

Polycystic ovary syndrome is associated with elevated plasma soluble CD40 ligand, a marker of coronary artery disease

Mesut Oktem, M.D.,^a Emel Ebru Ozcimen, M.D.,^a Ayla Uckuyu, M.D.,^a Ibrahim Esinler, M.D.,^b Baris Pamuk, M.D.,^c Nilufer Bayraktar, M.D.,^d Sevsen Kulaksizoglu, M.D.,^d and Hulusi B. Zeyneloglu, M.D.^a

^a Department of Obstetrics and Gynecology, ^c Department of Endocrinology, and the ^d Biochemistry Laboratory, Baskent University School of Medicine, Ankara, Turkey; and the ^b Department of Obstetrics and Gynecology, Hacettepe University School of Medicine, Ankara, Turkey

Objective: To determine the level of plasma soluble CD40 ligand (sCD40L) in patients with polycystic ovary syndrome (PCOS).

Design: Prospective study.

Setting: Baskent University School of Medicine in Turkey.

Patient(s): Thirty-one patients with PCOS and 31 non-PCOS (control) patients.

Intervention(s): Determination of plasma sCD40L and homocysteine levels.

Main Outcome Measure(s): Plasma sCD40L, fasting glucose, fasting insulin, homeostatic model assessment insulin resistance index (HOMA-IR), LH, FSH, E₂, total T, DHEAS, total cholesterol, high- and low-density lipoprotein cholesterol, triglyceride, homocysteine, and high-sensitivity C-reactive protein (hsCRP).

Result(s): The mean serum fasting insulin and HOMA-IR levels were significantly higher in the PCOS group. The mean serum homocysteine level was significantly higher in the PCOS group. Despite a trend for higher high-sensitivity C-reactive protein levels in the PCOS group, the difference did not reach statistical significance. The mean plasma sCD40L level in the PCOS group was significantly higher than that in the control group (5.14 ± 3.65 ng/mL vs. 3.45 ± 2.64 ng/mL, respectively).

Conclusion(s): Polycystic ovary syndrome is associated with elevated levels of sCD40L and homocysteine. (Fertil Steril® 2008; ■: ■–■. ©2008 by American Society for Reproductive Medicine.)

Key Words: Polycystic ovary syndrome, coronary artery disease, sCD40L, homocysteine, C-reactive protein

Polycystic ovary syndrome (PCOS) is the most common reproductive endocrine disorder, affecting approximately 5%–7% of reproductive-aged women (1). The diagnosis of PCOS requires the presence of at least two of the following three criteria: oligo- and/or anovulation, hyperandrogenism (clinical and/or biochemical), and ultrasonographic appearance of polycystic ovaries, after the exclusion of other etiologic factors (2).

It was previously shown that PCOS was associated with long-term health risks, including cardiovascular disease (3) and type 2 diabetes (4). The major cardiovascular risk factors

for patients with PCOS are the presence of insulin resistance (5), hyperandrogenism, and dyslipidemia (6).

To determine the risk of developing early-onset cardiovascular disease in patients with PCOS, several markers, such as left ventricular diastolic function (7), arterial stiffness (8), endothelial function (9), carotid wall thickness (10), and arterial calcification (11), have been studied. Unfortunately, at present there is no well-established marker to assess the risk of cardiovascular disease developing in patients with PCOS.

CD40 ligand (CD40L) is a member of the tumor necrosis family that, upon engagement with its receptor CD40, promotes processes that likely contribute to the initiation and progression of atherosclerosis, which is the main cause of cardiovascular disease. CD40L plays a role in the formation of atherosclerosis through endothelial cell activation, release of inflammatory cytokines and matrix-degrading enzymes, and tissue factor production (12). Several cell lines express CD40L, including lymphocytes and cells of the vascular system, such as endothelial cells, smooth muscle cells, monocytes, and platelets (12, 13). CD40L is cleaved in the serum

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Reprint requests: Mesut Oktem, M.D., Onur sokak, 38/09, Maltepe, Ankara 06570, Turkey (FAX: 90-312-2323912; E-mail: mesutoktem@hotmail.com).

and circulates as soluble CD40L (sCD40L). It was previously shown that more than 95% of circulating sCD40L is of platelet origin (14). Previous studies have found that individuals with hypercholesterolemia (15) and diabetes (16) have elevated sCD40L levels. Moreover, it was suggested that high levels of sCD40L may identify apparently healthy women at increased risk of having early-onset cardiovascular disease (17). To our knowledge, there is no study in the literature investigating sCD40L levels in patients with PCOS.

The aim of our study was to investigate plasma sCD40L levels in patients with PCOS and to determine the relationship between sCD40L and other known cardiovascular risk factors.

MATERIALS AND METHODS

Patients

Thirty-one consecutive patients with PCOS and 31 non-PCOS control patients matched for body mass index (BMI) and age were included in the study.

Polycystic ovary syndrome was diagnosed according to the criteria of the Rotterdam European Society of Human Reproduction and Embryology–American Society for Reproductive Medicine-sponsored PCOS consensus workshop group (2). Clinical hyperandrogenism was quantified by one examiner using the Ferriman-Gallwey scoring system, in which a score >8 indicates hirsutism. All women had normal renal, hepatic, and thyroid function. Women who had endocrinopathies (including diabetes mellitus, hyperprolactinemia, Cushing's disease, and congenital adrenal hyperplasia), a systemic disease (e.g., asthma), a collagen disorder, hypercholesterolemia, sickle cell anemia, a history of neoplasm, and those using any medication (e.g., insulin-sensitizing drugs, oral contraceptives, antiandrogens, statins, aspirin, corticosteroids, and GnRH agonists and antagonists) in the preceding 6 months were excluded. Furthermore, patients with hypertension, a family history of coronary artery disease and electrocardiographic changes suggestive of coronary artery disease, a history of angina or myocardial infarction, or a history of any known vascular, infectious, or inflammatory diseases were excluded from the study. All patients were nonsmokers.

For each patient, height and weight measurements were used to calculate BMI. Waist circumference was measured with the patient in the standing position and by placing a soft tape measure midway between the lowest rib and the iliac crest. Hip circumference was measured at the level of the major trochanters. The waist/hip ratio was calculated as waist circumference divided by hip circumference.

Informed consent was obtained from each patient. The study was approved by the Ethics Committee of Baskent University.

Laboratory Tests

All regularly menstruating patients were scanned on cycle days 3–5, whereas oligo/amenorrheic patients were scanned

3–5 days after progestin-induced withdrawal bleeding. A fasting blood sample was obtained from each patient in the morning between 8:00 and 9:00 AM. Serum levels of FSH, LH, and insulin were measured by a microparticle enzyme immunoassay method in an AxSYM autoanalyzer (Abbott, Wiesbaden, Germany). Serum homocysteine level was measured by a fluorescence polarization immunoassay method, also in an AxSYM autoanalyzer. Serum E₂, total T, and DHEAS levels were measured by a solid-phase competitive chemiluminescent enzyme immunoassay in an Immulite 2000 (Siemens Medical Solutions Diagnostics, Los Angeles, CA) using BioDPC (EuroDPC, Llanberies, Gwynedd, UK) reagents. Serum free T levels were measured by an RIA method (Diagnostic Systems Laboratories, Webster, TX). Although the validity of the use of the direct RIA method in clinical research has been called into question, direct RIA is widely available and frequently used in clinical research (18). Radioimmunoassay requires smaller volumes and less sample preparation. It needs no extractions with organic solvents and no chromatography when compared with equilibrium dialysis. In addition, this method is subject to quality control. The sensitivity of the kit that we used is 0.18 pg/mL. The intra-assay precision coefficient of variation (CV) is 3.7%, and the inter-assay precision CV is 7.3% (18). Serum total cholesterol, triglyceride, and high-density lipoprotein cholesterol (HDL-C) levels were measured spectrophotometrically in an Abbott Aeroset autoanalyzer using Abbott reagents (Abbott Laboratories, Abbott Park, IL). Serum low-density lipoprotein cholesterol (LDL-C) level was calculated according to the Friedewald formula. High-sensitivity C-reactive protein (hsCRP) level was measured by Aeroset/c8000 CRP Ultra reagent (Sentinel Diagnostics, Milan, Italy). Serum glucose level was measured by the hexokinase method in an Abbott Autoanalyzer using Abbott reagents.

All participants underwent a 75-g, 2-hour oral glucose tolerance test after 3 days on a carbohydrate-rich diet. The glucose/insulin ratio was calculated through the simultaneous testing of fasting glucose and fasting insulin. Insulin resistance was calculated using the homeostatic model assessment insulin resistance index (HOMA-IR), according to the formula $(\text{HOMA-IR} = [\text{fasting insulin } (\mu\text{IU/mL}) \times \text{fasting glucose } (\text{mg/dL})] / 22.5)$.

Plasma sCD40L levels were determined using a commercially available ELISA kit (BioSource International, Nivelles, Belgium) according to the manufacturer's instructions. The detection limit was 0.062 ng/mL. The overall inter-assay and intra-assay CVs were 6.8% and 4.0%, respectively.

Statistical Analysis

According to the data of a previous study, sample size calculations, assuming 80% power to detect 25%–30% change in sCD40L levels between groups, indicated the need for 27 patients in each group ($\alpha = 0.05$) (17).

The statistical analysis was performed using the Statistical Package for the Social Sciences for Windows 11.0 (SPSS,

Chicago, IL). Continuous variables were expressed as mean \pm SD and compared using an independent *t*-test between groups. Categorical data were expressed as numbers (percentages) and compared using the χ^2 test. Correlation analysis of plasma sCD40L levels and other parameters was performed using Pearson's bivariate correlation test. Logistic regression analysis (binomial logistic, enter method) was used to determine the association between plasma sCD40L and PCOS. The covariates were set as the following parameters: age, BMI, waist circumference, waist/hip ratio, fasting glucose level, 2nd-hour glucose level, fasting insulin level, fasting glucose/fasting insulin ratio, HOMA-IR, LH level, FSH level, LH/FSH ratio, E₂, total T, free T, DHEAS, total cholesterol, HDL-C, and LDL-C levels, LDL-C/HDL-C ratio, and triglyceride, homocysteine, and hsCRP levels. A *P* value of $<.05$ was considered statistically significant.

RESULTS

The basal characteristics of the PCOS and control groups are shown in Table 1. Age, body weight, BMI, waist circumference, and waist/hip ratio were comparable between the two groups. The Ferriman-Gallwey score, the rate of patients with oligo/amenorrhea, and the rate of patients with polycystic-appearing ovaries were significantly higher in the PCOS group than in the control group ($P<.01$) (Table 1).

The hormonal and biochemical features of the PCOS and control groups are shown in Table 2. The mean serum fasting glucose levels and 2nd-hour glucose levels were comparable between the two groups. However, mean serum fasting insulin ($P<.01$) and HOMA-IR levels ($P<.05$) were significantly higher and the mean fasting glucose/fasting insulin ratio was significantly lower in the PCOS group ($P<.01$). The total and free T levels and mean LH/FSH ratio were significantly higher in the PCOS group ($P<.05$). The mean serum triglyceride level in the PCOS group was higher compared with that in the control group ($P<.01$). However, the mean serum total cholesterol, HDL-C, and LDL-C levels and LDL-C/HDL-C ratio were comparable between the two groups. The mean serum homocysteine level was signifi-

cantly higher in the PCOS group than that in the control group ($P<.05$). Despite the trend for higher hsCRP levels in the PCOS group, the difference did not reach statistical significance ($P=.06$).

The mean plasma sCD40L level in the PCOS group was significantly higher than that in the control group (5.14 ± 3.65 ng/mL vs. 3.45 ± 2.64 ng/mL, respectively; $P<.05$; Table 2).

Bivariate analysis revealed that plasma sCD40L levels were correlated only with the LDL-C/HDL-C ratio in the PCOS group ($r = 0.356$, $P<.05$; Table 3).

Binomial logistic regression analysis revealed that the plasma sCD40L level was associated with PCOS independently of the parameters mentioned in the Statistical Analysis section above (odds ratio = 1.19, 95% confidence interval = 1.002–1.415, $P<.05$).

DISCUSSION

It has been suggested that plasma levels of sCD40L protein may be a marker of atherothrombotic potential because sCD40L is released during the early stage of atherogenesis through thrombus formation (19). It has also been suggested that sCD40L protein levels are higher in patients with metabolic syndrome and cardiovascular disease (20). It is obvious that patients with PCOS have an increased risk of developing metabolic syndrome, which is known to be a risk factor for cardiovascular disease. However, how patients with PCOS might develop metabolic syndrome and/or cardiovascular disease as they age is not well understood. In other words, there are no well-established markers for this prediction. To the best of our knowledge, no study in the literature has determined sCD40L levels in patients with PCOS. We noted in our study that the patients with PCOS were characterized by significantly elevated levels of sCD40L compared with non-PCOS controls (5.14 ± 3.65 ng/mL vs. 3.45 ± 2.64 ng/mL, respectively; $P<.05$). Moreover, plasma levels of sCD40L did not correlate with other cardiovascular risk parameters in patients with PCOS (Table 3).

TABLE 1

Baseline characteristic of the PCOS and control groups.

Characteristic	PCOS (n = 31)	Control (n = 31)	<i>P</i> value
Age (y)	25.6 \pm 3.5	25.6 \pm 3.8	NS
Body weight (kg)	64.4 \pm 7.5	64.2 \pm 5.7	NS
Body mass index (kg/m ²)	29.7 \pm 2.6	29.3 \pm 2.7	NS
Waist circumference (cm)	77.6 \pm 7.0	77.1 \pm 5.1	NS
Waist/hip ratio	0.80 \pm 0.04	0.80 \pm 0.04	NS
Patients with oligo/amenorrhea (%)	31 (100)	0	$<.01$
Patients with Ferriman-Gallwey score >8 (%)	25 (80.6)	0	$<.01$
Patients with polycystic ovaries (%)	26 (83.9)	6 (19.4)	$<.01$

Note: Values are mean \pm SD unless otherwise noted.

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TABLE 2

Hormonal and biochemical features of the PCOS and control groups.

Characteristic	PCOS (n = 31)	Control (n = 31)	P value
Fasting glucose (mg/dL)	89.9 ± 7.2	90.8 ± 5.5	NS
2nd-h glucose (mg/dL)	116.3 ± 19.7	109.3 ± 20.8	NS
Fasting insulin (μIU/mL)	11.5 ± 6.7	7.1 ± 2.8	<.01
Fasting glucose/fasting insulin ratio (%)	10.7 ± 7.1	15.0 ± 6.2	<.01
HOMA-IR	2.6 ± 1.5	1.6 ± 0.6	<.05
LH (mIU/mL)	9.2 ± 4.4	7.4 ± 2.3	.06
FSH (mIU/mL)	5.0 ± 2.1	6.1 ± 3.1	NS
LH/FSH ratio	2.1 ± 1.2	1.4 ± 0.5	<.05
E ₂ (pg/mL)	52.5 ± 28.9	56.3 ± 35.3	NS
Total T (ng/dL)	0.7 ± 0.3	0.5 ± 0.2	<.05
Free T (ng/mL)	2.3 ± 1.4	1.6 ± 0.7	<.05
DHEAS (ng/mL)	212.8 ± 98.6	174.3 ± 63.9	.07
Total cholesterol (mg/dL)	156.9 ± 23.4	161.8 ± 19.3	NS
HDL-C (mg/dL)	49.8 ± 21.1	49.1 ± 8.2	NS
LDL-C (mg/dL)	96.6 ± 21.9	97.3 ± 17.9	NS
LDL-C/HDL-C ratio (%)	2.1 ± 0.7	2.0 ± 0.5	NS
Triglyceride (mg/dL)	125.5 ± 15.6	83.5 ± 25.5	<.01
Homocysteine (mg/dL)	11.3 ± 4.8	9.0 ± 3.2	<.05
hsCRP (mg/dL)	2.9 ± 2.6	1.8 ± 1.8	.06
sCD40L (ng/mL)	5.1 ± 3.6	3.4 ± 2.6	<.05

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In previous studies it was shown that, in hypercholesterolemic patients, serum levels of sCD40L were higher than in healthy control patients (21). Levels of sCD40L were also elevated in patients with familial hypercholesterolemia (22). Recently, another study demonstrated that increased serum levels of sCD40L were seen in Asian Indian patients with impaired glucose intolerance, type 2 diabetes mellitus, metabolic syndrome, and insulin resistance (23). However, we know that most patients with PCOS are diagnosed at younger ages without any associated metabolic syndrome. Therefore, it is important to clarify whether patients with PCOS only (without impaired glucose intolerance, insulin resistance, type 2 diabetes mellitus, metabolic syndrome, and hypercholesterolemia) have elevated levels of sCD40L. In our study, levels of fasting insulin ($11.5 \pm 6.7 \mu\text{IU/mL}$ vs. $7.1 \pm 2.8 \mu\text{IU/mL}$; $P < .01$), HOMA-IR (2.6 ± 1.5 vs. 1.6 ± 0.6 ; $P < .05$), and triglyceride ($125.5 \pm 15.6 \text{ mg/dL}$ vs. $83.5 \pm 25.5 \text{ mg/dL}$; $P < .01$) were higher in the PCOS group compared with those in the control group. However, the ratio of fasting glucose/fasting insulin ($10.7\% \pm 7.1\%$ vs. $15.0\% \pm 6.2\%$; $P < .01$) was lower in the PCOS group than in the control group. Logistic regression analysis of our data revealed that elevation of sCD40L in the PCOS group was an independent factor. In other words, the PCOS group was associated with elevated levels of sCD40L independently of other factors that may be associated with PCOS, such as hyperinsulinemia and hypercholesterolemia. However, although the level of CD40L in the PCOS group was elevated compared with that in the control group, there is no established cutoff

value for plasma sCD40L. To calculate such a value, further studies with larger sample sizes are needed.

Elevated serum levels of hsCRP in patients with PCOS were demonstrated in earlier studies (24). The results of previous studies have suggested that hsCRP, rather than being only a marker of low-grade inflammation, directly promotes endothelial dysfunction and complements activation, therefore playing an active role in atherogenesis. In our study, there was a trend for higher hsCRP levels in the PCOS group, but the difference between the groups did not reach statistical significance ($P = .06$). Furthermore, we found no correlation between sCD40L and hsCRP. However, Lin et al. (25) demonstrated that the incubation of human umbilical vein endothelial cells with CRP resulted in a time- and dose-dependent increase in the cell-surface expression of CD40 and CD40L. Recently they also suggested a mechanism by which CRP activates the expression of CD40–CD40L through nuclear factor κ B, which has been implicated as a key mediator of atherosclerosis (26, 27). Despite these findings, some studies found no correlation between hsCRP and CD40L in patients with obesity (28) or coronary artery disease (29), and these results confirmed our findings.

In previous studies homocysteine levels were found to be elevated in patients with PCOS, suggesting that an alteration in homocysteine metabolism may play a role in the increased cardiovascular risk associated with PCOS (30, 31). In clinically stable patients with systemic lupus erythematosus, serum levels of homocysteine and CD40L were significantly

TABLE 3**Bivariate analysis of sCD40L with other parameters in PCOS patients.**

Variables	r	P value
BMI	0.253	NS
Waist/hip ratio	0.072	NS
Fasting glucose	0.091	NS
2 nd hour glucose	-0.040	NS
Fasting insulin	0.076	NS
Fasting glucose/fasting insulin ratio	-0.169	NS
HOMA-IR	0.077	NS
Total T	-0.249	NS
Free T	-0.033	NS
DHEAS	-0.198	NS
Total cholesterol	-0.091	NS
HDL-C	-0.276	NS
LDL-C	0.174	NS
LDL-C/HDL-C ratio	0.356	< .05
Triglyceride	-0.135	NS
Homocysteine	0.177	NS
hsCRP	0.191	NS

Note: Correlation given as Pearson coefficient.

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higher than those in control patients matched for age, sex, body mass index, and smoking status. It has been suggested that impaired endothelial function, as shown by decreased small artery elasticity, and an adverse profile of novel proatherogenic and prothrombotic vascular disease risk factors were prevalent in clinically quiescent systemic lupus erythematosus (32). In our study the homocysteine level was significantly higher in the PCOS group ($P < .05$) and was not correlated with sCD40L levels.

In our study, both the PCOS and control groups had a relatively high BMI, indicating that the patients are overweight ($29.7 \pm 2.6 \text{ kg/m}^2$ vs. $29.3 \pm 2.7 \text{ kg/m}^2$, respectively). Therefore, the results of our study reflect overweight patients only.

We used plasma level of sCD40L instead of serum level of sCD40L because many reports showed plasma level sCD40L as being more reliable to assess the risk of cardiovascular disease (33).

In conclusion, we noted that sCD40L levels were significantly higher in the PCOS group compared with the non-PCOS control group. Today the role of sCD40L in the prognosis of cardiovascular disease and metabolic syndrome is still under investigation, and like CRP, it is not exactly possible to call sCD40L an independent risk factor for cardiovascular disease. This study is the first to assess sCD40L in patients with PCOS; perhaps further studies with larger sample sizes and long-term follow-up will help to support our results.

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