




# Evaluation of cardiovascular risk marker in patients with polycystic ovary syndrome: Homocysteine

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## ABSTRACT

**Objective:** The aim of the study was to investigate homocysteine (Hcy) levels in polycystic ovary syndrome (PCOS) patients without insulin resistance (IR) and metabolic syndrome (MS).

**Material and Methods:** This retrospective study involved 64 patients aged between 18-35 years who applied to the tertiary clinic between March 2022 and March 2023. A total of 32 PCOS patients without IR and MS and 32 healthy women were enrolled in the study. All participants underwent history taking, clinical physical examination, gynecological ultrasonographic evaluation, and laboratory tests. Laboratory screening tests, including follicle-stimulating hormone (FSH), luteinizing hormone (LH), total testosterone (T), free T, sex hormone-binding globulin (SHBG), dehydroepiandrosterone sulfate (DHEAS), and low-density lipoprotein (LDL), were performed. Normal insulin sensitivity was defined based on fasting serum glucose, fasting insulin level, serum insulin response to an oral glucose tolerance test, and the homeostatic model of insulin resistance.

**Results:** There were no differences in terms of age, systolic and diastolic blood pressure, FSH, LH, total T, free T, SHBG, DHEAS, and LDL between the groups. PCOS patients had increased Hcy, waist-to-hip ratio (WHR), and body mass index (BMI) compared to the control group. Hcy was positively correlated with WHR and BMI. A strong correlation was found between Hcy and PCOS.

**Conclusion:** Elevated Hcy is associated with cardiovascular risk factors in PCOS patients without IR and MS.

**Keywords:** Cardiovascular risk factor, homocysteine, polycystic ovary syndrome.

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## INTRODUCTION

Polycystic ovary syndrome (PCOS) affects 5-10% of women of reproductive age. PCOS is an endocrine and metabolic dysfunction. It is a genetic disease with multifactorial etiology and a strong link with environmental factors. It is frequently associated with obesity and insulin resistance (IR).<sup>[1]</sup> Menstrual dysfunction, high androgen level, infertility, and IR are among the features of PCOS.<sup>[2]</sup>

PCOS is associated with cardiovascular risk.<sup>[3]</sup> Cardiovascular diseases (CVD) are the most important cause of morbidity and mortality. Atherosclerosis is the main cause of CVD. Inflammation plays an important role in atherosclerosis, and this has been widely accepted. Atherosclerosis is an inflammatory disease that produces an inflammatory response in the intima layer of the arterial wall.<sup>[4]</sup> CVD serum biomarkers such as high sensitivity C-reactive protein (hsCRP), homocysteine (Hcy), and adiponectin are abnormal in patients with PCOS.<sup>[5,6]</sup> In addition, it has been previously shown that there is a correlation between serum homocysteine level and blood pressure and body mass index (BMI).<sup>[7,8]</sup>

IR is a common pathogenetic factor in PCOS. 30–47% of patients with PCOS have IR accompanied by one or more of the metabolic syndrome (MS) criteria (obesity, hypertension, impaired glucose tolerance, low high-density lipoprotein cholesterol, hypertriglyceridemia).<sup>[9,10]</sup> The Framingham Offspring Study showed that hyperhomocysteinemia is associated with hyperinsulinemia and may be responsible for increased CVD risk.<sup>[11]</sup>

Hcy in normoinsulinemic PCOS patients without MS has not been fully evaluated. In this study, our aims were to evaluate the level of Hcy in PCOS patients without IR and MS.

## MATERIAL AND METHODS

Patients who applied to Kartal Lütfi Kırdar City Hospital's Gynecology and Obstetrics outpatient clinic or were hospitalized between March 2022 and March 2023 were included in the study. It is a retrospective, single-center study. The 64 women included in the study were between the ages of 18-35 and had a BMI <30 kg/m<sup>2</sup>. Of these women, 32 were PCOS without IR and MS and 32 were normoandrogenic ovulatory women, who attended the general gynecology clinic for routine gynecological examination. The study was approved by Bakırçay University Ethics Committee (dated April 5, 2023; decision number: 974). The study was conducted in accordance with the principles of the Declaration of Helsinki. Informed consent was obtained from all participants before the start of the study. The primary exclusion criteria were the presence of IR, MS, hyperprolactinemia, hypothalamic amenorrhea, premature ovarian failure, thyroid hormone dysfunction, congenital or acquired adrenal dysfunction, diabetes mellitus, hypertension, pregnancy, smoking and alcohol consumption, cocaine or opiates consumption, hyperlipidemia, history of major depression, age above 35 years, the presence of neoplastic disease, infectious diseases, autoimmune diseases, liver or kidney disease, using any psychoactive medication, diagnosed cardiovascular disease, and those with a family history of early coronary artery disease. Participants did not use hormonal drugs during the study period.

All participants underwent gynecological ultrasound evaluation. Mid-luteal phase progesterone measurements of less than 3 ng/

mL in regular menstrual cycles are indicative of oligo/anovulation. Clinical hirsutism was defined as a score of 8 and above according to the modified Ferriman-Gallwey scoring system. Biochemical hirsutism was defined as total testosterone (T) level >80 ng/dL or dehydroepiandrosterone sulfate (DHEAS) level >350 ng/dL.<sup>[12]</sup> The diagnosis of PCOS was made according to the 2003 Rotterdam Consensus Criteria.<sup>[13]</sup> BMI was used as an assessment measure of obesity. BMI (kg/m<sup>2</sup>) is calculated using weight and height (weight divided by height squared). Abdominal obesity was calculated as waist-hip ratio (WHR).<sup>[14]</sup> We included PCOS patients who were not diagnosed with hirsutism.

Insulin sensitivity was defined according to serum fasting plasma glucose, serum fasting insulin level, serum insulin response to oral glucose tolerance test (OGTT), and homeostatic model of insulin resistance (HOMA-IR). Fasting insulin and fasting plasma glucose levels were used for the calculation of HOMA-IR (insulin×glycemia in μmol/L/22.5). Patients with fasting insulin >25 μIU/mL, peak serum insulin >100 μIU/mL during OGTT, and HOMA-IR>4 were classified as IR. The 2-hour OGTT was <140 mg/dL in all participants.<sup>[15–18]</sup>

All participants with normal serum follicle-stimulating hormone (FSH), luteinizing hormone (LH), prolactin, thyroid function tests, spontaneous menstruation, or positive bleeding response to progesterone withdrawal were included in the study. All participants underwent clinical and ultrasonographic evaluation. Hormone and biochemical tests were performed by taking blood samples. Endocrine screening and laboratory parameters included serum assays for glucose, insulin, 75 g OGTT, prolactin, FSH, LH, thyroid function tests, estradiol, progesterone, free T, total T, sex hormone-binding globulin (SHBG), DHEAS, and low-density lipoprotein (LDL). All studies administered to the participants were performed between days 3 and 5 of the menstrual cycle. Fasting venous blood samples were taken after 12 hours of night fasting.

## Statistical Analysis

Statistical analyses were performed with IBM SPSS for Windows Version 25.0 software. Baseline characteristics of both groups were presented as mean±SD. Laboratory and anthropometric parameters of patients were compared by Student's t-test. Independent relationships between PCOS and Hcy, BMI, WHR were assessed by multiple linear regression analysis. Correlations between Hcy and BMI, WHR were assessed by Pearson correlation analysis, and the correlation between Hcy and PCOS status was assessed by Spearman's rank test. A statistical significance p-value of 0.05 was accepted.

## RESULTS

The clinical characteristics and laboratory parameters are shown in Table 1. There were no differences in terms of age, systolic and diastolic blood pressure, FSH, LH, free T, total T, DHEAS, LDL between the groups. SHBG levels were lower in PCOS patients compared to the control group, but this was not statistically significant. PCOS patients had significantly higher levels of BMI, WHR, Hcy than the control group (p<0.05). Hcy was positively correlated with BMI, WHR (p<0.05). There was also a strong association between Hcy and PCOS status in the study (p<0.05).

**Table 1: Clinical and laboratory parameters of women with normoinsulinemic PCOS and control group**

Parameters	PCOS (n=32)	Controls (n=32)
	Mean±SD	Mean±SD
Age (years)	28.1±4.0	27.5±4.0
Blood pressure (mmHg)	110.2±11.1 / 73.8±8.7	110.3±11.0 / 73.4±8.9
BMI (kg/m <sup>2</sup> )	28.9±1.8*	27.1±1.9*
WHR	0.85±0.0*	0.71±0.0*
FSH (mIU/mL)	4.7±2.1	4.8±2.1
LH (mIU/mL)	7.1±3.2	7.0±3.1
Free testosterone (ng/dL)	8.6±2.1	8.3±2.2
Testosterone (ng/dL)	60.0±10.7	57.6±10.5
SHBG (nmol/L)	59.5±11.9	61.2±12.1
DHEAS (µg/dL)	163.5±44.1	160.1±45.3
LDL (mg/dL)	107.3±15.5	104.5±16.1
Hcy (µmol/L)	10.51±2.9*	7.91±2.5*

\*: P<0.05; PCOS: Polycystic ovary syndrome; SD: Standard deviation; BMI: Body mass index; WHR: Waist to hip ratio; FSH: Follicle stimulating hormone; LH: Luteinizing hormone; SHBG: Sex hormone binding globulin; DHEAS: Dehydroepiandrosterone sulfate; LDL: Low density lipoprotein; Hcy: Homocysteine.

## DISCUSSION

PCOS has been shown to be correlated with cardiovascular disorders in the long term. There are data showing an increased incidence of CVD in patients with PCOS. An increased risk of atherosclerotic heart disease has been reported multiple times in patients with PCOS compared to healthy controls. Previous studies have investigated the relationship between PCOS and coronary artery disease, and most studies have found that PCOS patients have more extensive coronary artery disease than controls. It has also been reported that PCOS patients have a higher risk of myocardial infarction than controls.<sup>[19–22]</sup>

PCOS is associated with various factors such as insulin resistance, obesity, oxidative stress, dyslipidemia, increased serum hsCRP, and Hcy level. Hcy is a risk factor for CVD. There is evidence showing impaired Hcy metabolism in PCOS patients. Hcy is known to have atherogenic and prothrombotic properties; therefore, it contributes to cardiovascular morbidity and mortality.<sup>[23–25]</sup> The negative effects of Hcy on the cardiovascular system occur in various ways. The Hcy metabolite can combine with LDL cholesterol, thus forming atheroma plaque. Free radicals formed during the oxidation of Hcy can damage endothelial cells. Disruption of the nitric oxide mechanism as a result of the endothelium being exposed to Hcy for a long time and the proaggregation effect of Hcy are its negative effects. Hcy also causes an increase in smooth muscle cell proliferation and collagen production, activation of factor V and VIIa, inhibition of protein C and heparin sulfate, increase in blood viscosity, decrease in endothe-

lial antithrombotic activation, increase in fibrinopeptide-A, increase in prothrombin fragments 1/2, and disruption of endothelial tissue.<sup>[26]</sup>

The increase in obesity prevalence (increased BMI and WHR) may play an important role in the development of PCOS. Obesity may worsen the clinical, hormonal, and metabolic features of the disease in PCOS patients.<sup>[27]</sup> There is a correlation between Hcy and BMI.<sup>[28]</sup> Sagar Salehpour et al.<sup>[26]</sup> found increased Hcy levels in PCOS patients when compared with BMI-matched subjects. In our study, Hcy levels were compared among PCOS cases and controls; and serum Hcy levels showed a significant increase in PCOS patients. Elevated Hcy levels in PCOS patients correlated with an increase in BMI and WHR.

It has been previously shown that there is a strong association between hyperinsulinemia and an increased risk of CVD in patients with PCOS.<sup>[29]</sup> IR is associated with atherosclerotic processes.<sup>[30]</sup> The MS in women with PCOS is also associated with an increased risk of atherosclerosis, and this results in an increased risk of CVD.<sup>[31]</sup> The relationship between Hcy and IR has been shown previously.<sup>[32]</sup> Cardiovascular risk factors in patients with PCOS without IR and MS have not been adequately studied before. Therefore, we conducted our study on this group. Thus, we excluded the possible effects of these factors on cardiovascular risk.

In this study, we showed that Hcy was higher in PCOS without IR and MS compared to normoandrogenic ovulatory women. In our study, we demonstrated that BMI, WHR, and PCOS were closely associated with Hcy.

There were some limitations in our study. Initially, it was a single-center study. In addition, the study size was small, and future studies with larger groups are needed. In our study, we found that Hcy was higher in PCOS without IR and MS compared to controls.

## CONCLUSIONS

Our study demonstrated that Hcy levels are elevated in PCOS patients without IR and MS. This suggests that PCOS, even in the absence of IR and MS, may be associated with an increased cardiovascular risk, indicating a need for more intensive screening and treatment in these patients.

## Statement

**Ethics Committee Approval:** The Bakırçay University Clinical Research Ethics Committee granted approval for this study (date: 05.04.2023, number: 974).

**Author Contributions:** Concept – CT, TT, CÖK; Design – CT, TT, CÖK; Supervision – CT, TT, CÖK; Resource – CT, TT, CÖK; Materials – CT, TT, CÖK; Data Collection and/or Processing – CT, TT, CÖK; Analysis and/or Interpretation – CT, TT, CÖK; Literature Search – CT, TT, CÖK; Writing – CT, TT, CÖK; Critical Reviews – CT, TT, CÖK.

**Conflict of Interest:** The authors have no conflict of interest to declare.

**Informed Consent:** Written informed consent was obtained from patients who participated in this study.

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