CLINICAL STUDY

Oxidised low-density lipoprotein concentration – early marker of an altered lipid metabolism in young women with PCOS

Djuro Macut, Svetozar Damjanović, Dimitrios Panidis¹, Nikolaos Spanos¹, Biljana Glišić², Milan Petakov, David Rousso¹, Anargyros Kourtis¹, Jelica Bjekić³ and Nataša Milić⁴

Institute of Endocrinology, Diabetes and Metabolic Diseases, Clinical Center of Serbia, Dr Subotića 13, 11000 Belgrade, Serbia, ¹Division of Endocrinology and Human Reproduction, 2nd Department of Obstetrics and Gynecology, Aristotle University of Thessaloniki, Konstantinoupoleos 49, 54642 Thessaloniki, Greece, ²Institute of Medical Biochemistry, Clinical Center of Serbia, Višegradska 26, 11000 Belgrade, Serbia, ³Department of Endocrinology, CHC Bežanijska kosa, Autoput bb, 11000 Belgrade, Serbia and ⁴Institute of Medical Statistics, School of Medicine, Dr subotića 15, 11000 Belgrade, Serbia

(Correspondence should be addressed to D Macut; Email: macut@EUnet.yu)

Abstract

Objective: Women with polycystic ovary syndrome (PCOS) are assumed to be at increased risk for cardiovascular diseases. This study examined the variations in oxidised low-density lipoprotein (OxLDL) concentration in relation to insulin levels in young women with PCOS.

Design: Cross-sectional clinical study in tertiary cares research hospitals. A total of 179 women with PCOS (79 overweight) and 56 age- and body mass index-matched controls were examined.

Methods: Blood samples were collected in follicular phase of the cycle for the basal glucose, total-, high-density lipoprotein-cholesterol (HDL-C) and LDL-cholesterol, OxLDL, triglycerides, apolipoprotein-A1 (Apo-A1) and B (Apo-B), lipoprotein (a), insulin, testosterone and sex hormone-binding globulin (SHBG). Homeostatic model index (HOMA) and free androgen index (FAI) were determined.

Results: Overweight and normal weight women with PCOS had higher concentrations of OxLDL than their control counterparts ($P=0.007$ and 0.003 respectively). Both the basal insulin ($P=0.003$) and HOMA values ($P<0.001$) were significantly higher in overweight than normal weight patients. Testosterone and FAI were higher in patients than in the respective controls ($P<0.001$). The only independent predictor of increased OxLDL concentration in normal weight patients was Apo-B-to-Apo-A1 ratio ($P<0.001$, odds ratio (OR) 6.1; 95% confidence interval (CI) 2.3–16.4), while in obese PCOS, it was total cholesterol-to-high-density lipoprotein cholesterol ratio ($P<0.001$, OR 2.8; 95% CI 1.6–4.9).

Conclusion: Young normal weight and overweight PCOS women have similarly increased OxLDL levels. Our results may indicate the presence of primary alteration in lipid metabolism in patients with PCOS. To answer the question whether the alteration in LDL particle size can by itself pose a higher cardiovascular risk, a careful follow-up of these women is needed.

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Introduction

Polycystic ovary syndrome (PCOS) is a common reproductive endocrine disorder that is characterised by hyperandrogenism and chronic anovulation, affecting up to 10% of reproductive-aged women (1, 2). Today, PCOS is considered to be a metabolic disorder closely related to obesity, insulin resistance, hyperinsulinaemia, and atherogenic lipid profile (3–6).

Although the incidence of cardiovascular disease in PCOS is still a controversial issue (7–9), considerable attention in the past decade has been paid to this problem. Dyslipidaemia is the most common metabolic abnormality in PCOS, although the type and the extent of findings have not been defined. According to the National Cholesterol Educational Programme guidelines, prevalence of an abnormal lipid level is either

borderline normal or high, approaching almost 70% (5). In vast majority of patients with PCOS, using current cutoffs established by this programme, mean lipid values fall within the normal limits (3, 5, 6, 9). Additionally, insulin resistance has been associated with decreased levels of high-density lipoprotein cholesterol (HDL-C), increased levels of low-density lipoprotein cholesterol (LDL-C) and triglycerides (5, 8). Moreover, in these patients, the concentration of small dense LDL particles has been associated with three- to sevenfold increased relative risk of coronary artery disease (CAD) (10). Similarly, clinical signs of hyperandrogenism as hirsutism and acne appear to be associated with angiographic evidence of CAD in women with PCOS (11).

Recently, elevated levels of oxidised low-density lipoprotein (OxLDL) have been detected in patients with CAD (12–15), establishing the role of OxLDL in the

initiation and progression of atherosclerosis. In these studies, clinical predictors of elevated OxLDL levels were female sex, family history of premature cardiovascular disease, increased body mass index (BMI) and percent of body fat, as well as exercise less than four times per week (12). Furthermore, circulating levels of OxLDL were predictive for CAD with relatively high sensitivity and specificity (15).

Whether patients with PCOS have elevated levels of OxLDL is unknown. The aim of this study is to determine the values of OxLDL in these women and probably to disclose its association with insulin resistance as another major factor involved in the process of atherosclerosis.

Materials and methods

Materials

A total of 179 women with PCOS were examined. All patients came from the metropolitan areas of Belgrade and Thessaloniki, were Caucasians and had similar socio-cultural background. Subjects were recruited over a 12-month period from the outpatient clinics of every institution that conducted the study, where the patients were referred from different sources including self-referral, obstetrics-gynaecology clinics and primary care physicians for investigation of oligo- or amenorrhoea, fertility problem, hirsutism, or acne.

PCOS was defined according to the revised 2003 Rotterdam Consensus conference on diagnostic criteria for PCOS (16). Besides moderate oligo/amenorrhoea, our patients had elevated serum testosterone concentrations and polycystic appearance of their ovaries.

Exclusion criteria for all the subjects included: inability to comply with study requirements, the presence of impaired fasting glucose (fasting venous glucose ≥ 6.0 mmol/l), pregnancy, hypothyroidism, non-classical 21-hydroxylase deficiency, hyperprolactinaemia, Cushing's disease and androgen-secreting tumours excluded by appropriate tests, history of drug or alcohol abuse and a history of breast or uterine cancer, or cardiovascular disorder. No subjects had received any oral contraceptives, glucocorticoids, anti-androgens, ovulation induction agents, anti-diabetic and anti-obesity drugs, or other hormonal drugs for at least 3 months before the study.

In all examined subjects, BMI was calculated and waist-to-hip ratio (WHR) determined. The patients and controls were categorised with respect to BMI values to those having normal weight ($\text{BMI} \leq 25 \text{ kg/m}^2$) and those that were overweight ($\text{BMI} > 25 \text{ kg/m}^2$).

An age- and BMI-matched control group consisted of 56 women with no signs of hyperandrogenism, either clinical or biochemical, prior to the study. The normal ovulatory state was confirmed by transvaginal ultrasonography and plasma progesterone levels during the luteal phase of the cycle.

This study was approved by the Institutional Ethical Committees and written consent was obtained from all subjects.

Biochemical and hormonal testing

Women with PCOS were examined during early follicular phase (within the first 6 days after the onset of menstruation in the case of mild oligomenorrhoea) or at random in case of severe oligo- or amenorrhoea. Controls were investigated in early follicular phase. In all PCOS patients and controls, blood samples were collected in the morning subsequent to overnight fast. Basal serum levels of total cholesterol (TC), HDL-C, LDL-C, OxLDL, triglycerides, apolipoprotein-A1 (Apo-A1), apolipoprotein-B (Apo-B), lipoprotein(a) (Lp(a)), glucose, insulin, testosterone and sex hormone-binding globulin (SHBG) were measured, and TC-to-HDL-C ratio (TC/HDL-C), LDL-C-to-HDL-C ratio (LDL-C/HDL-C), Apo-B-to-Apo-A1 ratio (Apo-B/Apo-A1) and triglyceride-to-HDL-C ratio (triglyceride/HDL-C) were determined in all PCOS patients and respective controls. Samples for the determination of OxLDL, insulin, testosterone and SHBG were stored at -20°C until the analysis.

Assays

TC (mmol/l) and triglycerides (mmol/l) were measured by standard enzymatic methods (cholesterol: cholesterol oxidase, Randox, Belfast, UK; triglycerides: glycerol-3-phosphat oxidase, Randox, UK). HDL-C (mmol/l) was measured by direct method (Randox, UK). LDL-C (mmol/l) was determined by Friedewald formula (17). OxLDL (U/l) was measured by commercially available solid phase two-site enzyme immunoassay, sandwich ELISA (Mercodia AB, Uppsala, Sweden). The intra- and interassay coefficients of variation (CV) were 4.0 and 6.2% respectively. Apo-A1 (g/l), Apo-B (g/l) and Lp(a) (g/l) were determined by nephelometric method (Behring, Marburg, Germany). Plasma glucose (mmol/l) was determined by glucose oxidase method (Randox, UK), using the auto-analyser (Beckman, Brea, CA, USA). Plasma insulin (mU/l) levels were determined using two-site enzyme immunoassay, sandwich ELISA (Mercodia AB, Uppsala, Sweden). The intra- and interassay CV were 2.6 and 4.0% respectively. Serum testosterone (nmol/l) was measured by RIA (TESTO-CT2, CIS bio international, Gif-Sur-Yvette Cedex, France). The intra- and interassay CV was 4.5 and 5.1% respectively. SHBG (nmol/l) was measured by RIA (SHBG-RIACT, CIS bio international, Gif-Sur-Yvette Cedex, France). The intra- and interassay CV was 3.9 and 4.7% respectively. Free androgen index (FAI) was calculated from total testosterone and SHBG, using the formula $\text{FAI} = (100 \times T)/\text{SHBG}$, with both testosterone and SHBG expressed in nmol/l (18). $\text{FAI} > 8$ was considered significant.

Measures of insulin sensitivity

Insulin sensitivity was calculated by homeostatic model index (HOMA). HOMA was calculated using the formula $[\text{fasting insulin (mU/l)} \times \text{fasting glucose (mmol/l)}] / 22.5$ (19).

Data analysis

Results were presented as the mean $\pm$ s.d. Differences between groups were assessed by using ANOVA with Tukey's honestly significant difference test for pair-wise multiple comparisons or non-parametric Mann-Whitney U test, with Bonferroni correction for *P*-values for multiple comparisons, as appropriate. Associations between different variables were determined by using Pearson's Correlation Coefficient. Stepwise multivariate logistic regression analysis was used to identify independent predictors of elevated OxLDL levels. Only variables that were significant in univariate analyses entered the equation. *P* < 0.05 was considered significant.

Results

Clinical characteristics of patients

Clinical and biochemical characteristics of patients and control subjects are presented in Table 1. There was no difference in age, TC and LDL-C, Lp(a) and basal glucose

concentrations between control subjects and PCOS patients.

Other parameters of lipid metabolism were significantly different in women with PCOS than in healthy women. Concentrations of HDL-C were lower, while concentration of triglycerides was higher in overweight patients, but this was not the case in control subjects. In the controls, Apo-A1 was lower in overweight than in normal weight subjects, whereas in patient's group, the concentration of Apo-B was higher in overweight than in normal weight subjects. Thus, proatherogenic index Apo-B/Apo-A1 as well as TC/HDL-C and LDL-C/HDL-C ratios were significantly higher in overweight PCOS patients than in normal weight controls. In addition, overweight patients had significantly higher values of TC/HDL-C, LDL-C/HDL-C and triglyceride/HDL-C ratios in comparison to normal weight patients with PCOS. Women with PCOS had higher concentrations of OxLDL than control subjects.

Overweight women with PCOS were more insulin resistant than normal weight patients. They had higher basal insulin levels and HOMA values (Table 1). There was no difference between insulin resistance of normal weight and overweight control subjects. Normal weight control subjects were more insulin sensitive than overweight PCOS patients. Although basal testosterone concentrations between normal weight and overweight PCOS patients were similar (Table 1), significantly

Table 1 Clinical and biochemical features, and indices of insulin sensitivity in groups of PCOS patients and controls in relation to body mass index (BMI).

	PCOS patients		Controls		<i>P</i> value*
	Overweight (<i>N</i> = 79)	Normal weight (<i>N</i> = 100)	Overweight (<i>N</i> = 20)	Normal weight (<i>N</i> = 36)	
Age	24.9 $\pm$ 5.9	23.8 $\pm$ 4.0	25.7 $\pm$ 5.9	23.6 $\pm$ 4.4	0.121
BMI	30.2 $\pm$ 4.4 ^a	21.4 $\pm$ 2.02	28.9 $\pm$ 3.5 ^e	20.49 $\pm$ 1.9	<0.001
WHR	0.83 $\pm$ 0.07 ^a	0.76 $\pm$ 0.04	0.81 $\pm$ 0.07 ^e	0.75 $\pm$ 0.03	<0.001
TC	4.8 $\pm$ 0.9	4.6 $\pm$ 0.9	4.6 $\pm$ 0.6	4.4 $\pm$ 0.8	0.141
LDL-C	2.9 $\pm$ 0.9	2.7 $\pm$ 0.9	2.9 $\pm$ 0.6	2.5 $\pm$ 0.6	0.139
HDL-C	1.2 $\pm$ 0.3 ^a	1.4 $\pm$ 0.3	1.3 $\pm$ 0.3	1.5 $\pm$ 0.3	<0.001
TC/HDL-C	4.09 $\pm$ 1.4 ^{a,c}	3.2 $\pm$ 0.8	3.8 $\pm$ 1.4	3.03 $\pm$ 0.6	<0.001
LDL-C/HDL-C	2.5 $\pm$ 1.1 ^{a,f}	1.9 $\pm$ 0.8	2.2 $\pm$ 0.7	1.8 $\pm$ 0.5	<0.001
Triglycerides	1.4 $\pm$ 0.8 ^a	0.9 $\pm$ 0.6	1.2 $\pm$ 1.3	0.8 $\pm$ 0.2	<0.001
Triglyceride/HDL-C	1.3 $\pm$ 0.9 ^a	0.7 $\pm$ 0.5	1.4 $\pm$ 2.8 ^m	0.7 $\pm$ 0.5	<0.001
Apo-A1	1.6 $\pm$ 0.3	1.6 $\pm$ 0.3	1.5 $\pm$ 0.2 ^l	1.7 $\pm$ 0.3	0.014
Apo-B	0.9 $\pm$ 0.2 ^k	0.8 $\pm$ 0.2	0.8 $\pm$ 0.2	0.8 $\pm$ 0.1	0.007
Apo-B/Apo-A1	0.6 $\pm$ 0.2 ⁿ	0.5 $\pm$ 0.3	0.6 $\pm$ 0.1	0.5 $\pm$ 0.1	0.035
Lp(a)	0.2 $\pm$ 0.2	0.2 $\pm$ 0.2	0.2 $\pm$ 0.2	0.1 $\pm$ 0.1	0.475
OxLDL	65.4 $\pm$ 26.1 ⁱ	61.5 $\pm$ 26.8 ^h	44.8 $\pm$ 7.8	44.3 $\pm$ 8.5	<0.001
Basal glucose	4.9 $\pm$ 0.7	4.8 $\pm$ 0.6	4.8 $\pm$ 1.0	4.5 $\pm$ 0.7	0.126
Basal insulin	15.6 $\pm$ 10.2 ^{c,g}	10.8 $\pm$ 6.3	10.6 $\pm$ 5.8	7.8 $\pm$ 3.2	<0.001
HOMA	3.6 $\pm$ 2.6 ^{a,c}	2.4 $\pm$ 1.3	2.4 $\pm$ 1.5	1.6 $\pm$ 0.7	<0.001
Testosterone	3.3 $\pm$ 1.2 ^b	3.1 $\pm$ 1.0 ^d	1.6 $\pm$ 0.4	1.4 $\pm$ 0.7	<0.001
SHBG	27.5 $\pm$ 11.6 ^{b,j}	35.1 $\pm$ 13.4 ^d	45.1 $\pm$ 14.4	58.2 $\pm$ 22.4	<0.001
FAI	16.1 $\pm$ 12.2 ^{a,b}	10.2 $\pm$ 5.0 ^d	4.1 $\pm$ 2.2	3.0 $\pm$ 2.4	<0.001

**P* values for comparison of all four groups and for two-way comparison of patients and controls that were significant in the initial four-way comparisons.

^a*P* < 0.001 overweight PCOS vs normal weight PCOS, ^b*P* < 0.001 overweight PCOS vs overweight controls, *P* < 0.001 non-obese PCOS vs normal weight controls, ^e*P* < 0.001 overweight controls vs normal weight controls, ^l*P* = 0.001 overweight PCOS vs normal weight controls, ^g*P* = 0.003 overweight PCOS vs normal weight PCOS, ^h*P* = 0.003 non-obese PCOS vs normal weight controls, ⁱ*P* = 0.007 overweight PCOS vs overweight controls, ^j*P* = 0.01 overweight PCOS vs normal weight PCOS, ^k*P* = 0.013 overweight PCOS vs normal weight PCOS, ^m*P* = 0.021 overweight controls vs normal weight controls, ⁿ*P* = 0.034 overweight controls vs normal weight controls, ^o*P* = 0.04 overweight PCOS vs normal weight controls.

higher values of FAI were found in overweight patients. There was no difference in testosterone levels and FAI within control subjects. Significant differences between these parameters in control subjects and PCOS patients was found in our study.

Correlations in patients with PCOS

In all patients, BMI correlated positively with FAI ($r=0.38$; $P<0.001$), basal insulin ($r=0.41$; $P<0.001$), HOMA ($r=0.42$; $P<0.001$), testosterone concentration ($r=0.16$; $P=0.016$), TC/HDL-C ($r=0.41$; $P<0.001$), LDL-C/HDL-C ($r=0.33$; $P<0.001$), and triglyceride/HDL-C ($r=0.33$; $P<0.001$). OxLDL was in positive correlation with age ($r=0.22$; $P=0.006$), BMI ($r=0.14$; $P=0.036$), insulin ($r=0.33$; $P<0.001$) and testosterone ($r=0.27$; $P<0.001$) concentrations, HOMA ($r=0.31$; $P<0.001$) and FAI ($r=0.29$; $P<0.001$) indices. Indexes of cardiovascular risk, TC/HDL-C and Apo-B/Apo-A1 were in positive correlation with testosterone ($r=0.17$; $P=0.021$ and $r=0.26$; $P<0.001$) and FAI index ($r=0.18$; $P=0.026$ and $r=0.23$; $P=0.002$), while LDL-C/HDL-C was in positive correlation with testosterone concentration ($r=0.19$; $P=0.012$). Basal insulin levels and HOMA index were in positive correlation with testosterone concentration ($r=0.23$; $P=0.001$, for both), FAI index ($r=0.39$; $P<0.001$ and $r=0.36$; $P<0.001$), TC/HDL-C ($r=0.38$; $P<0.001$ and $r=0.4$; $P<0.001$), LDL-C/HDL-C ($r=0.34$; $P<0.001$ and $r=0.36$; $P<0.001$), Apo-B/Apo-A1 ($r=0.34$; $P<0.001$ and $r=0.32$; $P<0.001$) and triglyceride/HDL-C ($r=0.34$; $P<0.001$, for both).

In overweight PCOS patients, our analysis found the correlation of WHR with basal insulin and HOMA index ($r=0.29$; $P=0.024$), while in normal weight PCOS patients, it was in association with serum lipids: cholesterol ($r=0.41$; $P<0.001$), LDL-C ($r=0.39$; $P<0.001$), Apo-B ($r=0.35$; $P=0.001$), OxLDL ($r=0.27$; $P=0.009$), and lipid ratios of TC/HDL-C and LDL-C/HDL-C ($r=0.34$; $P=0.001$, for both).

Insulin was in correlation with TC/HDL-C ($r=0.5$; $P<0.001$), LDL-C/HDL-C ($r=0.52$; $P<0.001$) and Apo-B/Apo-A1 ($r=0.28$; $P=0.026$) in overweight PCOS patients. Similarly, HOMA index was in association with these indices ($r=0.47$; $P<0.001$, $r=0.5$; $P<0.001$ and $r=0.29$; $P<0.024$ respectively). LDL-C/HDL-C correlated with testosterone concentration significantly in overweight PCOS patients ($r=0.25$; $P=0.026$).

Logistic regression analysis

In both overweight and normal weight patients, similar variables were found to be statistically significant in univariate analyses. The only independent predictor of OxLDL in normal weight PCOS women, based on standard clinical criteria, was Apo-B/Apo-A1, while in

overweight patients, the independent predictor of disease was TC/HDL-C (Table 2).

Discussion

This study revealed that overweight and normal weight women with PCOS had similarly increased concentrations of OxLDL in comparison to healthy women. Independent predictors of elevated OxLDL levels in both overweight and normal weight patients were only pro-atherogenic lipid indices.

Difference of FAI and HOMA index values between overweight and normal weight PCOS women was not translated into different plasma concentrations of OxLDL in these patients. Obesity and atherogenic lipid profile are often found in women with PCOS (20). The most consistent alterations in lipid metabolism associated with metabolic syndrome are elevated triglycerides and lower HDL-C concentrations with increase of the triglyceride/HDL-C ratio (7, 9, 20–22) as it was the case with overweight patients and control subjects in our study. However, similar increase of OxLDL levels in -overweight and normal weight women with PCOS in this study supports the viewpoint that association of insulin-resistant syndrome and LDL particle size is not necessarily mediated by hyperinsulinaemia but via alterations in lipid metabolism itself (23).

It has been recently shown that young normal weight, non-dyslipidaemic and non-hypertensive women with PCOS have an early impairment of endothelial structure and function (24). Since, OxLDL was not measured in this study, these observations could be in line with our results, as young normal weight patients in our study had normal traditional lipid measures. The role of increased levels of small OxLDL particles in atherogenesis has been extensively studied only in patients with cardiovascular disease (13–15, 21, 25). These patients showed consistency in proving upregulation of OxLDL production and this is concordant with our results (21). It is likely that LDL atherogenicity increases simultaneously with reduction of LDL particle size (26). Our results are in line with findings of Dejager *et al.* (27), who showed that women with PCOS had reduced size of LDL-C particles in

Table 2 Variables independently associated with OxLDL*.

Independent variables	Odds ratio (95% Confidence Interval)	P value
Normal weight patients: Apo-B/Apo-A1	6.1 (2.3–16.4)	<0.001
Overweight patients: Total cholesterol/HDL-C	2.8 (1.6–4.9)	<0.001

*In univariate logistic regression analyses, the following variables were statistically significant: WHR, testosterone, FAI, SHBG, total cholesterol/HDL-C, LDL-C/HDL-C, triglyceride/HDL-C, basal insulin and HOMA.

comparison with normal cycling women. The influence of increased OxLDL concentrations on early atherogenesis in women with PCOS has not been studied. Thus, careful follow up of these women is needed to prove causality between the increased susceptibility to atherosclerosis and reduced diameter of LDL particles (23).

It is likely that obesity may cause aggravation of the existing functional hyperandrogenism and alterations in traditional lipid measures by increasing the insulin resistance (28), as it has been demonstrated in our study as well. However, complete normalisation of testosterone levels by long-acting GnRH agonist does not influence at all the insulin resistance in women with PCOS, consequently demonstrating that hyperandrogenaemia does not play a role in their insulin resistance (29). Besides impact on clinical characteristics of the syndrome, the existing insulin resistance of PCOS may also be involved in other metabolic disorders, since higher prevalence of type 2 diabetes within families of obese (54.8%) and non-obese (24.2%) women with PCOS has been demonstrated (30).

In conclusion, our results provide the evidence about the presence of primary alteration in lipid metabolism in young patients with PCOS. Whether the elevation of OxLDL in these young women is associated with silent phase of atherosclerosis remains to be elucidated.

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