



Evaluation of oxidative stress and inflammation in patients with polycystic ovary syndrome

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Objective

Polycystic ovary syndrome (PCOS) is a heterogeneous endocrine and metabolic disorder characterized by hyperandrogenism, hyperinsulinemia, and insulin resistance. The prevalence of PCOS is increasing worldwide. Although the etiology of this disease is currently unknown, it is thought to be closely related to inflammation and oxidative stress. Our study aimed to compare patients have PCOS to healthy volunteers and assess the changes in oxidative stress and inflammatory parameters in these patients.

Methods

Thirty patients between the ages of 18-45 diagnosed with PCOS and 30 healthy volunteers with the same demographic characteristics were included in this study. Clinical parameters were measured using immunoassays. Oxidative stress biomarkers, total oxidant (TOS), total antioxidant (TAS), total thiol (TT), and native thiol (NT) levels were measured using photometric methods according to Erel's method. The dynamic disulfide level (DIS) and oxidative stress index (OSI) were calculated using mathematical equations. Among the inflammatory parameters, values for interleukin (IL)-1 β , IL-6, and tumor necrosis factor- α (TNF- α) were measured photometrically using commercially purchased kits.

Results

Moreover, TT and NT levels were lower in patients with PCOS compared to those in the healthy group statistically significantly ($P<0.001$). In addition, TAS, TOS, OSI, DIS, IL-1 β , IL-6, and TNF- α levels were identified to be significantly higher in the patients with PCOS than those in the healthy group ($P<0.001$).

Conclusion

Evaluation of oxidative stress and clinical parameters used in the follow-up may be beneficial for the disease.

Keywords: Inflammation; Oxidative stress; Polycystic ovary syndrome; Reactive oxygen species; Disulfide

Introduction

Polycystic ovary syndrome (PCOS) is an endocrine and metabolic disease with a prevalence between 6-21% [1]. PCOS is associated with hyperandrogenism, hirsutism, oligomenorrhea, amenorrhea, and anovulation. Moreover, anxiety and depression are also among the symptoms of this disease [2]. The Rotterdam criteria are used to diagnose the disease of disease when at least two or three of the scale criteria are present [3].

Received: 2024.01.22. Revised: 2024.03.01. Accepted: 2024.04.30.

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The prevalence of insulin resistance, a condition where tissues do not respond adequately to insulin despite elevated insulin levels in the bloodstream, ranges from 44% to 70% in patients with PCOS. Insulin resistance is one of the causes of metabolic disorders, as well as endocrine and reproductive abnormalities in the aforementioned patients [4]. Regulation of menstruation and hyperandrogenism along with insulin-sensitizing drugs that control insulin resistance are utilized for long-term treatment [5].

Oxidative stress is the imbalance between oxidants and antioxidants in the body. Reactive oxygen species (ROS) destructively affect the human body owing to the imbalance caused by the excessive production of oxidants [6]. Recent studies suggested that inflammation, oxidative stress, and genetic mechanisms are underlying causes of PCOS [2]. Oxidative stress is generally observed in infertile women with PCOS, regardless of metabolic anomalies. PCOS is closely associated with chronic inflammation and oxidative stress [7]. In our study, we aimed to compare and clinically evaluate oxidative stress and inflammatory markers in patients compared to those in healthy volunteers.

Materials and methods

Thirty patients aged between 18 and 45 who visited gynecology and obstetrics polyclinics and were diagnosed with PCOS along with a control group of 30 people with similar demographic characteristics and no chronic illnesses were included. The number of volunteers in each group was calculated to be at least 30, based on a power analysis with 80% power at the $\alpha=0.05$ significance level. According to the 2003 Rotterdam criteria [8] for the patient group, volunteers who met two of the following conditions were included: oligo- or anovulation, clinical findings, and detection of polycystic ovaries by ultrasonographic examination. Patients with diabetes mellitus, Cushing's syndrome, androgen-secreting tumors, and endocrinopathies, including late-onset 21-hydroxylase deficiency, infectious diseases, hypertension, thyroid dysfunction, hyperprolactinemia, chronic liver disease, use of drugs that affect sex hormones and lipid profiles, or altered insulin secretion and function, were excluded from the study. The inclusion criteria for the healthy control group were as follows: age between the ages of 18 and 45, no history of chronic disease, no use of any routine medica-

tion, and no smoking or alcohol addiction. Individuals with chronic illnesses, medication use, and substance addiction were excluded from the control group. In addition to these criteria, patients eligible for the study signed an informed consent form. Ethics committee approval was obtained from the University of Health Sciences Turkey, Hamidiye Scientific Research Ethics Committee (decision number 22/16) on 2021 January 14.

1. Collection of blood samples

Blood samples collected from patients in sterile biochemistry tubes with a gel clot activator were centrifuged for 10 minutes at $3,000 \times g + 4^{\circ}C$ to separate the serum. Separated serum samples were stocked until biochemical analysis at $-80^{\circ}C$.

2. Clinical parameters

Serum thyroid-stimulating hormone, follicle-stimulating hormone, luteinizing hormone, and prolactin (PRL) levels were measured using an immunoassay (Advia Centaur XP; Siemens, Erlangen, Germany).

3. Oxidative stress parameters

Oxidative stress biomarkers, total antioxidant (TAS), total oxidant (TOS) [9], total thiol (TT), and native thiol (NT) levels [10] were measured photometrically based on Erel's method. The dynamic disulfide level (DIS), oxidative stress index (OSI), and percentages of DIS/TT, DIS/NT, and NT/TT were calculated using mathematical equations.

4. Inflammatory parameters

The levels of inflammatory biomarkers interleukin (IL)-1 β (E0143Hu, Bioassay Technology Laboratory [BT Lab] Zhejiang, China), IL-6 (E0090Hu, BT Lab), and tumor necrosis factor (TNF- α ; E0088Hu, BT Lab) were measured photometrically with enzyme-linked immunosorbent assay kits.

5. Statistical analysis

All statistical analyses were performed using the SPSS version 25.0 program (IBM Corp., Armonk, NY, USA). Parametric data are expressed as mean \pm standard deviation. A P -value <0.05 was considered statistically significant. Additionally, a student's t -test was used to calculate the difference between two parameters for normally distributed variables. Data that did not demonstrate a normal distribution were analyzed us-

ing the Mann-Whitney *U*-test.

Results

The volunteers' mean age was 31.0±7.94 years in the study. Routine clinical biochemical findings in the PCOS group are displayed in Table 1.

TAS was increased significantly in the patient group compared to the levels in the healthy control group as one of the antioxidant biomarkers in Table 2. The TT and NT levels were

Table 1. Age and clinical hormonal findings of the patients with PCOS in the study

	PCOS
Age (yr)	27.44±3.77
FSH (mIU/mL)	5.45±1.75
LH (mIU/mL)	7.29±3.02
LH/FSH	1.46±0.76
TSH (mIU/mL)	2.09±0.73
PRL (mIU/mL)	18.71±8.40

Values are presented as mean±standard deviation.

PCOS, polycystic ovary syndrome; FSH, follicle-stimulating hormone; LH, luteinizing hormone; TSH, thyroid-stimulating hormone; PRL, prolactin.

significantly lower in the patient group than those in the control group ($P<0.001$). The oxidant biomarkers TOS, OSI, and DIS were significantly higher in the patient compared to those in the healthy group ($P<0.01$). The DIS/NT and DIS/TT rates were significantly higher in the patients with PCOS than the rates in the healthy group ($P<0.001$). The NT/TT ratio was significantly reduced ($P<0.001$). The levels of inflammatory biomarkers were higher in the PCOS group than the levels in the healthy control group.

Correlation analysis was performed between clinical findings, oxidative stress, and inflammation parameters. A statistically significant positive correlation was observed between PRL levels and TT ($r=0.570$; $P=0.004$) and DIS ($r=0.598$; $P=0.002$) levels.

Discussion

ROS are oxygen-derived molecules primarily generated in the mitochondria during aerobic respiration in the body. They are produced by enzymatic and non-enzymatic reactions under both physiological and pathological conditions [11]. Superoxide anion (O_2^-), hydrogen peroxide (H_2O_2), hydroxyl radical (HO), and singlet oxygen (O_2) are the most important reactive oxygen derivatives. Additionally, ROS are unstable

Table 2. Comparison of oxidative stress and inflammatory biomarkers in healthy control group and patients with PCOS in the study

	Control	PCOS	<i>P</i> -value
TAS (mmol Ascorbate eq/L)	0.98±0.12	1.21±0.14	<0.001 ^{b)}
TOS (μmol H_2O_2 eq/L)	9.78±1.03	13.53±1.95	<0.001 ^{b)}
OSI (AU)	10.15±1.97	11.26±1.53	<0.01 ^{a)}
TT (μM)	631.71±43.91	513.53±64.28	<0.001 ^{b)}
NT (μM)	424.61±42.23	274.77±58.68	<0.001 ^{b)}
DIS (μM)	103.55±24.45	119.38±36.31	<0.01 ^{a)}
DIS/TT (%)	16.31±3.37	23.09±5.82	<0.001 ^{b)}
DIS/NT (%)	24.91±7.3	38.15±25.92	<0.001 ^{b)}
NT/TT (%)	67.36±6.74	53.80±11.65	<0.001 ^{b)}
IL-1β (pg/mL)	65.83±35.76	134.06±56.57	<0.001 ^{b)}
IL-6 (pg/mL)	139.18±50.35	164.86±46.99	<0.01 ^{a)}
TNF-α (pg/mL)	143.85±32.91	189.36±75.33	<0.01 ^{a)}

Values are presented as mean±standard deviation.

PCOS, polycystic ovary syndrome; TAS, total antioxidant status; TOS, total oxidant status; OSI, oxidative stress index; TT, total thiol; NT, native thiol; DIS, disulfide; IL, interleukin; TNF-α, tumor necrosis factor-α.

^{a)} $P<0.01$.

^{b)} $P<0.001$ compared to healthy control.

due to the presence of unpaired electrons [6,11]. Therefore, they interact with biological molecules, including proteins, carbohydrates, lipids, and DNA, in the cell and cause damage. Although ROS are normally involved in simple cellular events, such as cellular proliferation, differentiation, and signal transduction, each cell has a specific capacity for ROS [12]. Antioxidants are exogenous or endogenous molecules that function to prevent cell damage caused by an excess of ROS in cells. Antioxidant systems are classified as enzymatic or non-enzymatic. This enzymatic system includes superoxide dismutase, glutathione-S-transferase, glutathione peroxidase, glutathione reductase, catalase, and cytochrome oxidase, which safeguard cells against oxidative stress [13]. The non-enzymatic system includes metal-binding proteins, bilirubin, glutathione, and vitamins A, C, and E. This system neutralizes radicals and oxidants [14]. The main purpose of this system is to protect cellular substances from damage caused by ROS generated during aerobic respiration [15]. TAS indicates the total antioxidant capacity of the body against free radicals [16]. In previous studies, TAS levels were significantly higher in a healthy control group than those in a PCOS group [17]. In addition, Verit and Erel [18] demonstrated that TAS levels are significantly higher in patients with PCOS than the levels observed in controls. In this study, TAS levels were significantly elevated in patients with PCOS. This phenomenon is believed to balance the increasing levels of oxidants in the body during PCOS.

Oxidative stress is a metabolic imbalance between endogenous and exogenous free radicals and the antioxidants produced against them [19]. Free radicals consist of unpaired electrons. These electrons attempt to reach a stable state with other electrons in the cell and can react very quickly to form non-radical substances. Free radicals are produced endogenously, mainly in the mitochondria, and exogenously in response to ultraviolet rays and various chemicals [20]. The presence of oxidants due to insufficient or excessive antioxidant capacity in the body causes damage, such as membrane damage, loss of organelle function, decrease in metabolic efficiency, loss of electrolytes, and mutations [21]. Although TOS measures the levels of all oxidants in the body, the main indicator of oxidative load is the OSI [22]. Verit and Erel [18] discovered that TOS levels were significantly increased in non-obese and normal insulinemic patients. In a study conducted by Zhang et al. [23] TOS and OSI levels were significantly higher in patients with PCOS compared to those in the

healthy control group. In this study, the TOS and OSI were also significantly higher in patients compared to those in the healthy controls.

Thiol groups, organic compounds containing sulfhydryl (-SH) group, play a critical role in preventing oxidative stress in cells. Thiols represent a resistance mechanism against oxidation owing to the -SH group. A balance exists between thiol and disulfide levels, which is an oxidized form, as per normal [24]. The thiol-disulfide balance plays an important role in regulating transcription and cellular signal transfer mechanisms, as well as antioxidant detoxification, defense, enzyme activities, and apoptosis. Thiol-disulfide homeostasis is a parameter used to measure oxidative stress [25]. Aydin et al. [26] identified no significant differences between the NT, TT, DIS, DIS/NT, DIS/TT, and NT/TT levels in patients with PCOS and those in healthy volunteers. Yildirim et al. [27] reported lower disulfide levels and higher thiol levels in non-obese and obese patients with PCOS than the levels in healthy controls. Biyik et al. [28] demonstrated that NT levels were low in patients with PCOS; however, TT, DIS, DIS/NT, and DIS/TT levels were similar in both patients with PCOS and healthy controls. These results demonstrate that thiol/disulfide levels also change depending on the antioxidant levels in patients with PCOS [27]. In this study, NT, TT, and NT/TT levels were significantly lower in patients with PCOS compared to those in the healthy controls, whereas DIS, DIS/TT, and DIS/NT levels were significantly elevated. PRL can regulate both the reduction of glutathione disulfide (oxidized glutathione) to reduced glutathione and the biosynthesis of new glutathione [29]. In this study, we observed a positive correlation between protein levels and TT and DIS and established that PRL affects the main thiol metabolism in the cell.

In PCOS, health issues such as obesity, insulin resistance, metabolic syndrome, and type 2 diabetes mellitus are associated with adipose tissue accumulation in the visceral mesenteric regions. Globally 40-50% of women with PCOS are obese, 25-30% have impaired glucose tolerance, 50% have insulin resistance, and 8% have type 2 diabetes mellitus [30]. Current studies have revealed that inflammatory biomarkers such as TNF- α , IL-1 β , and IL-6 are identified to be higher in women with PCOS compared to those in healthy volunteers, depending on body mass index and age. Although the cause remains unknown, PCOS is thought to be related to obesity, insulin resistance, or high androgen levels. In addition, studies have demonstrated that insulin resistance in patients with

PCOS is associated with increased levels of inflammatory mediators in the blood [31]. In obesity, the increased production of adipose tissue-derived proinflammatory cytokines and chemokines causes low-grade inflammation. IL-1 β is a cytokine that is synthesized mainly from activated macrophages in humans and causes effects such as B lymphocyte maturation and proliferation, fever, and acute phase protein synthesis [32]. IL-6 is a cytokine released from T and B cells, monocytes, fibroblasts, endothelial cells, and various tumor cells, and is involved in hematopoiesis, inflammation, and cell differentiation [33]. TNF- α is released from immune system cells, adipocytes, and endothelial cells; mainly acts on lipid metabolism, coagulation, insulin resistance, and endothelium [34]. Even though an increase in TNF- α levels was observed in non-obese patients with PCOS in one study [35], another study stated that elevated TNF- α and IL-6 in patients with PCOS were related to excessive secretion from obesity-induced adipose tissue, suggesting PCOS had no impact [36]. In one study, the serum IL-6 level in obese patients with PCOS was lower than the serum IL-6 level in people who did not have a disease other than obesity. Therefore, the relationship between PCOS and IL-6 cannot be fully determined [37]. In several studies, IL-1 β levels were lower in the control group compared to those in the patients with PCOS [38]. In this study, inflammation markers TNF- α , IL-6, and IL-1 β levels were lower in the healthy control group than those in women with PCOS.

The limitations of our study include the small sample size, the small number of clinical parameters examined in the patient group, and the lack of data on clinical parameters in the healthy control group.

Oxidative stress and inflammation levels in patients with PCOS were examined, and both oxidative stress and inflammation were increased in patients with PCOS compared to those in the healthy control group. The evaluation of inflammatory parameters and oxidative stress, as well as the clinical parameters used in the follow-up of the disease, may be beneficial in terms of the disease.

Conflict of interest

The authors declare no conflict of interest.

Ethical approval

Ethics committee approval was obtained from the University of Health Sciences Turkey Hamidiye Scientific Research Ethics Committee (decision number 22/16) on January 14, 2021.

Patient consent

All volunteers in the study signed an informed consent form.

Funding information

None declared.

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