Original Study

Infertility and the Presence of Insulin Resistance Are Associated With Increased Oxidative Stress in Young, Non-obese Turkish Women With Polycystic Ovary Syndrome

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A B S T R A C T

Study Objective: To investigate the relationship between both insulin resistance and fertility and the oxidant/antioxidant system in young, non-obese patients diagnosed with polycystic ovary syndrome (PCOS).
Design: Case-control study.
Setting: Department of Obstetrics and Gynecology, Ege University, Izmir, Turkey.
Participants: PCOS patients without insulin resistance (IR-) (n = 33), PCOS patients with insulin resistance (IR+) (n = 27), and healthy controls (n = 30). Patients with PCOS and regular sexual intercourse were further divided into infertile (n = 14) and fertile (n = 15) groups.
Main Outcome Measures: The malondialdehyde (MDA) and thiol levels as well as the catalase (CAT) and superoxide dismutase (SOD) enzyme activities.
Results: Both IR+ and IR- PCOS patients had higher MDA levels and lower thiol levels when compared to the controls (each $P < .001$). However, only IR- patients had significantly higher SOD (3700.81 ± 410.13 vs 2614.19 ± 611.80 U/g Hb; $P < .001$) and CAT (7565.06 ± 628.27 vs 6819.61 ± 539.2 U/g Hb; $P < .001$) activities when compared to the controls. Infertile PCOS patients had significantly higher MDA levels (347.5 ± 22.8 vs 278.6 ± 42.6 nmol/g Hb, $P < .001$) and lower thiol levels (498.5 ± 56.2 vs 568.5 ± 38.6 μmol/l, $P = .001$) when compared to fertile patients.
Conclusions: The results of this study demonstrated an imbalance in the oxidative-antioxidative system of PCOS patients. This imbalance was worse in IR+ and infertile PCOS patients.
Key Words: Polycystic ovary syndrome, Fertility, Insulin resistance, Oxidant-antioxidant status

Introduction

Polycystic ovary syndrome (PCOS) is a syndrome that causes various degrees of menstrual irregularity, hirsutism, acne, and obesity. PCOS is the most common endocrinopathy in women of reproductive age with a prevalence of 5%-10%.[1] This syndrome most frequently emerges between the ages of 15 and 30 and is a result of impairments in the interactions between the central nervous system, pituitary gland, ovaries, adrenal glands, and extra-glandular tissues that follows a chronic course and negatively affects the quality of life over years.[2] Moreover, PCOS may occur concurrently with hyperandrogenism, hyperinsulinemia, and glucose intolerance, leading to conditions such as infertility, recurrent spontaneous abortions, hyperlipidemia, type 2 diabetes mellitus, hypertension, coronary atherosclerosis, endometrial hyperplasia, and endometrial cancer.[3] Although PCOS is seen in obese women by 30%-75%, central adiposity, a higher adipose rate, and insulin resistance may also occur in non-obese patients.[4]

Oxidative stress is believed to play a role in endometriosis,[5] unexplained infertility,[6] male factor infertility,[7] ovulatory regulation, and impairment of oocyte quality in the human reproductive system.[8] Moreover, researchers have investigated the role of oxidative stress in the pathogenesis of PCOS and related complications.[9] Oxidative stress is defined as impairment of the balance of antioxidant defense mechanisms and reactive oxygen species (ROS). This imbalance leads to increased ROS, which damages the cell membrane lipids, leading to lipid peroxidation by formation of malondialdehyde (MDA). The harmful effects of an increased oxidative load are counteracted by antioxidant enzymes such as superoxide dismutase (SOD) and catalase (CAT) that convert ROS to less harmful molecules. Thiols are also antioxidant compounds and play an important role in antioxidant defense. In the case of an oxidant-thiol interaction, the oxidant is neutralized to less toxic products through the consumption of thiols. When excessive ROS production overpowers the body's natural antioxidant defense system, it may create an unstable environment for the reproductive cells and tissues.[10,11] Therefore; it is important to assess the oxidant/antioxidant status in PCOS and infertile women.

In the present study, we aimed to investigate the relationship between insulin resistance and fertility and the oxidant-antioxidant status in young, non-obese PCOS patients. Although it has been shown that increased oxidative

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stress may be associated with anovulation,[12,13] to our knowledge, this is the first study in the literature evaluating the relationship between the oxidant-antioxidant system and the fertility status of PCOS patients after 1 year of regular sexual intercourse.

Materials and Methods

This study was conducted between 2009 and 2012 at the Department of Obstetrics and Gynecology at Ege University School of Medicine after approval from the ethics committee. Written informed consent was obtained from all patients. The diagnosis of PCOS was based on the presence of 2 of the 3 following criteria: (1) the presence of oligo or amenorrhea, (2) clinical and/or laboratory confirmed hyperandrogenism, (3) polycystic ovaries on ultrasonography and exclusion of related disorders.[14] The inclusion criteria were as follows: age between 20 and 30 years; no major medical disorders (endocrinologic or neoplastic); and the presence of clinical and laboratory findings of hyperandrogenism in the absence of the other pathologies, such as ovarian, adrenal, pituitary, or congenital adrenal hyperplasia. Patients who were smokers, had a body mass index (BMI) > 30 kg/m^2, or were taking medication to treat PCOS were not included in the study. The patients were divided into the following study groups: PCOS patients with insulin resistance (IR+, n = 27), PCOS patients without insulin resistance (IR-, n = 33), and healthy control women (n = 30). All control participants had regular menstrual cycles lasting 25-35 days and a luteal range serum progesterone level consistent with ovulation (>5 ng/ml). The control patients also had normal serum androgen levels.

Blood samples were collected on the 3rd day of the spontaneous or gestagen-induced menstrual cycles. Venous blood was taken from the forearm following 8 hours of fasting between 8:00 and 10:00 in the morning. The data obtained from the patients included age, past medical history, family history, weight, height, and social history.

Insulin resistance was calculated using the HOMA index (fasting glucose $\times$ fasting insulin/constant). Because the fasting glucose value was measured in mg/dL, the constant was set at 450. The HOMA-IR level of the control group was 1.76 ± 0.81. The cut-off HOMA-IR value for this study was 1.93, which was the upper level of the 95% confidence interval of the mean value in the healthy group.

Patients were excluded from this study if they had the following characteristics: use of contraceptive methods or any medication for ovulation, male factor infertility, or not having regular sexual intercourse. The following 2 subgroups were created: patients with PCOS who did not become pregnant despite 1 year of unprotected intercourse (n = 14) and PCOS patients who became pregnant without therapy (n = 15). The oxidant-antioxidant status, insulin resistance, and fertility were evaluated in both groups.

Measurements

Erythrocyte Lysate Preparation

After blood samples were centrifuged at 4°C at 3,000 rpm for 10 minutes, plasma samples were separated as aliquots and stored at -80°C until the analysis. After removal of plasma, the packed erythrocytes were washed 3 times with 9 g/L NaCl solution and hemolyzed with ice-cold distilled water (1/5, v/v).

Measurement of Malondialdehyde Levels

The malondialdehyde measurements were performed using the method described by Yagi.[15] This method is based on the spectrophotometric measurement of the red color in the thiobarbituric acid-MDA complex at 532 nm after the erythrocyte lysate is deproteinized with trichloroacetic acid. The results were calculated as nmol/g of hemoglobin (Hb).

Catalase Activity

After the erythrocyte lysate was diluted to 1:100 with phosphate buffer, the CAT activity was measured with a Shimadzu UV-1208 spectrophotometer (Kyoto, Japan) using the method described by Aebi.[16] This method is based on the decomposition of hydrogen peroxide by catalase. The results were calculated as U/g Hb.

Superoxide Dismutase Activity

Superoxide dismutase was measured using a Shimadzu UV-1208 Spectrophotometer and the method described by Sun et al.[17] This method is based on the inhibition of nitroblue tetrazolium reduction with xanthine-xanthine oxidase used as a superoxide generator. The specific activity was calculated as U/g Hb.

Measurement of Thiol Levels

The thiol levels were determined by the method described by Hu et al,[18] which is based on the thiol-disulfide interchange reaction between thiols and 5,5′-dithio-bis-(2-nitrobenzoic acid). The evaluation was performed using a standard curve for glutathione. Sample absorbances were measured at 412 nm. Plasma protein carbonyl content levels were measured using the method described by Reznick and Packer.[19] Sample absorbances were measured at 360 nm using the molar extinction coefficient of 2,4-dinitrophenyl hydrazine and a Folin reagent. Thiol levels were recorded as micromoles per liter.

Statistical Analysis

Statistical analyses were performed with the SPSS 15 package for Windows (SPSS, Chicago, IL). Variables were investigated using visual (histograms, probability plots) and analytical methods (Kolmogorov-Smirnov/Shapiro-Wilk test) to determine a normal distribution. Continuous data (presented as the mean $\pm$ SD) were analyzed using Student t test and 1-way analysis of variance (ANOVA) followed by post hoc tests. The chi-square and Fisher exact tests were used to compare the proportions of different groups. The correlation coefficients and their significances were calculated using the Pearson correlation test. A P-value $< .05$ was considered to be statistically significant.