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Original Article

Evaluating indicators of oxidative stress and their relationship with Insulin Resistance in polycystic ovary syndrome

Beitolah Alipour[1], Farzaneh Shahrdami[1],ElhamRoohelhami[1],BahramRashidkhani[2], Sara Edalati[1]

[1]Department of Community Nutrition, Nutrition Faculty, Tabriz Medical University, Tabriz, Iran - Email: elham.roohelhami@gmail.com; [2] Department of Community Nutrition, Faculty of Nutrition Sciences and Food Technology, ShahidBeheshti University of Medical Sciences, Tahran, Iran

Summary. *Background:* Polycystic ovary syndrome (PCOS) is the most frequent female disorder in the reproductive years. It is characterized by amenorrhea or oligomenorrhea, hyperandrogenism and obesity. Due to the unknown pathophysiology of PCOS and contradictory results of multiple studies on oxidative stress and relationship between oxidative stress and insulin resistance (IR), we aimed to evaluate indicators of oxidative stress and their relationship with insulin resistance in women with polycystic ovary syndrome. *Method:* This case-control study was accomplished over 90 patients diagnosed with PCOS from the Moheb Yas comprehensive Women hospital in Tehran (March-November 2015) based on the Rotterdam criteria. Additionally, 90 healthy individuals were recruited and matched according to age and BMI by simple sampling. At the beginning of the study, weight, height, waist circumference (WC), hip circumference (HC), fasting blood sugar (FBS), fasting insulin, total antioxidant capacity (TAC), malondialdehyde (MDA) were measured by standard methods. In addition, body mass index (BMI) and insulin resistance (IR) were calculated. *Results:* Insulin and IR were statistically higher in the PCOS group in comparison to the control group ($p<0.001$, $p<0.001$) In contrast, TAC was statistically lower in the PCOS group in comparison to the control group ($p<0.001$). However, there were no statistically significant differences for FBS and MDA between the two groups. Additionally, Insulin and IR had a significant positive correlation with anthropometric variables. *Conclusion:* According to the results obtained from this study, imbalance of oxidative status/antioxidant defenses in PCOS patients indicate that oxidative stress is probably significant in the pathogens of PCOS. Thus, recommending patients to use abundant sources of anti-oxidants may be efficient in ameliorating symptoms of PCOS.

Key words: Polycystic ovary syndrome, Oxidative stress, Insulin resistance, Total antioxidant capacity, Malondialdehide

Introduction

Polycystic ovary syndrome (PCOS) is the most common endocrine disorder in women in the reproductive years and the most significant cause of anovulatory infertility (1, 2) . The worldwide prevalence of PCOS is 6-10% and affects one in 15 women (3) In Tehrani's study, the prevalence of PCOS was 14.6% based on the Rotterdam criteria in the Iran (4). Symptoms include oligo-/amenorrhea, hyperandrogenism, hirsutism and acne. According to the latest manifestation of Rotterdam 2003, PCOS is described as having at least two out of three indicators of PCOS including oligo- or anovulation, clinical and/or biochemical

signs of hyperandrogenism and sonographic results of having polycystic ovaries (5) (6, 7). Risk factors such as insulin resistance, dyslipidemia and oxidative stress in these patients increase the risk of diabetes mellitus type II and cardiovascular disorders. In these patients increased level of oxidative stress is detected long before dysfunction of the arterial endothelial (8).

Free radicals are atoms or molecules that due to having unpaired electrons circulate in the body and damage macromolecules including lipids, proteins, DNA and carbohydrates. There is a distinctive system in the body for defeating the damage obtained from free radicals called the "antioxidant defense system". In an ordinary situation, there is a balance between the production of free radicals and the antioxidant defense system in a healthy person. But if under any circumstance this balance is impaired, this condition is called oxidative stress (9).

Studies have revealed that oxidative stress status is higher among women diagnosed with PCOS than women with a normal situation. Although high oxidative stress indicators are significantly related to obesity, insulin resistance, hyperandrogenism and chronic inflammation and considering oxidative stress as one of the possible causes of PCOS, it is not easy to say if PCOS causes excess oxidative stress or the consequences of PCOS increases oxidative stress (10). In several studies, including the study of Mohammadin et al with the aim of evaluating and comparing oxidative and antioxidant status in PCOS women with healthy subjects, it was observed that antioxidant status was significantly lower in PCOS women in comparison to the control group (11). Also in the study by Unni et al in 2015, it was shown that erythrocytes MDA and SOD were significantly higher in PCOS women in comparison to the control group which revealed that oxidative stress was higher among PCOS women (12). Desai et al (13) showed that oxidative stress can induce insulin resistance in non-obese women with PCOS.

There is a positive correlation between low levels of oxidative stress and high levels of oocyte maturation in PCOS and infertile women. Therefore, we can say that by reducing oxidative stress, antioxidants can improve PCOS precursors. Additionally, antioxidants are related to the apoptosis process in the ovary mesenchyme and can outbreak a series of events with different mechanisms. Antioxidants are associated with the ordinary growth and accurate function of interstitial cells (7). Thus, due to the lack of similar studies in Iran, our objective was evaluating the relationship between oxidative stress and PCOS, oxidative stress and IR, and also the effect of antioxidants on reducing the symptoms of PCOS.

Methods

Subjects

In the present case-control study, 90 patients diagnosed with PCOS were recruited from the gynecology and infertility clinic of the Moheb Yas comprehensive Women hospital in Tehran, Iran using simple sampling (March-November 2015). Patients were chosen after diagnoses by a physician and based on the Rotterdam criteria. Features of diagnosis are: amenorrhea (no cycle for > 6 mo) or oligomenorrhea (< 8 cycle length > 45d or both), clinical (hirsutism, acne, obesity) or biochemical evidence for hyperandrogenemia (total testosterone >54 ng/dL or free testosterone >9.2 pg/mL), polycystic ovaries on ultrasound test (presence of of >12 follicles in each ovary, with each follicle measuring 2–9 mm in diameter; increased ovarian volume > 10 mL or a combination) (5). The study method was described to patients and written informed consent was obtained from all participants. The control group were women with regular menstrual cycle, without hirsutism and endocrine dysfunctions recruited from the same hospital and matched for age and body mass index BMI (Kg/cm2). The age range was between 18 to 35 years and BMI less than 40. At the beginning of the study, demographic questionnaires were completed for all subjects. Weight was measured with light clothes by Seca electronic scale (0.1 kg delicacy) and height was measured by Seca stadiometer (0.1cm delicacy). Before measuring weight, the weight scale was calibrated by a standard scale and the mean number of two times measuring was registered. Waist circumference (WC) was recorded as the smallest measurement between the iliac crest and the lateral costal margin and hip circumference (HC) was the largest measurement over the buttocks, using a non-elastic tape.

Exclusion criteria were pregnancy, lactation,

smoking, using alcoholic drinks, thyroid dysfunction, infectious disorders, liver and coronary diseases, diabetes, hyperprolactinemia, Cushing's syndrome and also using any kind of vitamin or mineral supplement, glucocorticoids, anti-obesity, anti-diabetes, anti-pregnancy and reducing lipid drugs.The study protocol was approved by the Ethics committee of Tabriz University of Medical Sciences.

Serological analysis

Analyses were performed in the Endocrinology and Metabolism Research Center-Cellular and Molecular Bioloy of Shahid Beheshti Medical University (SBMU) by a specialized medical technician. Blood samples were obtained after an overnight fasting in the morning and then centrifuged at 3000× *g* for 15 min then the serum was extracted and finally stored at -70°C until biochemical assays.

The chemical photometric method was used for analyzing total antioxidant capacity (TAC) and malondialdehyde (MDA) using Biocore Germany assay kits (Sensitivity: 0.1 μM). Glucose analyzes was performed using enzymatic methods (Parsazmun Kit, Tehran, Iran). Insulin was measured by a Human recombinant enzyme-linked immunosorenti assay (ELISA) method using commercial kits (Monobind, ELISA kit, USA. Assay sensitivity was 0.75 μIU/ml). Insulin resistance was calculated by the homeostasis model assessment (HOMA) as follows: insulin (microunits/mL) multiplied by glucose (mg/dL) and dividing the product by 405.

Statistical analysis

Data were analyzed using Statistical Package for the Social Sciences (SPSS ver.16) (SPSS Inc, Chicago, IL, USA) software. Data normality wase valuated by Kolmogorov-Smirnov test and expressed by mean and standard deviation or median (25^{th}- 75^{th} percentiles) in tables. Independent sample T-test and Mann-Whitney U-test were performed to compare means and median values between two groups. Pearson correlation or Spearman rank correlation tests were used to identify associations. Additionally, Linear Regression test was used for adjusting confounders. All statistical tests were two-sided with a P-value <0.05 considered statistically significant.

Results

In this study, 180 women participated and were divided into two groups. The case group consisted of 90 women diagnosed with PCOS and 90 healthy women were in the control group. Mean age was 28/41±4.36 years in the PCOS group and 27.84±3.18 years in the control group. Demographic and anthropometric data are presented in table 1. Based on table 1, there were no statistically significant differences between the two groups for age, weight, height, BMI, weight circum-

Table1. Age and Anthropometric characteristics of study subjects

Variable	PCOS (n=90)	Control (n=90)	P†
Age (years)	28.41 (4.36)	27.84 (3.18)	0.321
Weight (Kg)	70.42 (13.50)	68.45 (10.81)	0.280
Height (cm)	161.86 (6.53)	161.8 (6.22)	0.944
BMI (kg/m²)	24.85 (5.20)	26.43 (6.17)	0.217
WC (cm)	85.17 (12.77)	84.40 (10.42)	0.655
HC (cm)	106.55 (11.49)	103.66 (9.24)	0.064

PCOS, PolyCystic Ovarian Syndrome; BMI, Body Mass Index; WC, waist circumference, HC, Hip Circumference ; †P based on Independent sample T-test. Data are presented as mean ± SD

Table 2. The comparison of biochemical variables between study groups

Variable	PCOS (n=90)	Control (n=90)	P†
FBS (mg/dl)	100 (92-108)	102 (95-109)	0.125
Insulin (μIU/ml)	12.14 (8.07)	7.13 (2.66)	0.001>
Insulin Resistance (HOMA-IR)	2.97 (1.98)	1.82 (0.69)	0.001>
TAC (U/ml)	10.07(9.13-11.04)	11.06(9.54to12.46)	< 0.001
MDA (μM)	5.70 (0.98)	5.57±1.13	0.432

PCOS, PolyCystic Ovarian Syndrome; FBS, Fasting Blood Sugar ; TAC, Total Antioxidant Capacity; MDA, Malondialdehyde. †P value based on Independent sample T-test or Mann–Whitney U test. Data are expressed as mean (SD) or medians (25^{th} percentile –75^{th} percentile).

ference (WC) and hip circumference (HC).

Table 2 indicates the comparison of biochemical variables between the two groups. There was no statistically significant difference between the two groups for fasting blood sugar (FBS) (p=0.12) and MDA (p=0.43). However statistically significant differences were observed for fasting insulin (p<0.001), insulin resistance (p<0.001) and TAC (p<0.001).

The correlation between insulin and IR and anthropometric variables such as weight, BMI, WC, HC are presented in table 3. According to table 3, weight, WC, HC and BMI had a significant positive correlation with insulin and IR in both groups.

In this study, TAC and MDA were compared between two groups with the Linear Regression test and considering IR as the confounder factor. After adjusting for IR, there was a statistically significant difference for TAC between two groups (p=0.001). However, there was no statistically significant difference for MDA between the two groups (p=0.608).

Discussion

Total anti-oxidant capacity is defined as the ability of the serum to quench free radicals and protect against molecular damage of the cell structure. Antioxidants such as uric acid, albumin, vitamin E, vitamin C, β-carotene, thiol groups, α-tocopherol and bilirubin are present in the plasma in high concentrations. Indeed, TAC is sensitive to changes in plasma antioxidant levels, degree of oxidative stress and is a cumulative index of plasma antioxidant status (14, 15).

In the present study there was a statistically significant decrease in the TAC level in the PCOS group in comparison to the control group (p<0.001). In other studies, different findings were observed (16). In a case-control study by Mohammadin et al in 2009 in Saudi Arabia, TAC was measured in 35 women with infertility disorders (diagnosis based on Rotterdam criteria) and 30 healthy women. According to this study, TAC was significantly lower in the case group in comparison to the control group (p<0.001) (11). In 2005, Yilmiz and colleagues measured TAC in 50 PCOS women with BMI less than 25 and 35 healthy women which were matched for age and weight. Similar to our study, a significant decrease was also observed in the TAC level in the PCOS group (p=0.005) (17).

In contrast, in the study of Cakir et al in 2011 among 52 women with PCOS and 36 healthy women matched for age and BMI different results were observed. In this study, there were no statistically significant differences for oxidative stress indicators such as total anti-oxidant status (TAS) and total oxidant status (TOS) in both groups. This finding is mainly due to

Table 4. The comparison of oxidative stress variables after adjusting IR between PCOS group and control group

Variable	B(SE)	ß	P†
TAC (U/ml)	**1.489(0.455)**	**-0.256**	**0.001**
MDA (μM)	**0.088(0.171)**	**0.041**	**0.608**

Linear regression analysis with TAC and MDA as dependent variables and group and Insulin Resistance (IR) as independent variables in patients with PCOS and control group

Table3. Correlation analyses between Insulin, IR (Insulin Resistance) and Anthropometric characteristics in Polycystic Ovarian Syndrome patients and control groups

	PCOS (n=90)		Control(n=90)	
	Insulin (μIU/ml)	**IR (HOMA-IR)**	**Insulin (μIU/ml)**	**IR (HOMA-IR)**
Weight (Kg)	P = <0.001 r = 0.403	P = <0.001 r =0.401	P = 0.016 r = 0.254	P = 0.006 r = 0.287
BMI (kg/m²)	P = <0.001 r = 0.389	P = <0.001 r = 0.367	P = 0.005 r = 0.291	P = 0.003 r = 0.309
WC(cm)	P = <0.001 r =0.373	P = <0.001 r = 0.376	P = 0.005 r = 0.296	P = 0.003 r = 0.305
HC(cm)	P = 0.003 r = 0.311	P = 0.003 r = 0.311	P = 0.002 r = 0.328	P = 0.001 r = 0.356

BMI, Body Mass Index; WC, waist circumference, HC, Hip Circumference

abnormal BMI range (24.9 in the PCOS group and 22.63 in the control group) and participant's young age (23.61 in the PCOS group and 25.50 in the control group) (18). In a recent study in 2016 by Zhang et al, 544 women diagnosed with PCOS and 468 healthy women were detected. It was observed that TAS was significantly lower and TOS was significantly higher in the PCOS group in comparison to the control group (19).

Malonyldialdehyde (MDA) is produced enzymatically by the breakdown of unstable hydroperoxides during peroxidation of unsaturated fatty acyls (20). Measurement of MDA levels in plasma or serum provides a convenient in vivo index of lipid peroxidation and represents a non-invasive biomarker of oxidative stress often clinically employed to investigate radical-mediated physiological and pathological conditions (21). In the present study, MDA was higher in the PCOS group in comparison to the healthy group, however, it was not statistically significant (p=0.432). In a study by Macut et al in 2011, oxidative stress factors were measured in 34 women with PCOS and 23 healthy women. In consistent to our study, MDA was insignificantly higher in the PCOS group in comparison to the healthy group. In this study it was observed that, non-obese women with PCOS were less likely to have oxidative stress. In our study, similar results were also seen (22).

In a study by Karadeniz et al in 2011, 98 women with PCOS and 92 healthy women were studied. Sample size and matching factors between the two groups were similar to our study and there was no significant difference in MDA level between the two groups (23). Controversy, in the study of Fan et al in 2011, MDA level was significantly higher in the PCOS group (n=291) in comparison to the healthy group (n=281) (p<0.001) (24). In the study of Bayram and colleagues, MDA and IR were significantly higher in the PCOS group (25).

In the present study, we considered IR as the confounding factor to compare oxidative stress factors such as MDA and TAC between the two groups. After adjusting IR between the two groups (Linear Regression test), there was a significant decrease in TAC and a slight increase in MDA in the PCOS group in comparison to the healthy group. Thus, it seems that other factors such as disease feature, over weight and obesity, hyperandrogenism and etc. are responsible for increasing oxidative stress in patients diagnosed with PCOS (10).

Additionally, BMI and other anthropometric factors such as WC and HC had a significant and positive correlation with insulin and IR in both groups. These findings are in consistent to the results of That hapudi's study. Which over weight, obesity and WC were the main causes of oxidative stress in PCOS patients (26).

In conclusion, a significant increase in insulin and IR and a significant decrease in TAC were observed in this study. Also, there were no significant differences in FBS and MDA between the two groups. Thus, it seems that further larger sample size studies are crucial in order to evaluate oxidative stress factors in patients with PCOS. Also studying PCOS patients with normal BMI range could help evaluate oxidative stress factors despite considering obesity risk factors.

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Correspondence:
Elham Roohelhami
Department of Community Nutrition, Nutrition Faculty, Tabriz Medical University, Tabriz, Iran.
Email: elham.roohelhami@gmail.com
Tel: +98 9126770378
Fax: +98 413 3340634