance of oxidative/antioxidative status in women with PCOS. Impaired metabolic profile and increased cardiovascular risk factors associated with PCOS.

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