ORIGINAL ARTICLE

Increased serum C-reactive protein levels in normal weight women with polycystic ovary syndrome

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Abstract

Background: The clinical spectrum of polycystic ovary syndrome (PCOS) includes components of the metabolic syndrome, such as central obesity, insulin resistance, dyslipidemia, arterial hypertension and, even, disturbances of the clotting mechanism. All these disorders are epidemiologically related to cardiovascular disease, most probably through low-grade intravascular chronic inflammation. The aim of this study was to evaluate the serum concentrations of high sensitivity C-reactive protein (hsCRP), a non-specific marker of low-grade inflammation and a predictive marker for cardiovascular disease, in normal weight women with (PCOS).

Patients and Methods: One hundred and eighty-eight (188) normal weight [body mass index (BMI) < 25 kg/m^2] women with PCOS were included in the study. Forty-three (43) normal weight women without PCOS (normal ovulation without clinical or biochemical hyperandrogenemia) served as controls. Serum samples for luteinizing hormone, follicle-stimulating hormone, prolactin, total testosterone, Δ_4-androstenedione, 17α-hydroxy-progesterone, sex hormone-binding globulin (SHBG), insulin, glucose and hsCRP were collected in early follicular phase (third to sixth day) of a menstrual cycle in the control group or during a spontaneous bleeding episode in the PCOS group.

Results: Normal weight women with PCOS had higher concentrations of serum hsCRP as compared to normal weight women without PCOS (mean ± standard error of the mean 0.55 ± 0.08 versus 0.27 ± 0.08 mg/dL, p = 0.001).

Conclusions: As normal weight women with PCOS are characterized by elevated serum concentrations of hsCRP, they have to be considered as carrying at least one marker of low-grade inflammation. Hippokratia 2011; 15 (4): 323-326

Keywords: polycycstic ovary syndrome; high-sensitivity C-reactive protein; inflammation; cardiovascular disease.

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The polycystic ovary syndrome (PCOS) is a common endocrine disorder among the women of reproductive age, which is characterized by chronic anovulation and biochemical or clinical hyperandrogenism. In addition, the clinical spectrum of PCOS includes components of the metabolic syndrome, such as central obesity, insulin resistance, dyslipidemia, arterial hypertension and, even, disturbances of the clotting mechanism[1]. All these disorders are epidemiologically related to clinical conditions, such as cardiovascular disease, most probably through low-grade intravascular chronic inflammation[2].

C-reactive protein (CRP) is an acute-phase protein, which is measured in the serum and is widely used in the routine clinical practice for the monitoring of bacterial infections, as well as the efficacy of the antimicrobial therapy[3]. CRP serves not only as a marker of severe infection but also of low-grade chronic inflammation, as it constitutes a useful screening marker of intravascular inflammatory processes[4].

During the last decade, several research groups [5-34] studied the concentrations of serum CRP in women with PCOS in an attempt to link PCOS with cardiovascular disease. The majority of these studies were characterized by small populations and exclusion of normal weight women; whenever normal weight women were included, they were not studied separately. The results of these studies were conflicting. In most of them, women with PCOS had higher serum concentrations of CRP compared to healthy controls[5-26], whereas, in fewer studies, there was no difference between the groups[27-34]. One study[8] that compared obese and normal weight women with PCOS concluded that elevated CRP concentrations might be related to obesity rather than PCOS. In limited number of studies[22,26], normal weight women [body mass index (BMI) < 25 kg/m^2] were studied separately concluding that normal weight women with PCOS have higher serum CRP concentrations compared to normal weight controls; still the

studies involved relatively small numbers of women (21 and 40, respectively).

The main aim of this cross-sectional study was to evaluate the serum concentrations of high sensitivity CRP (hsCRP) in a large population of normal weight women with PCOS, in an attempt to isolate the effect of obesity on CRP. A secondary aim was to identify possible correlation of hsCRP with the hormonal profile of women with PCOS.

Materials and Methods

Patients. One hundred and eighty-eight normal weight (BMI < 25 kg/m^2) women with PCOS (age range 18 - 38 years) were recruited from the outpatient clinics of a tertiary referral hospital ("Hippocration" General Hospital, Thessaloniki, Greece). The diagnosis of PCOS was set according to the criteria proposed by the Rotterdam ESHRE/ASRM-sponsored PCOS Consensus Workshop Group[35]. Forty-three normal weight women (age range 18 - 38 years) without PCOS served as controls. All controls had normal ovulating cycles (menstrual cycle length 28 ± 2 days; mid-luteal phase serum progesterone > 10 μg/L in two consecutive cycles) and no biochemical or clinical signs of hyperandrogenemia. In addition, none of the PCOS and control women had symptoms of any type of infection during the last two weeks or any type of chronic inflammatory disease in their past or present medical history. Declaration of Helsinki protocol was followed.

Sample size / Power analysis. According to the literature, we set these *a priori* parameters: mean hsCRP concentration 0.5 mg/dL for the PCOS group and 0.25 mg/dL for the control group, standard deviation of hsCRP concentration 0.5 mg/dL for both groups, PCOS to control ratio 4:1, type α error 0.5 and type β error 0.2 (power 80%). According to these assumptions, we had to involve 160 women with PCOS and 40 women as controls.

All women were recruited between March 2006 and April 2009. The study was approved by the Institutional Ethics Committee; informed consent was obtained from all women.

Hormonal and biochemical measurements. Blood samples were collected in early follicular phase (third to sixth day) of a menstrual cycle in the control group or during a spontaneous bleeding episode in the PCOS group. The samples were collected at 09:00 after an overnight fast. Studied parameters included serum concentrations of hsCRP, follicle-stimulating hormone (FSH), luteinizing hormone (LH), total testosterone, prolactin, Δ_4-androstenedione (Δ_4-A), dehydro-epiandrosterone sulfate (DHEA-S), 17α-hydroxy-progesterone (17α-OH-P), sex hormone-binding globulin (SHBG), glucose and insulin.

Serum levels of hsCRP were measured by a nephelometric technique (N high sensitivity CRP, Dade Behring). The assay sensitivity was 0.017 mg/dL and normal range < 0.5 mg/dL. Within-assay and between-assay coefficients of variation (CV) were 4.1 – 7.0% and 6.0 – 9.2%, respectively. LH, FSH, prolactin, total testosterone, Δ_4-A, DHEA-S and 17α-OH-P were measured with RIA methods; SHBG was measured with IRMA. All assay kits were available commercially: FSH, LH and prolactin from Nichols Institute Diagnostics; total testosterone, Δ_4-A, DHEA-S, SHBG and 17α-OH-P from Diagnostic Systems Laboratories. Serum insulin concentrations were measured with ELISA (Mercodia AB). Finally, plasma glucose concentrations were measured with the glucose oxidase technique on an automated analyzer (Roche/Hitachi 902; Roche Diagnostics).

Statistical analysis. The Kolmogorov-Smirnov test was used to test the normality of distribution; parameters that did not fit the Gaussian distribution were log-trans-

Table 1: Clinical characteristics and basic serum hormone concentrations of the studied women.

	PCOS	Controls	p value
Number of women	188	43	
Age	22.6 ± 0.3	25.9 ± 0.6	0.807
BMI (kg/m^2)	21.5 ± 0.1	21.4 ± 0.3	0.976
FSH (IU/L)	5.5 ± 0.1	8.6 ± 1.8	0.001
LH (IU/L)	7.9 ± 0.4	5.9 ± 0.3	0.020
Prolactin (μg/L)	14.5 ± 0.6	12.6 ± 1.0	0.307
Testosterone, total (ng/dL)	92.4 ± 2.1	37.8 ± 1.5	0.001
Δ_4-androstenedione (μg/L)	3.0 ± 0.8	1.6 ± 0.7	< 0.001
DHEA-S (mg/L)	2997 ± 82	1651 ± 84	0.001
17α-hydroxy-progesterone (μg/L)	1.10 ± 0.03	0.70 ± 0.04	< 0.001
SHBG (nmol/L)	32.2 ± 1.1	56.9 ± 4.1	< 0.001
Glucose (mg/dL)	88.9 ± 0.9	84.4 ± 1.6	0.025
Insulin (mIU/L)	14.6 ± 1.5	7.8 ± 0.8	0.001
Glucose : Insulin ratio	10.6 ± 0.4	13.4 ± 0.8	0.003
hsCRP (mg/dL)	0.55 ± 0.08	0.27 ± 0.08	< 0.001

PCOS: polycystic ovary syndrome; BMI: body mass index; SHBG: sex hormone-binding globulin; hsCRP: high sensitivity C-reactive protein. Values are given as mean ± standard error of the mean.

formed. Descriptive data are presented as mean ± standard error of the mean (SEM). For comparison of quantitative variables independent-samples t test were performed. Bivariate correlation analysis (calculation of the Pearson coefficient after log-transformation) was used to assess the correlation between serum hsCRP concentration and other study variables. Statistical significance was set at 5%. All p values were two-tailed. All analyses were performed with SPSS software (Version 14; SPSS, Inc, Ill, USA).

Results

Patient and control data. The anthropometric, hormonal, and metabolic parameters of the women studied are summarized in Table 1. As there were no significant difference in age or BMI between women with PCOS and controls, no adjustments were done for these parameters.

Comparison of hsCRP between PCOS and control. Serum hsCRP concentrations were significantly higher in women with PCOS as compared to controls (mean ± SEM 0.55 ± 0.08 versus 0.27 ± 0.08, p = 0.001). As expected, women with PCOS had significantly higher serum concentrations for LH (p = 0.002), total testosterone (p = 0.001), Δ_4-A (p < 0.001), 17α-OH-P (p < 0.001), DHEA-S (p = 0.001), glucose (p = 0.025), insulin (p = 0.001) and glucose-to-insulin ratio (p = 0.003).

Correlation of hsCRP with study variables. Calculation of the Pearson coefficient showed that serum hsCRP was positively but weakly correlated to LH ($R^2 = 0.045$), total testosterone ($R^2 = 0.032$), DHEA-S ($R^2 = 0.017$), Δ_4-A ($R^2 = 0.020$) and glucose ($R^2 = 0.062$) (Figure 1).

Discussion

The main aim of this study was to evaluate the serum concentrations of hsCRP in a large population of normal weight women with PCOS. We found that women with PCOS have higher hsCRP concentrations as compared to normal weight women without PCOS.

The studies that have been conducted during the last decade in order to evaluate the serum concentrations of CRP in women with PCOS failed to come into a safe conclusion[5-34]. On the contrary, our study provided a clear answer having three methodological advantages. First, it had the power to answer the research question, as an adequate number of women with PCOS and controls were recruited. Second, we studied exclusively women of normal weight in an attempt to exclude the known effect of obesity on serum CRP concentrations[36]. Theoretically, women of normal weight have reduced risk for cardiovascular disease as compared to overweight or obese women[37]. Nevertheless, even these young women have a marker of low- grade inflammation, suggesting that PCOS *per se* constitutes a state that could be associated to cardiovascular disease. Third, in our study, the group of women with PCOS was uniform as the diagnosis of PCOS was, in all cases, in accordance to the 2003 revised criteria[35].

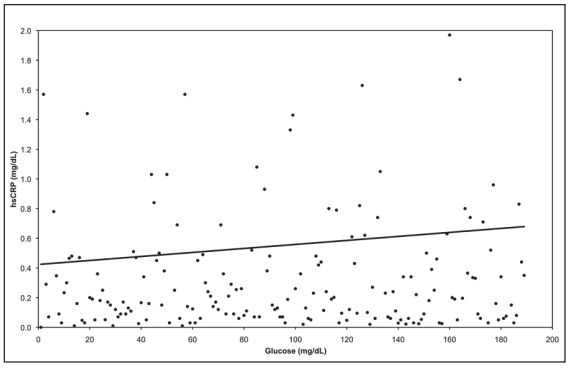

Figure 1: Scatter plot of serum hsCRP versus serum glucose concentrations in women with PCOS. hsCRP: high sensitivity C-reactive protein; PCOS: polycystic ovary syndrome.

A secondary aim was to identify possible correlation of hsCRP with the hormonal profile of women with PCOS. We found significant positive correlation between hsCRP and production of strong (total testosterone) and weak androgens (DHEA-S and Δ_4-A). Of course, this study was not designed to investigate if this correlation is a causative one (i.e. high androgen concentrations result in high hsCRP ones or vice versa) or it is due to a third, confounding factor.

In conclusion, this study showed that normal weight women with PCOS have higher concentrations of serum hsCRP as compared to normal weight women without PCOS. Given this fact, normal weight women with PCOS have to be considered as carrying at least one marker of low-grade inflammation. As the association of the latter with cardiovascular disease has been already established, further studies have to be focused on a possible association between PCOS and cardiovascular disease.

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