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Association of Interleukin -18 and CD40 in obese polycystic ovary syndrome

Ruaa A.jasem ,Jinan .M.Al-saffar and Hasan F.Al-Azzawie*
Dept. of Biotechnology/College of science/University of Baghdad

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Corresponding Author

Hasan F.Al-azzawie.E.mail
hazzawie2@yahoo.com

Abstract

This study was designed to examine the association between Interleukin -18, leptin, homocysteine, plasma CD40L levels and insulin resistance in women with obese PCOS and its relationship to endocrine, clinical and metabolic parameters and to know the expanded role of these factors in reproduction might impact our understanding of PCOS. Seventy five women with obese PCOS and fifty healthy, age and body mass index (BMI) matched controls were enrolled in this controlled clinical study. Homocystine,C-reactive protein(hs-CRP),Interleukin-18,Leptin,CD40L,fasting glucose and insulin, homeostatic model assessment insulin resistance index(HOMA-IR),LH,FSH,E_2,and Testosterone hormones in addition to ,lipid profile were performed. The levels of homocysteine,Interleukin-18, leptin,CD40L, hs-CRP, fasting insulin and HOMA-IR levels were significantly higher in the obese PCOS group in comparison with the non obese control group($p<0.05$). The mean serum triglyceride level in the PCOS group was higher compared with control group ($P<0.01$), a signifantly increase in levels of homocysteine, sCD40L, hs-CRP, interleukin- 18 Leptin and (HOMA-IR), in obese PCOS patients may be exactly possible to call CD40L associated with hs-CRP a dependent risk factors for cardiovascular disease.This study is the first to assess relation and proathrogenic and proinflammatory markers in obese Iraqi women with PCOS.

INTRODUCTION

Polycystic ovary syndrome (PCOS) is a complex and heterogeneous disorder, affecting approximately 7 per cent of women in reproductive age (Diamanti-Kandarakis et al., 2008). It is characterized by chronic anovulation, hyperandrogenemia, altered LH: FSH ratio (>2) and polycystic ovaries. The syndrome is a major cause of anovulatory infertility (Dunaif A 2010). The aetiopathogenesis of this syndrome still remains elusive but likely to be multifactorial consisting of genetic and environmental components. Insulin resistance (IR) is now known to be intrinsic to this disorder, present in approximately 50-70 percent of these women independent of obesity, and contributing in a major way to its pathogenesis (Legro et al., 2005). Women with PCOS are frequently obese which contributes an extrinsic component of IR. It is known that IR progresses towards the development of compensatory hyperinsulinemia, which drives hyperandrogenemia in these women (Azziz et al.,2004). Excess androgen levels lead to menstrual disturbances, development of ovarian cysts, hirsutism and other related disorders. IR also increases the risk for development of glucose intolerance, type 2 diabetes mellitus (T_2DM), hypertension, dyslipidaemia and cardiovascular abnormalities in these women (Poretsky et al., 1999; Maitra et al .,2001).

Leptin, the gene product of the obese gene, is thought to provide the central nervous system with Feed-back information about fat storage of the body (Hang et al., 1994). Thus, leptin is thought to be a part of the regulation of appetite, food intake and the lipid metabolism (Janeckova .,2010). Obese humans present with hyperleptinaemia as an indicator of leptin resistance which plays a major role in the pathogenesis of obesity (Considine et al., 1996).

Polycystic ovary syndrome is among the most common endocrine disorders, affecting more than 7% of women of reproductive age(Asuncion et al .,2000;Azziz et al., 2004).An association of PCOS with peripheral insulin resistance, compensatory hyperinsulinemia and alterations in ß-cell function as the cause of its predisposition to

develop a metabolic syndrome (type 2 diabetes mellitus, hypertension, lipid disorders, and obesity) has been established (Dunaif., 1989).According to the majority of studies, most PCOS women are insulin resistant and overweight or obese(Loverro et al., 2002; Garlichs et al .,2001; Varo et al .,2003;Ahn et al., 2004). In mice, a genetic defect in the ob gene results in severe obesity and type 2 diabetes mellitus, as well as in infertility (Dunaif., 1989).

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 (Schonbeck et al .,2001). Several cell lines express CD40L, including lymphocytes and cells of the vascular system, such as endothelial cells, smooth muscle cells, monocytes, and platelets (Miller et al., 2004;[Novo et al .,2005). CD40L is cleaved in the serum and circulates as soluble CD40L (sCD40L). It was previously shown that more than 95% of circulating sCD40L is of platelet origin (Apridonidze et al .,2005). Previous studies have found that individuals with hypercholesterolemia and diabetes (Semb et al .,2003) have elevated CD40L levels. Moreover, it was suggested that high levels of sCD40L may identify apparently healthy women at increased risk of having early-onset cardiovascular disease (Gokulakrishnan et al., 2006). To our knowledge, there is no study in the literature investigating sCD40L levels in obese patients with PCOS.

Women with PCOS have a clustering of cardiovascular risk factors, such as obesity, lipid abnormalities, impaired glucose tolerance and hypertension. Recent data have shown not only an
increased prevalence of cardiovascular disease (CVD) but also higher cardiovascular morbidity in women with PCOS(Gokulakrishnan et al., 2004; Schachter et al.,2003 Lin et al., 2004 ; Lin et al., 2005. In recent years homocysteine, which is an amino acid containing thiol, has been described as an independent risk factor for CVD (Schachter et al., 2003;Azar et al .,2005 ;Guldiken et al .,2007). Plasma homocysteine levels have been shown to correlate with blood pressure (Yilmaz et al., 2005), body mass index and insulin resistance (Lee et al .,2006 ; Weber et al .,2007). Since hypertension, obesity and hyperinsulinemia are frequently encountered features of PCOS, it seems logical to hypothesize that elevated homocysteine levels could be another feature of PCOS and this feature may contribute to increased prevalence of CVD in women with PCOS. This study was designed firstly to evaluate homocysteine concentrations and the relationship between homocysteine and insulin resistance in normal weight and obese women with PCOS and healthy control group. Secondly to investigate plasma sCD40L levels in patients with PCOS and to determine the relationship between sCD40L and other known cardiovascular risk factors. Thirdly to investigate the possible roles of IL-18 in the pathogenesis of PCOS, and its correlation with IR, obesity and hyperandrogenism, and the role of CRP levels as marker of cardiovascular risk in obese PCOS women.

MATERIALS AND METHODS

The study was carried out on 125women (75 obese PCOS subjects and 50 voluntary BMI, and age matched healthy women with normal menstrual cycle as controls). Clinical diagnosis of PCOS was made according to The Rotterdam ESHRE/ASRM-Sponsored PCOS Conference Workshop Group., when either oligomenorrhea (cycles lasting longer than 35 days) or amenorrhea(less than two menstrual cycles in the past 6months) and either clinical signs of hyperandrogenism (hirsutism or obvious acne or alopecia and/or an elevated total testosterone) were found, and other pituitary, adrenal or ovarian diseases could be excluded. PCOS as well as control subjects had not taken any medication known to affect carbohydrate metabolism or endocrine parameters for at least 3 months before entering the study .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. Blood sampling was done in control group during the early follicular phase (3rd–5th menstrual day). In the study group because the patients were often amenorrheic, blood samples were withdrawn at random but after sonographic exclusion of dominant follicular activity. After obtaining fasting blood samples serum was separated, then portion of the sample was used for insulin and glucose estimation and the rest was immediately frozen (−70◦C) until hormonal assays were performed. Fasting blood sample was obtained from each patient in the morning between 8:00 and 9:00 AM. Serum levels of FSH, LH, and homocysteine. Serum E2, total T, DHEAS and insulin were measured by ELISA kits.(Diagnostic Systems Laboratories,). Serum total cholesterol, triglyceride, and high-density lipoprotein cholesterol (HDL-C) levels were measured spectrophotometrically. Serum low-density lipoprotein cholesterol (LDL-C) level was calculated according to the Friedwald formula. High-sensitivity C-reactive protein (hsCRP) level was measured with immunometric assay kit. Serum glucose level was measured by the hexokinase method using Randox 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. Serum glucose level was measured by the hexokinase method using Randox 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 (HOMA-IR¼ [fasting insulin (mIU/mL.) - fasting glucose (mg/dl)] /22.5).Plasma sCD40L levels were determined using a commercially available ELISA kit (Biotech) according to the manufacturer's instructions.

RESULTS AND DISCUSSION

The basal characteristics of the obese 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 obese PCOS group than in the control group (P<.01) (Table 1).The hormonal and biochemical features of the obese 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<0.01) and HOMA-IR levels (P< 0.05) were significantly higher and the mean fasting glucose/fasting insulin ratio was significantly lower in the obese PCOS group (P<0.01). The total and free testosterone levels and mean LH/FSH ratio were significantly higher in the obese PCOS group (P<0.05).The mean serum triglyceride level in the obese PCOS group was higher compared with that in the control group (P<0.01). 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 higher in the PCOS group than that in the control group (P<0.05), in addition to the mean plasma sCD40L level in the PCOS group was significantly higher than that in the control group (6.44 ±2.8 ng/mL vs. 2.25 ±0.64 ng/mL, respectively; P<0.05; Table 2).

Table 1: Baseline characteristic of the PCOS and control groups

Characteristic	PCOS (n =75)	Control (n = 50)	P value
Age (y)	27.4 ± 3.5	25.9 ±3.2	NS
Body weight (kg)	69.2 ±7.3	68.3 ± 6.8	NS
Body mass index (kg/m2)	29.9 ± 2.7	29.7 ± 2.8	NS
Waist circumference (cm)	8.6±6.4	77.3 ±4.9	NS
Waist/hip ratio	0.82±0.07	0.80 ±0.03	NS
Patients with oligo/amenorrhea (%)	75 (100)	0	<0.001
Patients with Ferriman-Gallwey score >8	70(95)	0	<0.001
Patients with polycystic ovaries (%)	65 (92.4)	7 (22.6)	<0.001

The total and free testosterone levels and mean LH/FSH ratio were significantly higher in the obese PCOS group (P<0.05). The increased level of Dihydroandrostenedione (DHAS) strongly correlates with the clinical degree of hyperandrogenism. It seems that DHES could be a crucial diagnostic and predictive factor among women with menstrual disorders or presence of polycystic ovaries on ultrasound examination .The elevated levels of the indexes that correlate with the degree of insulin resistance such as the beta cell function index (HOMA-β) or insulin level in 30th min, after 75 g glucose load, are met more often among obese women with BMI > 30 kg/m2, without coexisting hyperandrogenism or presence of polycystic ovaries on ultrasound examination. There were noticed elevated levels of total testosterone, androstenedione, and significantly higher levels of triglyceride. It may point out the increased risk of cardiovascular and metabolic diseases.

The mean plasma sCD40L level in the obese PCOS group was significantly higher than that in the control group (6.44 ±2.8 ng/mL. vs. 2.25 ± 0.64 ng/mL. respectively; P<0.05; Table 2). Elevated serum levels of hsCRP in patients with obese PCOS were demonstrated in earlier studies (Boulman et al 2004). The results of previous studies have suggested that hsCRP, rather than being only a marker of low-grade inflammation, directly promote 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 obese PCOS group, and the difference between the groups reached statistical significance (P<0.01), furthermore, we found a positive correlation between sCD40L and hsCRP(r = 0.67).

However, Lin et al, 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 (Lin et al 2004).

Recently they also suggested a mechanism by which CRP activates the expression of CD40–CD40L through nuclear factor k B, which has been implicated as a key mediator of atherosclerosis (Lin et al 2005). 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 (Weber et al 2007). In conclusion, we noted that sCD40L levels were significantly higher in the obese 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 obese patients with PCOS; perhaps further studies with larger sample sizes and long-term follow-up will help to support our results.

Table 2: Hormonal and biochemical features of the obese PCOS and control groups.

Parameter	Obese PCOS (n = 75)	Control (n=50)	P value
Fasting glucose (mg/dL)	93.9 ±7.2	81.8 ± 5.5	NS
2nd-h glucose (mg/dL)	126.3 ± 19.7	100.3 ± 14.8	NS
Fasting insulin (mIU/mL)	11.6 ± 6.6	7.1 ± 2.8	<0.01
Glucose/ insulin (%)	10.6 ± 7.2	15.0 ± 6.2	<0.01
HOMA-IR	3.7 ± 1.5	1.7 ± 0.8	<0.05
LH (mIU/mL)	10.2 ± 4.4	7.4 ± 2.3	NS
FSH (mIU/mL)	5.3 ± 2.1	6.1 ± 3.1	NS
LH/FSH ratio	2.1 ± 1.2	1.4 ± 0.5	<0.05
E2 (pg/mL)	59.5 ± 28.9	56.3 ± 25.3	NS
Total T (ng/dL)	0.74 ± 0.24	0.5 ± 0.2	<0.05
Free T (ng/mL)	2.4 ± 1.5	1.6 ± 0.7	<0.05
DHEAS (ng/mL)	212.8 ± 98.6	174.3 ± 63.9	NS
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)	135.5 ±15.6	83.5 ± 25.5	<0.01
Homocysteine (mg/dL)	11.3 ± 4.8	9.0 ± 3.2	<0.05
hsCRP (mg/dL)	4.49 ± 2.0	0.4±0.1	<0.01
sCD40L (ng/mL)	6.44 ±2.8	2.25 ±0.64	<0.05
Interleukin - 18 pg/ml	233 ±44	108±22	<0.05
leptin mg/ml	76.41± 5. 56	40.85 ± 2.90	<0.05

Our data showed that serum homocysteine levels were significantly higher in obese PCOS women than controls. Homocysteine has a well-known role in cardiovascular morbidity and mortality. It has primary atherogenic and prothrombotic properties. Homocysteine promotes leukocyte recruitment by up regulating monocyte chemo attractant protein-1 and interleukin-8 expression and secretion. The metabolite of homocysteine can combine with LDL-cholesterol to produce foam cells and atherosclerotic plaques. Homocysteine increases smooth muscle cell proliferation and enhances collagen production. Prothrombotic effects of homocysteine include attenuation of endothelial cell tissue plasminogen activator binding sites, activation of factor VIIa and V, inhibition of protein C

and heparin sulfate, increased fibrinopeptide A and Prothrombin fragments 1 and 2, increased blood viscosity, and decreased endothelial antithrombotic activity due to changes in thrombomodulin function. Free radicals formed during the oxidation of reduced homocysteine may directly injure endothelial cells. Marked platelet aggregation may be secondary to the proaggregatory effects of homocysteine.

Prolonged exposure of endothelial cells to homocysteine impairs the production of nitric oxide. Hyperhomocysteinemia has been linked to myocardial infarction and recurrent coronary events, adverse outcomes after angioplasty, carotid artery stenosis, recurrent venous thrombosis, osteoporosis, dementia and silent brain infarct (Yilmaz et al .,2005 ; Lee et al., 2006). The levels of homocysteine in the PCOS population compared with controls have been studied with conflicting results. Because of the higher rate of hyperhomocysteinemia in obese PCOS subjects with significantly elevated fasting insulin, we suggest that it may be secondary to the higher prevalence of insulin resistance in obese PCOS patients (Weber et al .,2007).

Badawy et al., (2006), in their prospective case-control study on ninety PCOS women which used a cutoff level of 11 µmol/L for a normal homocysteine level, found that 41.1% of PCOS patients and 2.9%of the control group had high homocysteine levels, which demonstrated the effect of insulin resistance on homocysteine levels (Gokulakrishnan et al .,2006). In another similar study, the authors found that mean plasma homocysteine levels were significantly higher in the insulin-resistant PCOS patients as compared with non-insulin-resistant PCOS patients (Schechter et al., 2003).

Patients with obese PCOS showed significantly higher concentrations of leptin (76.41± 5. 56 ng/ml) vs. control (40.85 ± 2.90 ng/ml, $p<0.01$), and LH (11.2 ± 4.4) vs. control (7.4 ± 2.3, $p<0.05$) in plasma (Table 2).We observed a marked difference in insulin level between patients with obese PCOS (11.5mIU/ml) vs. control (7.1±2.8mIU/ml).Furthermore, we analyzed the relationships between leptin, with BMI, insulin, insulin resistance and LH in PCOS and control groups. Significant correlations were observed between leptin and BMI ($r =0.78$, $p=0.000$), insulin resistance (IR) ($r=0.53$, $p=0.004$) and insulin($r=0.93$, $p=0.000$) in obese PCOS patients.

Leptin levels are increased in obesity and may play a role in the development of insulin resistance (Janeckova R., 2010). Our study showed a significant high total leptin in obese PCOS patients as it is compared with controls (Table 2) ($p<0.05$). Total leptin levels correlated significantly with BMI in both PCOS women and controls. Leptin correlated with other metabolic parameters including insulin resistance and insulin level in both groups. Similar results were found in PCOS patients from different countries (Garlichs et al .,2001; Varo et al .,2003; Yilmaz et al., 2005). Significant correlation of total leptin with HOMA IR reflected a degree of insulin resistance in both controls and test groups (Table 2). We can place our results along the large group of studies, which declare an important role of leptin in pathogenesis of insulin resistance(Apridonidze et al., 2005;Diamanti-Kandarakis et al .,2008; Dunaif A., 2010). A considerable portion of circulating leptin is free form which is affected by the degree of adiposity and nutritional state (Gennarelli et al .,1998). Gennarelli G and co-workers demonstrated that Free leptin index is correlated with total energy intake and inversely correlated with energy intake from dietary fat. They speculated that the macronutrient composition of the diet influences serum concentrations of free leptin. The authors hypothesized that the increase of free leptin levels represents a compensatory mechanism to overcome insulin resistance (Gennarelli et al .,1998).

Serum levels of IL-18 were significantly higher in the obese PCOS group than in the control group. Serum level of IL-18 in the obese PCOS group was positively related to BMI, IR index and T. so we concluded that IL-18 level was increased in obese PCOS patients, and correlated with insulin resistance, obesity and hyperandrogenism. The mean level of IL-18 230 ±44 pg/mL in the obese PCOS compared with 108 ±22 pg/ml in the control group. Serum IL-18 concentrations were increased in PCOS patients irrespective of the presence or absence of IR, and obese PCOS women with IR presented with increased IL-18 level than PCOS without IR. PCOS patients presented with increased LH, Testosterone , HOMA-IR and LH/FSH levels, especially in PCOS group with IR, and there was a statistically significant difference ($P < 0.05$) (Table 2).Serum IL-18 concentrations was correlated with BMI ($r = 0.688$, $P = 0.000$), HOMA-IR ($r = 0.599$, $P = 0.000$), T ($r = 0.602$, $P = 0.000$) and LH/FSH ($r = 0.468$, $P = 0.000$) .

It has been suggested that plasma levels of sCD40L protein may be a marker of atherothrombotic potential because CD40L is released during the early stage of atherogenesis through thrombus formation (Novo et al., 2005). It has also been suggested that sCD40L protein levels are higher in patients with metabolic syndrome and cardiovascular disease (Apridonidze et al., 2005). 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 Iraqi patients with obese PCOS. We noted in our study that the obese patients with PCOS were characterized by significantly elevated levels of sCD40L compared with

controls (6.44 ±2.8 ng/mL. vs. 2.25 ±0.64 ng/mL., respectively; P<0.05).Levels of sCD40L were also elevated in patients with familial hypercholesterolemia(Semb et al.,2003).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 (Gokulakrishnan et al., 2006). 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 obese 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 ± 6.7 mIU/mL vs. 7.1 ± 2.8mIU/mL; P<0.01), HOMA-IR (3.7 ±1.5 vs. 1.7 ± 0.8; P<0.05), and triglyceride (135.5 ± 15.6 mg/dL vs. 83.5 ± 25.5 mg/dL; P<0.01) were higher in the obese PCOS group compared with those in the control group. However, the ratio of fasting glucose/fasting insulin (10.7% -7.1% vs. 15.0% - 6.2%; P<.01) was lower in the obese PCOS group than in the control group. Logistic regression analysis of our data revealed that elevation of sCD40L in the obese 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 (Boulman et al., 2004). The result 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, and the difference between the groups reached statistical significance (P<0.01). Furthermore, we found a positive correlation between sCD40L and hsCRP. Recently they also suggested a mechanism by which CRP activates the expression of CD40–CD40L through nuclear factor k B, which has been implicated as a key mediator of atherosclerosis (Lin et al 2005). Despite these findings, some studies found no correlation between hsCRP and CD40L in patients with obesity or coronary artery disease and these results confirmed our findings (Weber M et al., 2005).

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 CVD risk associated with PCOS (Badawy et al., 2006). In clinically stable patients with systemic lupus Erythematosus, serum levels of homocysteine and CD40L were significantly 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 proathrogenic and prothrombotic vascular disease risk factors were prevalent in clinically quiescent systemic lupus Erythematosus (Lee AB et al., 2005).In our study the homocysteine level was significantly higher in the PCOS group (P<0.05) 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 (29.9 ± 2.8kg/m2 vs. 29.8 ± 2.9 kg/m2, respectively). Therefore, the results of our study reflect overweight patients only. 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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