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Research Article

How to Effect of Metformin Adding to Cyproterone Acetate+Ethinyl Estradiol Treatment on Antioxidant Status in Polycystıc Ovary Syndrome?

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Abstract

Objectives: Polycystic Ovary Syndrome (PCOS) is the most commonly seen endocrinopathy that affects the women of reproductive age. We aimed to determine relationship between insulin resistance and oxidant/antioxidant status in women with PCOS and evaluate the effect of metformin combined with cyproterone acetate+ethinyl estradiol on oxidative stress in patients with PCOS.

Methods: 30 women with PCOS and 20 healty controls enrolled in the study. Women with PCOS divided into two groups according to insulin resistance. Superoxide dismutase, Glutathione peroxidase, Glutathione, Malonyldialdehyde, Myeloperoxidase level results were compared both in PCOS and control groups.

Results: Malonyldialdehyde was statistically significant in PCOS patients compared to the control group. MDA levels increased as the waist circumference increased. MDA and MPO enzymes were found to be significantly low after treatment in both groups compared to the pretreatment levels.

Conclusion: An increase in oxidant status was found in women with PCOS according to control groups. Oxidative stress parameters were decreased in both groups but with addition of metformin treatment there is no effect on oxidant and antioxidant status in polycystic ovary syndrome.

Keywords: Insulin resistance, Insulin sensitizer, polycystic ovary syndrome

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Polycystic ovary syndrome (PCOS) is a heterogeneous disease seen in women of reproductive age characterized by clinical and/or biochemical findings of hyperandrogenemia, polycystic appearance of the ovaries and oligo-anovulation. [1] In addition to reproductive problems patients with PCOS have the risk of obesity, hyperinsulinemia, impaired glucose tolerance, dyslipidemia and hypertension. In recent studies, oxidative stress is reported to play a role in many important pathological processes including aging, atherosclerosis, diabetes and pathogenesis of ischemia-reperfusion damage. This study was planned to determine oxidant and antioxidant status in PCOS patients and is there any difference between two groups according to insulin resistance. The aim of this study is to evaluate the effect of metformin treatment to be combined with cyproterone acetate+ethinyl estradiol on oxidative stress in patients with PCOS.

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Methods

30 patients with PCOS who are admitted to Internal Medicine and Endocrinology clinics of Pamukkale University Medical Faculty Hospital are included in the study. Accordingly Rotterdan criteria which comprise i) oligo-anovulation, ii) clinical and/or biochemical signs of hyperandrogenism, iii) presentation of polycystic ovaries by pelvic ultrasonography (USG) were evaluated. Those with a history of medication use affecting insulin secretion or activation and lipoprotein metabolism, hypertension, smoking, cardiovascular disease history in the family, diabetes, infectious diseases and Cushing syndrome or any endocrinological disease involving androgen secreting tumors, late onset 21-hydroxilase deficiency, thyroid dysfunction and hyperprolactinemia are excluded from the study. 20 volunteer women, selected from patients referring to Pamukkale University Medical Faculty Hospital, Gynecology and Obstetrics clinic and who are assessed to be normal according to ultrasonography and hormonal evaluation without any medication history composed the control group. After admission to the hospital their medical histories were taken in detail and general physical examination was performed. Gynecological and pelvic USG examinations of the study and control groups were carried out at Pamukkale University Medical Faculty Hospital Gynecology and Obstetrics clinic. Age, BMI, waist circumference (WC), waist/hip ratio (WHR), systolic (SBP) and diastolic (DBP), Ferriman-Gallwey hirsutism scores were evaluated in the study and control groups. In all patients OGTT, serum total testosterone, sex hormone binding globulin (SHBG), dehydroepiandrosterone sulfate (DHEAS), luteinizing hormone (LH), follicle stimulating hormone (FSH), glucose, insulin, triglyceride (TG), total cholesterol (TC), high density lipoprotein cholesterol (HDL) were checked and low density lipoprotein cholesterol (LDL) levels were determined. Insulin resistance was defined by means of calculations according to the homeostasis model assessment (HOMA-IR) score (fasting insulin concentration (mIU/L) x fasting glucose (mmol/L)/22.5).[5] Blood samples were taken at the time of the diagnosis and oxidative stress markers were assessed. PCOS cases with identified insulin resistance were taken to prospective evaluation by adding metformin to the cyproterone acetate–ethinyl estradiol treatment. Polycystic ovary syndrome cases with positive insulin resistance (n=15) were treated with metformin+cyproterone acetate+ethinyl estradiol; those without insulin resistance (n=15) were treated with cyproterone acetate+ethinyl estradiol. Biochemical analyses and antropometric assessments of the patients before the treatment and four months after the treatment were evaluated.

Biochemical Analyses

Malonyldialdehyde (MDA): Serum levels of MDA were defined with the Ohkawa method.[2] 0.5 ml serum 1.5 ml thiobarbituric acid (%0.8) was mixed with 1.5 ml acetic acid (pH 3.5, %20), 0.2 ml sodium dodecyl sulfate (%8.1) and 0.5 ml distilled water. After the mixing process all samples and standards were heated at 100 C for 1 hour. Absorbance was recorded at 532 nm and compared with the results of MDA standards. Results were expressed in nmol/ml.

Superoxide Dismutase (SOD): Superoxide dismutase (SOD) activity was evaluated according to the Winterbourn et al.[3] method. After mixing with ethanol and chloroform and the hemolysis, the samples were centrifuged and supernatant was used in identifying the SOD activity. 0.05 ml of supernatant was mixed with 0.2 ml of EDTA+NaCN, 0.1 ml of nitro-blue tetrazolium (NBT) (1.5 mM), 0.05 ml of riboflavin (0.12 mM) and 2.6 ml of phosphate tampon (0.02 M). Test tubes containing the reaction mixture were shaken and mixed, and kept at room temperature under fluorescent light for 15 minutes. SOD activity was identified spectrophometrically by means of evaluating the spectrophotometric inhibition of the reduction of nitro-blue tetrazolium (NBT) at 560 nm. The results were presented in U/gHb.

Reduced Glutathione (GSH): GSH identification is performed by means of the modified form of the procedure defined by Moron et al.[4] Deproteination solution (sodium chloride, metaphosphoric acid, EDTA and distilled water) is mixed with 0.4 ml filtrate 1.6 ml Na_2HPO_4 (0.3 M) solution and 0.2 ml Ellman reactive (DTNB; dithiodinitrodibenzoic acid, sodium citrate, distilled water) after hemolysis and filtration. Absorbance was recorded at 412 nm and results were expressed in µmol/gHb.

Myeloperoxidase (MPO): Knigth et al.'s[5] method was used in order to assess myeloperoksidaz (MPO) activity. Blood samples were centrifuged and used for defining supernatant enzyme activity. 0.05 ml of serum was mixed with 1.45 ml 100 mM of phosphate tampon containing H2O2 (%0.01) and o-dianisidine (%1). MPO activity was detected on the basis of the o-dianisidine reduction feature of the enzyme. Reduced o-dianisidine level was identified at 460 nm wave length by spectrophometer. Results were presented in U/L.

Statistical Analysis

SPSS program was used for the statistical analysis of the data. All the data are presented in average±standard deviation interval. Statistical analyses were performed by utilizing Mann-Whitney, Wilkoxon tests, repeated measures analysis of variance and Spearman correlation analysis.

Results

30 patients with PCOS and 20 healthy volunteers as the control group were included in our study. At the beginning of our study the differences between the patients with PCOS and the control group in terms of their anthropometric data and biochemical analyses were evaluated. Demographic and biochemical characteristics of the patients and the control group are presented in Table 1. According to the analysis there were statistically significant differences in the FGS score, insulin value, LDL, uric acid value and MDA in PCOS patients compared to the control group. A positive relation was defined between the MDA level and waist circumference which is an indicator of visceral obesity. MDA levels increased as the waist circumference increased. Demographic and biochemical characteristics of insulin resistant group was compared with the non-resistant group are presented in Table 2. There were statistically significant differences in the insulin resistant group in terms of body mass index, waist circumference, SHBG and uric acid levels. No significant difference was detected for oxidative stress enzyme levels between insulin resistant and non-resistant groups of PCOS patients. Pretreatment and post treatment values of PCOS patients with insulin resistance in presented in Table 3 and without insulin re-

Table 1. Comparison of PCOS and control groups

	PCOS (n=30)	Control (n=20)	p
Age (year)	23±6	23±4	NS
SBP (mmHg)	103±11	103±11	NS
DBP (mmHg)	70±9	73±10	NS
BMI (kg/m^2)	25±6	24±1	NS
waist circumference (cm)	83±12	76±6	NS
FGS	15±8	5±1	0.001
Glucose (mg/dl)	91±7	89±10	NS
Insulin (µIU/ml)	10±7	6±1	0.016
HOMAIR	2.3±1.7	1.4±0.3	NS
T. cholesterol (mg/dl)	167±33	152±27	NS
HDL cholesterol (mg/dl)	45±10	46±8	NS
LDL cholesterol (mg/dl)	101±28	87±23	0.036
Triglyceride (mg/dl)	103±47	94±42	NS
Testosterone (ng/ml)	50±25	38±13	NS
SHBG (nmol/L)	36±39	35±18	NS
DHEAS (µg/dl)	254±110	241±107	NS
Uric acid (mg/dl)	3.8±1	2.3±0.7	0.001
MDA (mmol/l)	2.73±0.42	2.39±0.38	0.007
GSH (µmol/gHb)	3.32±1.19	4.15±2	NS
SOD (U/gHb)	778±530	886±382	NS
MPO (U/L)	456±526	301±331	NS
FSH (Mıu/ml)	5.7±1.6	7±1.3	0.005
LH (mIU/ml)	6.5±3.2	4.8±1.3	0.029
Estrogen (pg/ml)	56±8	43±9	NS

p<0.05 is accepted as statistically significant; NS: Not significant.

Table 2. Comparison of insulin resistant and non-resistant groups among patients diagnosed with PCOS

	Insulin resistance (-) PCOS (n=15)	Insulin resistance (+) PCOS (n=15)	p
Age (year)	23±4	23±8	NS
SBP (mmHg)	101±10	106±10	NS
DBP (mmHg)	69±9	72±10	NS
BMI (kg/m^2)	22±4	29±5	0.001
waist circumference (cm)	77±10	91±10	0.001
FGS	15±7	16±9	NS
Glucose (mg/dl)	89±7	93±7	NS
Insulin (µIU/ml)	6.7±3	15±8	0.001
HOMAIR	1.4±0.7	3.5±1.9	0.001
T. cholesterol (mg/dl)	171±37	163±26	NS
HDL cholesterol (mg/dl)	46±9	43±11	NS
LDL cholesterol (mg/dl)	106±29	96±27	NS
Triglyceride (mg/dl)	93±52	115±39	NS
Testosterone (ng/ml)	49±20	51±31	NS
SHBG (nmol/L)	43±43	27±31	0.016
DHEAS (µg/dl)	245±90	266±133	NS
Uric acid (mg/dl)	3.4±1	4.3±0.9	0.016
MDA (mmol/l)	2.6±0.41	2.8±0.43	NS
GSH (µmol/gHb)	3.3±1.3	3.2±1.06	NS
SOD (U/gHb)	805±449	744±630	NS
MPO (U/L)	514±382	382±335	NS

p<0.05 is accepted as statistically significant; NS: Not significant.

Table 3. Comparison of pretreatment and post-treatment values of PCOS patients with insulin resistance receiving Cyproterone Acetate + Ethinyl Estradiol + metformin treatment

	Pretreatment	Post treatment 4th month	p
SBP (mmHg)	106.50±11.50	98.70±8.80	0.006
DBP (mmHg)	72.50±10.40	66.60±7.40	NS
BMI (kg/m^2)	29.30±6.70	29.40±6.70	NS
waist circumference (cm)	91.20±11.80	90.10±11.00	NS
FGS	17.80±9.70	13.60±9.70	0.034
Glucose (mg/dl)	93.60±8.70	89.50±9.40	0.05
Insulin (µIU/ml)	15.30±8,70	15.40±7.50	NS
HOMAIR	3.50±2.00	3.20±1.50	NS
T. cholesterol (mg/dl)	166.70±26.50	178.40±37.30	0.048
HDL cholesterol (mg/dl)	44.30±12.80	47.20±13.70	NS
LDL cholesterol (mg/dl)	98.70±27.70	106.10±27.50	0.048
Triglyceride (mg/dl)	117.70±41.80	128.20±60.80	NS
Testosterone (ng/ml)	51.77±34.85	48.83±28.40	NS
SHBG (nmol/L)	30.10±34.94	32.10±34.50	0.05
DHEAS (µg/dl)	253.15±142.40	303.32±169.22	NS
Uric acid (mg/dl)	4.20±0.94	3.90±1.03	NS
MDA (mmol/l)	2.80±0.45	1.14±0.64	0.001
GSH (µmol/gHb)	3.30±1.11	3.80±1.92	NS
SOD (U/gHb)	821.30±653.42	530.40±251.62	NS
MPO (U/L)	446.20±331.50	148.32±247.20	0.005

p<0.05 is accepted as statistically significant; NS: Not significant.

Table 4. Comparison of pretreatment and post treatment 4th month values of PCOS patients with no insulin resistance who received Cyproterone Acetate + Ethinyl Estradiol treatment

	Pretreatment	Post treatment 4th month	p
SBP (mmHg)	100.20±10.30	98.70±7.40	NS
DBP (mmHg)	70.40±9.40	64.60±8.40	0.046
BMI (kg/m^2)	23.20±4.10	23.30±4.70	NS
waist circumference (cm)	79.20±11.30	79.40±11.80	NS
FGS	14.50±5.60	14.40±6.50	NS
Glucose (mg/dl)	91.70±6.40	89.40±7.30	0.049
Insulin (µIU/ml)	7.10±3.40	6.40±2.30	NS
HOMAIR	1.50±0.86	1.30±0.57	NS
T. cholesterol (mg/dl)	177.40±33.40	192.70±35.20	0.048
HDL cholesterol (mg/dl)	47.30±10.20	51.40±13.30	NS
LDL cholesterol (mg/dl)	111.70±28.40	120.40±30.35	NS
Triglyceride (mg/dl)	93.56±45.46	105.76±39.20	NS
Testosterone (ng/ml)	49.60±22.20	39.66±14.43	NS
SHBG (nmol/L)	39.20±40.10	71.80±68.70	0.005
DHEAS (µg/dl)	253.10±93.40	241.30±98.40	NS
Uric acid (mg/dl)	3.37±1.10	3.17±0.72	NS
MDA (mmol/l)	2.74±0.45	1.10±0.26	0.001
GSH (µmol/gHb)	2.97±1.08	3.54±1.64	NS
SOD (U/gHb)	799.60±440.45	634.50±141.42	NS
MPO (U/L)	674.72±720.65	156.50±175.82	0.002

p<0.05 is accepted as statistically significant; NS: Not significant.

sistance presented in Table 4. An assessment was made to see whether the oxidative stress regressed or not as a result of the treatment. MDA and MPO enzymes were found to be significantly low after treatment in both groups compared to the pretreatment levels. There was no significant difference between the treatment groups at the end of the fourth month in terms of both biochemical findings and oxidative stress markers.

Discussion

Polycystic ovary syndrome is a common endocrinological disorder which is characterized by hyperandrogenemia and anovulation in premenstrual women. Since 1980s together with the identification of the relation between PCOS and insulin resistance the definition of the disease has gained a different dimension. Burghen et al.[6] showed for the first time that insulin levels were higher in PCOS cases. The resistance at the peripheral insulin receptor level in PCOS is thought to be associated with the decrease in postreceptor tyrosine autophosphorylation.[7] In this study at the beginning when we compared PCOS with healthy premenopausal women with similar age and weight we observed significantly high values for basal insulin levels. HOMA-IR index was found to be 2.3±1.7 in cases with PCOS and 1.4±0.3 in the control group. The data were not at significant levels probably due to the low number of cases.

In our study we observed higher serum MDA levels in polycystic ovary cases compared to the control group. MDA is one of the lipid peroxidation products. MDA causes cross-binding and polymerization of membrane components. This processes can change the intrinsic membrane characteristics such as deformation, ion transport, enzyme activity, and aggregation of cell surface components.[8] In our study serum MDA levels of PCOS were found to be significantly higher than those in the control group. This finding supports the assumption that PCOS cases are directly under high oxidative stress. The study of Fenkçi et al.[9] demonstrates an increase in total oxidant stress and a decrease in antioxidant system. We detected high serum LDL levels in cases with PCOS in comparison with the control group. In our study, we demonstrated that FGS scores were higher in PCOS cases as a clinical indication of hyperandrogenism. When the values of the group receiving cyproterone acetate+ethinyl estradiol treatment at the time of the diagnosis and at the post-treatment fourth month were compared, FGS scores clearly regressed to statistically significant levels. The MDA level, which is an indicator of lipid peroxidation, and the MPO level, which is a part of the antioxidant system decreased. It was also detected that there was a positive correlation between the MDA level and waist circumference.

Oxidative stress is also involved in the pathogenesis of atherosclerosis. Free oxygen radicals are available in the oxidative mechanisms of lipoproteins. Together with the increase of free radicals cholesterol biosynthesis and esterification increase cholesteryl ester hydrolysis in the cell decreases and cholesterol excretion from the cell decreases. All these events are reversed by the antioxidant system. In our study we identified higher serum LDL levels in PCOS cases in comparison with the control group. LDL cholesterol is known as the most important lipid fraction that has a tendency for oxidation during the development of foam cells which cause endothelial damage. Oxidized LDL levels elevated with the increased oxidative stress can be considered as a reason for the increased atherosclerosis in these cases. Despite higher average triglyceride levels of PCOS cases in our study, they did not reach significant levels. While there was no difference detected between TG and HDL levels in our study. Fenkçi et al.[10] identified high TG and low HDL levels in patients with polycystic ovary syndrome in their study. In such cases changes in the lipid metabolism may also have an effect on the development of cardiovascular risk profile.

We assessed our cases in two groups according to the pres-

ence of insulin resistance. Cases with an HOMA-IR index 2.7 and above were categorized as PCOS cases with insulin resistance. It was observed that PCOS cases with positive insulin resistance had significantly high BMI and waist circumference levels. Uric acid level was found to be high in patients with positive insulin resistance. Sex hormone binding globulin levels were found to be significantly lower. Polycystic Ovary Syndrome cases with positive insulin resistance although they had higher average values in both free testosterone (49±20ng/dl 51±31) and DHEAS (245±90 266±133) when compared to the PCOS cases with negative insulin resistance. This explains the effect of insulin resistance on the severity and development of hirsutism. Cases receiving Cyproterone Acetate+Ethinyl Estradiol+metformin had significant decrease in FGS scores, which is an indicator of clinical hyperandrogenemia.

Conclusion

An increase in oxidant status was found in women with PCOS according to control groups. There was no difference in terms of oxidative stress indicators between insulin resistant and non-resistant groups but those two groups were analyzed again after four months and oxidative stress indicators decrease with the treatment.

Disclosures

Ethics Committee Approval: The study was approved by the Local Ethics Committee.

Peer-review: Externally peer-reviewed.

Conflict of Interest: None declared.

Authorship Contributions: Concept – B.Y.T.; Design – B.Y.T.; Supervision – M.S.F.; Materials – Ş.T.; Data collection &/or processing – Ö.Ö.; Analysis and/or interpretation – Y.E.; Literature search – G.F.Y.; Writing – B.Y.T.; Critical review – M.S.F.

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