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Examination of Oxidative Stress Level in Adolescents with Polycystic Ovary Syndrome by Biochemical Parameters

Polikistik Over Sendromlu Adolesanlarda Oksidatif Stres Düzeyinin Biyokimyasal Parametrelerle İncelenmesi

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ABSTRACT

Polycystic ovary syndrome (PCOS) is one of the common endocrine disorders among women. Its prevalence increases up to 15% based on the Rotterdam criteria. It has been extensively studied for over 10 years that, among many factors, oxidative stress (OS) is a potential factor in the etiology of PCOS. This study aims to investigate the role of OS in the pathogenesis of PCOS. An examination was made on 30 patients with PCOS and 30 healthy adolescent and young adult women aged between 15-22 years. The diagnosis of PCOS was established considering the recommendations of the last Amsterdam ESHRE/ASRM conference. Basal hormone levels in the early follicular phase, fasting glucose and insulin values, serum lipid profiles, serum albumin, ischemia-modified albumin (IMA), total thiol, native thiol, and disulfide levels were recorded as the patients' main parameters. Albumin, IMA, thiol, and disulfide levels were compared between the PCOS and control groups. The albumin level was statistically significantly higher in the PCOS group than in the control group. A negative correlation was detected between serum CRP levels and serum albumin, native thiol, and total thiol levels. Total and native thiol values were statistically significantly lower in the BMI overweight group. In conclusion, in the present study in which OS markers in 30 PCOS patients and 30 healthy adolescent and young adult women were examined, the serum albumin level was statistically significantly higher in the PCOS group compared to the control group, and IMA, total and native thiol and disulfide levels did not differ between the groups. For a clear understanding of the place of OS in the diagnosis of PCOS, it should be supported by studies involving larger patient groups and adult women of reproductive age.

Keywords: Oxidative Stress, Polycystic Ovary Syndrome, Adolescent

ÖZ

Polikistik over sendromu (PCOS), kadınlar arasında görülen yaygın endokrin bozukluklardan biridir. Prevalansı Rotterdam kriterleri baz alındığında %15'e kadar çıkmaktadır. Birçok etken arasında oksidatif stresin (OS), PCOS etiyolojisinde yer alan potansiyel bir faktör olduğu 10 yılı aşkın bir süredir yoğun bir şekilde çalışılmaktadır. Bu çalışmadaki amaç, PCOS patogenezinde OS'nin rolünün daha iyi anlaşılmasını sağlamaktır. Çalışmada yaşları 15-22 arasında değişen 30 PCOS'lu hasta ve 30 sağlıklı adolesan ve genç erişkin kadınlar yer almıştır. PCOS tanısı son Amsterdam ESHRE/ASRM konferansı önerileri dikkate alınarak koyuldu. Erken folliküler fazdaki bazal hormon düzeyleri, açlık glukoz ve insülin değerleri, serum lipid profilleri, serum albümin, iskemi modifiye albümin (İMA), total tiyol, native tiyol, disülfid düzeyleri hastaların ana parametreleri olarak kaydedildi. PCOS ve kontrol grubu arasında albümin, İMA, tiyol, disülfid düzeyleri karşılaştırılmıştır. Albümin seviyesi PCOS grubunda kontrol grubuna göre istatistiksel olarak anlamlı yüksek bulunmuştur. Serum CRP düzeyleri ile serum albümin, native tiyol, total tiyol düzeyleri arasında negatif korelasyon saptanmıştır. Total ve native tiyol değerleri VKİ aşırı kilolu olan grupta istatistiksel olarak anlamlı derecede düşük çıkmıştır. Sonuç olarak 30 PCOS, 30 sağlıklı adolesan ve genç erişkin dönemdeki kadınlardaki OS belirteçlerinin incelendiği çalışmada, serum albümin düzeyi PCOS grubunda kontrol grubuna göre istatistiksel olarak anlamlı yüksek bulundu. İMA, total ve native tiyol, disülfid düzeyleri gruplar arasında farklılık tespit edilmedi. OS'in PCOS tanısındaki yerinin net olarak anlaşılması için daha geniş hasta gruplarının ve reprodüktif dönemdeki erişkin kadınların dahil edildiği çalışmalarla desteklenmesi gerekmektedir.

Anahtar Kelimeler: Oksidatif Stres, Polikistik Over Sendromu, Adolesan

Ethical approval was obtained (Date: 16.01.2019, Decision No:12/2019) from Dr. Zekai Tahir Burak Education and Research Hospital.

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INTRODUCTION

Polycystic ovary syndrome (PCOS) is one of the most seen endocrine disorders among women. Its prevalence increases up to 15% based on the Rotterdam criteria.[1] It first emerges at early reproductive ages and usually involves symptoms and signs such as oligo-anovulation, hyperandrogenism, and the presence of polycystic ovaries.[2] However, in a recently published review, pelvic ultrasound is not recommended for diagnosis of PCOS within 8 years post menarche.[3] Impaired insulin secretion and insulin resistance (IR) (50-70%) were detected in a significant number of women with PCOS, and it is believed that increased IR is one of the leading factors that cause some metabolic disorders such as hypertension (HT), dyslipidemia, metabolic syndrome, impaired glucose tolerance, and type 2 diabetes mellitus (DM). In recent years, there has been a prevailing opinion that the diagnosis and treatment of PCOS disease should be started in adolescence. Therefore, adolescent girls with progressive hirsutism, treatment-resistant acne, menstrual irregularity, and obesity should be evaluated more carefully in terms of PCOS. Although the pathophysiology of PCOS has been partially elucidated despite many studies, it is observed to occur as a combination of genetic predisposition and environmental factors. Apart from environmental factors, many candidate genes are involved in the etiology of PCOS.[4] Studies have revealed that PCOS is a disorder of ovarian steroidogenesis. It is thought that chronic inflammation, hyperandrogenemia, insulin resistance, hypoxia, and OS are some of the factors involved in the pathogenesis of PCOS.[5-7]

It has been extensively studied for over 10 years that, among many factors, OS is a potential factor in the etiology of PCOS. OS is defined as an imbalance between free radicals that are formed as a result of pathologically or physiological metabolism in living organisms and the antioxidant system, which has a counteracting role against free radicals.[8] It is thought that OS leads to diseases by causing toxic effects on carbohydrate, protein, lipid, and DNA metabolism.[9] A study determined that PCOS was associated with OS, free oxygen radicals (superoxide, hydrogen peroxide, hydroxyl radical) increased, and antioxidant levels and antioxidant enzyme activities decreased in PCOS.[10]

In the presence of OS, human serum albumin (HSA) modifies and turns into IMA form with reduced affinity for metals such as copper, nickel, and cobalt.[11] IMA is a metabolic variant resulting from ischemic conditions in serum albumin. Increased free levels of IMA are being studied as a new marker for OS, endothelial dysfunction, and hypoxia in some endocrine disorders.[12, 13] Moreover, there are studies in the literature with different views about free IMA levels in PCOS. While some studies found high IMA levels in PCOS, other studies, on the contrary, did not show a difference in free IMA levels in patients with PCOS when compared with control groups. If it is accepted that increased IMA levels indicate an elevated OS status in PCOS, clinicians may consider antioxidant supplementation as an alternative approach to prevent OS and related potential complications in PCOS patients in addition to the existing clinical management.[14] Dynamic thiol/disulfide homeostasis has also recently been identified as a marker of OS and has been demonstrated that support antioxidant defense, apoptosis, and detoxification.[15] Thiol is an organic component containing an –SH group and has an important role in the antioxidant system. The first target of reactive oxygen radicals (ROS) is sulfur-containing amino acids' –SH groups, and the first action of ROS is converting –SH groups into reversible disulfide bonds by oxidation. Disulfide bonds can be reduced to thiol groups again, and thiol/disulfide homeostasis is ensured in this way.[16]

This case-control study was designed to compare serum-free IMA and thiol-disulfide levels in adolescent and young adult girls with PCOS and healthy adolescent and young adult girls. This study will provide us with a better understanding of the role of OS in the pathogenesis of PCOS.

MATERIALS AND METHODS

Thirty PCOS patients and 30 healthy adolescent and young adult women, who were aged between 15-22 years and applied to a tertiary health center, were included in the study between April 2019 and August 2019.

Ethical Approval

Ethical approval was obtained (Date: 16.01.2019, Decision No:12/2019) from Dr. Zekai Tahir Burak Education and Research Hospital, and the study was conducted in accordance with the Helsinki Declaration.

Patient Selection and Study Protocol

The diagnosis of PCOS was established considering the recommendations of the last Amsterdam ESHRE/ASRM conference. Those who met all of the Rotterdam criteria were included in the PCOS group. Pelvic ultrasonography was performed on each patient, and it was determined whether the ovaries appeared polycystic. Those with menstruation over 45 days were considered oligomenorrhoeic, and those who did not menstruate in 3 consecutive cycles were considered amenorrheic. Patients with a modified Ferriman-Gallwey score of 8 and above were diagnosed with hirsutism and accepted to have clinical hyperandrogenism. The free testosterone level above 3.18 pg/ml and/or DHEA-S level above 358 ug/ml was considered as biochemical hyperandrogenemia. The patients included in the study did not have any chronic or acute systemic disease, endocrinological disorder, did not use an oral contraceptive or any other drug. Patients' information and examination results were recorded by preparing standard questions. The purpose and content of the study were explained to the patient and control groups, and written consent documents were obtained.

All patients underwent general physical and pelvic examination, their detailed anamnesis was taken, and sociodemographic characteristics were recorded. Patients' age, body mass index (BMI), waist circumference, hip circumference measurements, waist/hip ratios, menarche age, menstrual patterns, hirsutism grades, basal hormone levels in the early follicular phase, fasting glucose and insulin values, serum lipid profiles, serum albumin, IMA, total thiol, native thiol, and disulfide levels were recorded as the patients' main parameters. Basal hormone levels were measured on the 2nd or 3rd day of menstruation. Fasting blood glucose and insulin levels were evaluated from morning blood after 8 hours of fasting.

A UniCel DxI 800 Immunoassay System (Beckman Coulter, Fullerton, CA, USA) was used to detect serum levels of basal hormones and insulin. Insulin resistance was calculated using the hemostatic model formula (HOMA) (fasting serum insulin (mU/mL) x fasting plasma glucose (mmol/L)/22.5). This value above 2.5 was defined as insulin resistance. Serum DHEA-S, 17-OH progesterone, and free testosterone levels were measured by radioimmunoassay. Serum lipid and glucose levels were analyzed by the AU680 Chemistry System (Beckman Coulter, Fullerton, CA, USA).

Thiol and disulfide levels were analyzed by a newly developed method by Erel O.[15] In summary, reducible disulfide bonds were reduced to free functional thiol groups. Half of the difference between total and native thiol provides the dynamic disulfide amount. After the amount of native thiol and disulfide was determined, the native thiol/disulfide ratio was measured. Measurement analyses were performed blindly to patients' clinical information and course, and results were not available to the treating clinician, study group, or researchers during the study period.

Statistical Evaluation

The Statistical Package for the Social Sciences (SPSS) 22 program was used in the data analysis. The conformity of the data to the normal distribution was examined by considering the Kolmogorov-Smirnov test and the Shapiro-Wilk test. Parametric

methods were used in the analysis of normally distributed variables, otherwise, non-parametric methods were used. The independent samples t-test and the Mann-Whitney U test were used in the comparison of two independent groups. The comparison of categorical data was tested by Pearson's chi-square and Fisher's exact tests. Spearman's and Pearson's correlation tests were used to examining the correlations of the variables with each other. While normally distributed nominal data are shown in the tables as mean ± standard deviation, non-normally distributed data are expressed as median ± (minimum-maximum). Categorical data are presented as n (number) and percentage (%). The data were examined at a 95% confidence level, and a p-value below 0.05 was considered significant.

RESULTS AND DISCUSSION

The study was carried out between April 2019 and August 2019 on a total of 60 cases aged between 15-22 years. Albumin, IMA, thiol, and disulfide levels were compared between the PCOS and control groups. The albumin level was found to be statistically significantly higher in the PCOS group compared to the control group (P: 0.025). Total and native thiol levels were higher and IMA levels were lower in the PCOS group, and this difference was not statistically significant (Table 1).

Table 1. Intergroup Comparison of Oxidative Stress Markers

	PCOS	Control	P value
Albumin	4.32± 0.09	4.26 ± 0.1	0.025
Albumin adjusted IMA	0.94± 0.23	0.96± 0.21	0.756
IMA	0.94± 0.24	0.97 ± 0.2	0.624
Native Thiol	423.08 ± 29.86	407.22± 35.02	0.064
Total thiol	465.01 ± 29.52	451.2± 36.74	0.114
Disulfide	20.97± 4.88	21.99± 3.44	0.352

The correlation between oxidative stress markers and inflammatory markers was compared. A negative correlation was detected between serum CRP levels and serum albumin, native thiol, and total thiol levels. The p-value for albumin was 0.021, the p-value for native thiol was 0.001, and the p-value for total thiol was 0.003 (Table 2).

Table 2. Correlation between Oxidative Stress Markers and Inflammatory Markers

	NLR	CRP	HOMA-IR
Albumin	R: 0.038	**R:-0.420 ***	R:-0.263
Albumin adjusted IMA	R:0.110	R:0.036	R:-0.037
IMA	R:0.107	R:0.073	R:-0.016
Native Thiol	R:0.231	**R:-0.587 ****	R:-0.317
Total Thiol	R:0.174	**R:-0.523 ***	R:-0.256
Disulfide	R:-0.180	R:0.214	R:0.195

**p<0.05 ** p<0.001*

NLR: Neutrophil-lymphocyte ratio; CRP: C-reactive protein HOMA-IR: Homeostatic model assessment for insulin resistance

Patients in the PCOS group were classified as normal (<25) and overweight (>=25) according to BMI. OS parameters were compared between these two groups. Native thiol was detected as (429.9 ± 27.7) in the BMI normal group and (407.17 ± 30.09) in the BMI overweight group, the total thiol was found as (473 ± 26.73) in the BMI normal group and (446.39 ± 28.54) in the BMI overweight group, and total and native thiol values were found to be statistically significantly lower in the BMI overweight group (Table 3).

Table 3. Comparison of Oxidative Stress Markers According to BMI

	BMI Normal N:21	BMI owerweight N:9	P value
Albumin	4.33± 0.08	4.29 ± 0.13	0.504
Albumin adjusted IMA	0.91± 0.24	1.03 ± 0.2	0.104
IMA	0.9 ± 0.24	1.04 ± 0.21	0.094
Native thiol	429.9± 27.7	407.17± 30.09	**0.050**
Total thiol	473± 26.73	446.39± 28.54	**0.032**
Disulfide	21.55± 4.9	19.61 ± 4.85	0.326

Increased OS and secondary chronic inflammation in PCOS patients are among the factors that have been frequently emphasized in the pathophysiology of PCOS in recent years. A wide range of endocrine and metabolic disorders such as obesity, hyperinsulinemia, and dyslipidemia may be responsible for PCOS-related oxidative stress. A meta-analysis published in 2013 found increased serum levels of free reactive radicals (such as superoxide, hydrogen peroxide), homocysteine, malondialdehyde, asymmetric dimethylarginine, and superoxide dismutase in PCOS patients. This meta-analysis stated that oxidative stress played a role in the pathophysiology of PCOS.[17] In a recent study that evaluates serum copper and magnesium and total antioxidant capacity (TAC) levels in PCOS patients, TAC was significantly lower in PCOS patients than those in the controls. Copper and magnesium seem to contribute to oxidative stress and insulin resistance in PCOS patients.[18] ROS affect atherogenesis from many aspects, including endothelial activation, matrix remodeling, and oxidized LDL formation. Physiologically, ROS have an intracellular messenger function, and protein thiols are among their main targets. ROS cannot be measured directly in serum because they are intracellular molecules. MDA is activated and cell membrane lipids are harmed by increased ROS by causing lipid peroxidation. Additionally, catalase and SOD which are antioxidant enzymes, eliminate these harmful effects of ROS. Therefore, these enzyme levels indicate increased ROS. It was found that in PCOS MDA and SOD, catalase levels increase. Moreover, they found a negative correlation between low thiol and serum MDA and thiol in PCOS.[19]

It was emphasized that abdominal obesity affects OS in PCOS and non-PCOS groups in literature.[20] OS is emphasized more in overweight patients with PCOS, but this alone is not a sufficient factor. Suresh et al. revealed that OS, IR, and testosterone levels are correlated in women with functional hyperandrogenism.[21] Özler et al. stated that hyperandrogenism played a significant role in determining cardiovascular disease (CVD) risk in overweight PCOS patients. In their study, Özler et al. revealed significantly lower total thiol and disulfide levels in overweight adolescent PCOS patients compared to normal weight PCOS patients and the control group. This study suggested that low serum total thiol levels may contribute to future CVD in obese adolescent PCOS patients.[22] In their study, Turan et al. measured higher MDA and lower thiol levels in IR and infertile PCOS patients compared to the control group. They concluded that the oxidative-antioxidative system imbalance was more severe in the infertile and IR group.[19] In the study in which Başkol G. et al. investigated OS in PCOS patients, xanthine oxidase levels were higher in the PCOS group, but they could not detect a significant difference in serum thiol, glutathione peroxidase, and NO levels.[23]

BMI>25 was found to be associated with high IMA levels in PCOS.[14] However, Beyazıt et al. did not find a significant correlation between IMA level and BMI in their study. Nevertheless, they detected a positive correlation between IMA level and free and total testosterone levels.[24] The detection of oxidative stress in adolescents and young girls with PCOS without signs of chronic inflammation suggests that it may be

a marker in early pathophysiological development before patients gain weight and before chronic inflammation begins. Samy et al. showed that BMI was an important determinant of CRP levels and chronic inflammation.[25] Higher IMA levels in patients with metabolic syndrome, supporting the correlation between increased IMA levels and microvascular dysfunction. While some studies detected high IMA levels in PCOS other studies found no significant difference.[24,26] Considering the relationship of increased OS in the development of potential complications in PCOS, it is reasonable to measure serum IMA level as a simple marker of increased OS in PCOS. In our opinion, it is incorrect to evaluate the correlation of serum IMA level with diagnostic features, at least in adolescent PCOS cases. The different results in these studies may originate from differences in the data in the population (BMI, IR). It can be more clinically significant to measure the IMA/albumin ratio. Although serum IMA levels were higher in the PCOS group in the study conducted by Çakır et al., the results were not statistically significant. TAS and TOS levels were also found to be similar. In this study, it was claimed that OS markers were not valuable markers in the early and weak PCOS group.[27] Beyazit et al. determined higher serum IMA levels in infertile PCOS patients compared to the group with unexplained infertility and the normal healthy group. A positive correlation was revealed between only serum-free testosterone levels and IMA levels.[24]

In the present study, we found serum total thiol, native thiol, and disulfide levels to be similar in the group with and without PCOS ($p>0.05$). In our study, the serum albumin level was statistically significantly higher in the PCOS group ($p= 0.025$). We detected no difference between the groups in albumin-adjusted IMA and IMA levels ($p>0.05$). However, upon comparing these OS markers between the group with normal BMI and the overweight group, we found that serum total thiol and native thiol levels were significantly lower in the group with high BMI. Although the albumin level was lower in the group with high BMI compared to the control group and the albumin adjusted IMA and IMA levels were higher, the difference was not statistically significant.

When the anthropometric characteristics of the PCOS and control groups in our study were compared, 30 of the 60 cases in total were PCOS patients, and 30 were healthy individuals. A study carried out in Turkey stated that high BMI might be the most important factor in the development of metabolic disorders in adolescents with PCOS.[28] According to a recent review diet is an effective, acceptable, and safe intervention for relieving IR, and professional dietary advice should be offered to all PCOS patients.[29] In our study, the age of menarche, body mass index, waist circumference, hip circumference, and waist/hip ratio of PCOS patients were found to be statistically significantly higher compared to the control group. Of the adolescents with PCOS included in our study, 30% were overweight (BMI>25). These findings were also consistent with the literature.

CONCLUSION AND RECOMMENDATIONS

The diagnosis of PCOS in adolescence is different from the criteria used for women of reproductive age. The group with risk factors such as irregular menstruation, hirsutism, and obesity should be analyzed well. However, incorrect and unnecessary diagnosis should be avoided. The lack of studies on adolescents, the absence of specific criteria to define PCOS in early adolescence, not knowing the normal range of biochemical markers in adolescence, and the relatively narrow sample size of our study constitutes the limitations of the present study and are likely to have affected our study results.

As a result, in this study in which we examined OS markers in 30 PCOS and 30 healthy adolescent and young adult women, the serum albumin level was statistically

significantly higher in the PCOS group than the control group, and IMA, total and native thiol and disulfide levels did not differ between the groups. To understand the place of OS in the diagnosis of PCOS clearly, it should be supported by studies involving larger patient groups and adult women of reproductive age.

Conflict of Interest

Authors declare that there is no conflict of interests regarding the publication of this paper.

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