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HIGH-SENSITIVITY C-REACTIVE PROTEIN IN PATIENTS WITH POLYCYSTIC OVARY SYNDROME

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Abstract

Objective: *To assess the inflammation evaluated by high sensitivity C Reactive Protein (hsCRP) in women with polycystic ovary syndrome (PCOS) compared with healthy women without PCOS.* ***Methods:*** *This was a retrospective, case control, observational study. The study group included 31 patients with PCOS and 29 healthy patients matched for age and body mass index (BMI) but without PCOS (control group). PCOS was diagnosed using Rotterdam criteria.* ***Results:*** *Patients with PCOS had higher mean hsCRP levels compared with healthy controls: 3.89±2.75 mg/l in PCOS group and 2.61±1.81 mg/l in the control group, p=0.04. The difference was no longer significant after adjustment for BMI. In the PCOS group hsCRP was positively correlated with BMI, waist circumference, visceral fat area, body fat mass and glycated hemoglobin (HbA1c).* ***Conclusion:*** *hsCRP levels are increased in patients with PCOS and are correlated with obesity, fat accumulation and not with the presence of PCOS per se.*

key words: *polycystic ovary syndrome, inflammation, obesity*

Background

Polycystic ovary syndrome (PCOS) is one of the main causes of endocrine-dependent infertility, with a prevalence of 6.5-10% among women of reproductive age [1-3]. PCOS is often associated with obesity, mainly abdominal obesity [4] and insulin resistance [5]. The link between obesity and reproductive problems in women has been studied for a long time and is confirmed by numerous epidemiological and clinical studies [6]. It has been demonstrated that visceral fat is the most significant variable correlating with metabolic dysfunction in women with PCOS [7]. A recent review showed that visceral adipose tissue is involved in the secretion of various adipokines and vasoactive substances associated with an increased risk of metabolic and cardiovascular diseases [8].

High sensitivity C reactive protein (hsCRP) is a marker of low-grade subclinical inflammation. Increased levels of this protein are associated with abdominal obesity,

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diabetes mellitus and cardiovascular diseases. Many studies have demonstrated that CRP levels have important prognostic implications for patients [9]. For example, individuals with elevated CRP levels have an increased risk of cardiovascular events. The JUPITER study showed that reducing CRP levels can mitigate this risk [10]. A number of previous studies reported that PCOS is associated with increased hsCRP levels [11]. It is now clear that PCOS is a proinflammatory state and emerging data suggest that chronic low-grade inflammation underpins the development of metabolic disturbances and ovarian dysfunction in this disorder [12,13]. Recent evidence indicates a condition of low-grade chronic inflammation in PCOS that could be considered one of the potential links between PCOS and long-term metabolic (type 2 diabetes) and cardiovascular complications [11]. Many studies have demonstrated a positive relationship between CRP values and insulin resistance, body weight and fatty mass in women with PCOS [14,15]. These findings are further supported by the observation that 6 months of metformin treatment, an insulin sensitizing drug, is followed by a significant reduction in circulating levels of CRP [14,16,17].

The objective of this study was to assess the inflammation level evaluated by hsCRP in patients with PCOS compared with healthy women without PCOS, and to identify factors associated with hsCRP levels.

Materials and Methods

Study Design and Study Subjects

This was a retrospective, case control, observational study. Patients with PCOS addressing to a private healthcare network in Cluj-Napoca between 2009 and 2011 were identified through a retrospective review of the medical records and were included in the PCOS group. PCOS was diagnosed using the Rotterdam criteria for PCOS (presence of two out of three: oligo-ovulation or anovulation, clinical and/or biochemical signs of hyperandrogenism or polycystic ovaries, and exclusion of other etiologies) [18]. Only patients without any personal history of diabetes, dyslipidemia, arterial hypertension or cardiovascular disease were included in this study.

Healthy female patients matched for age and body mass index (BMI) but without PCOS were identified and included in the control group. Only patients with available results for the hsCRP were included in the study.

The following clinical, anthropometric characteristics, personal history and laboratory investigation results were collected from patients' files:

- Age, sex
- Weight, height, waist, hip circumference
- Body mass index (BMI) was calculated using the formula: weight (kg)/ height (m^2)
- Parameters describing body composition: visceral fat area (VFA), body fat mass (BFM) were measured by bioelectric impedance, using the InBody 720 analyser (Biospace, Korea)
- Laboratory analysis: fasting plasma glucose (FPG), total cholesterol, HDL cholesterol, LDL cholesterol, triglycerides (TG), insulinemia, hsCRP, HbA1c
- Personal history of dysglycemia, dislipidemia, arterial hypertension.

The study was conducted in accordance with the Declaration of Helsinki and approved by the Institutional Ethics Committee.

Statistical analysis

Statistical analysis was carried out using the SPSS-PC 13.0 (SPSS Inc., Chicago, IL, USA). Distribution of variables was tested with Kolmogorov - Smirnov test. Results were reported as means ± standard deviation (SD) for continuous variables with normal distribution, median (1^{st} and 3^{rd} quartile) for continuous variables with a distribution that differs from normal distribution and % for dichotomial data. T-test and Mann-Whitney tests (as appropriate) were used to compare data. According to variables distribution, Pearson or Spearman correlation coefficient was calculated. General linear model was used to adjust the mean value of the hsCRP levels for BMI. The level of significance was set at 5% ($p < 0.05$) in all analyses.

Results

Overall, 140 female patients diagnosed with PCOS were identified. Of these, in the current analysis were included 31 patients with available hsCRP results and without previous treatment (PCOS group). 29 healthy patients matched for age and BMI were included in the control group.

Anthropometric and metabolic characteristics of the patients included in the study are presented in Table 1.

Table 1. Metabolic and anthropometric characteristics of patients with PCOS and healthy controls.

Parameter	PCOS group (n=31)	Control group (n=29)	p-value
Age (years)	32.26 ± 8.29	33.96 ± 8.54	0.43
BMI (kg/m^2)	30.69 (26.90; 37.50)	29.70 (29.18; 36.26)	0.59
Waist circumference (cm)	98.80 (92.35; 118.00)	98.00 (86.50; 111.00)	0.52
FPG (mg/dl)	87.42 ± 12.02	87.65 ± 11.44	0.94
HbA1c (%)	5.38 ± 0.79	5.58 ± 0.38	0.39
Total cholesterol (mg/dl)	200.89 ± 47.81	186.35 ± 39.28	0.21
HDL cholesterol (mg/dl)	54.47 ± 14.90	54.08 ± 12.09	0.91
LDL cholesterol (mg/dl)	115.01 ± 42.66	112.29 ± 29.92	0.78
Triglyceride (mg/dl)	154.96 ± 81.53	99.90 ± 60.93	0.005
VFA (cm^2)	118.63 ± 32.92	116.58 ± 31.69	0.81
BFM (kg)	39.05 ± 18.41	35.67 ± 15.86	0.45

n = number of patients in each group; BMI = body mass index; FPG = fasting plasma glucose; HbA1c = glycated hemoglobin; VFA = visceral fat area; BFM = body fat mass

There was no statistically significant difference between the PCOS and control group in terms of waist circumference, BMI, body composition (VFA, BFM) and parameters evaluating glucose metabolism (FPG and HbA1c) ($p > 0.05$ in all cases). Additionally, both groups displayed similar values of lipid parameters: ($p > 0.05$), except for triglycerides: 154.96 mg/dl in PCOS group and 99.0 mg/dl in control group, $p = 0.005$.

Patients with PCOS had higher mean hsCRP levels compared with healthy controls: 3.89 ± 2.75 mg/l in PCOS group and 2.61 ± 1.81 mg/l in control group, $p = 0.04$ (Figure 1). After adjustment for BMI by using general linear model, this difference was no longer significant: 3.22 ± 0.76 mg/l in PCOS

group and 3.33 ± 0.83 mg/l in control group, p = 0.60.

Correlation coefficients between hsCRP levels and other parameters in patients with PCOS are showed in Table 2. In this group hsCRP was positively correlated with BMI (ρ = 0.547, p = 0.001), waist circumference (ρ = 0.517, p = 0.003), VFA (r = 0.585, p = 0.001), BFM (r = 0.479, p = 0.006) and HbA1c (r = 0.389, r = 0.03).

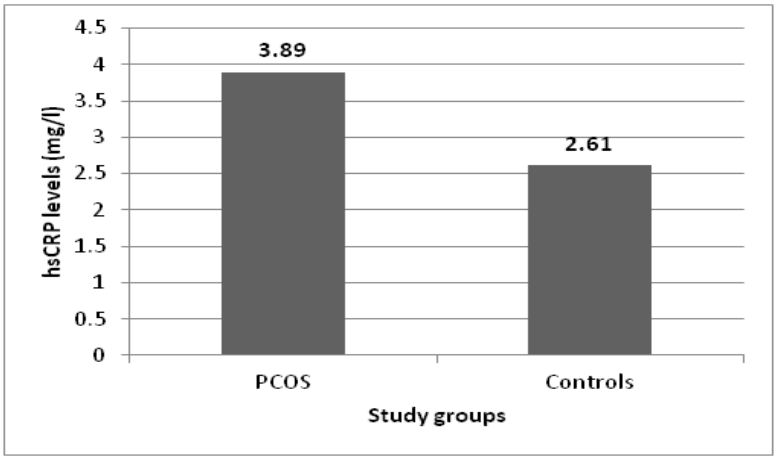

Figure 1. High sensitivity C reactive protein levels in patients with PCOS and controls.

Table 2. Correlation of hsCRP with other parameters in patients with PCOS.

Parameter	Correlation coefficient	p
BMI	**0.547***	**0.001**
Waist	**0.517***	**0.003**
VFA	**0.585**	**0.001**
BFM	**0.479**	**0.006**
FPG	**0.299**	**0.102**
HbA1c	**0.389**	**0.031**
Total cholesterol	**0.085**	**0.651**
HDL cholesterol	**0.140**	**0.454**
LDL cholesterol	**0.042**	**0.822**
Triglycerides	**0.031**	**0.869**

*Spearman correlation coefficient (calculated because BMI and waist circumference presented distribution that differs from normal distribution); Pearson correlation coefficient was calculated for all other variables

Discussion

Women with PCOS are known to be at increased risk for insulin resistance, impaired glucose tolerance, and type 2 diabetes mellitus. Also, women with PCOS are often obese, obesity being strongly associated with insulin resistance and chronic low-grade inflammation [19-21]. Circulating levels of the proinflammatory cytokine tumor necrosis factor-α (TNFα) are elevated in obesity, and are also elevated in PCOS independent of obesity [22,23]. In fact, the discovery of TNFα elevations in PCOS served as the initial indication that PCOS is a proinflammatory state. It seems there is a genetic basis for the chronic low-grade inflammation observed in PCOS. Several proinflammatory genotypes of genes encoding TNFα and the type 2 TNF receptor, as well as interleukin-6 (IL-6) and its signal transducer are associated with PCOS [24,25]. CRP is an acute phase reactant

produced by the liver following stimulation by IL-6 which is a cytokine produced by mononuclear cells and adipose tissue. It seems that IL-6 is directly responsible for stimulating hepatic C-reactive production [12,19]. Alterations in circulating CRP levels reflect an exacerbation or amelioration of inflammation, making it a useful measurement of inflammatory load in clinical practice [26-28]. Among the many biomarkers of inflammation proposed for diagnostic use, CRP measured by a highly sensitive assay (hs-CRP) has generated considerable attention. A hsCRP test measures low levels of CRP using laser nephelometry. The test gives results with a sensitivity of 0.04 mg/L [29]. In individuals without acute infections or inflammatory diseases (e.g. rheumatoid arthritis), levels of hsCRP remain stable over long periods of time. In addition hsCRP requires no special precautions for sampling, and has a relatively long half-life without the diurnal variation that plagues certain other biomarkers [30,31]. Numerous prospective cohort studies indicate that hsCRP predicts incident myocardial infarction, stroke, and cardiovascular death even after full adjustment for the traditional Framingham covariates [32].

We found that in this population of women with PCOS, hsCRP values were higher compared with healthy controls. In the same time, some anthropometric measures of obesity and abdominal obesity, as well as HbA1c, positively correlated with hsCRP.

Our findings in regard to CRP levels in women with PCOS are similar to those found in a study of Engin-Ustun et al, showing that CRP level was significantly higher in women with PCOS than in controls. However, in this study the difference could not be attributed to age, BMI, waist-to-hip ratio (WHR), and lipid profile [33].

A meta-analysis published in 2011 that included 31 clinical trials and 2359 women with PCOS showed that CRP levels are 96% higher in women with PCOS than in healthy subjects [24]. The results were similar after excluding five studies with mismatches in body mass, frequency of obesity, or both between women with PCOS and controls (102% relative increase in PCOS compared with controls; 95% CI, 73%– 131%; z = 6.93; P<0.0001; Egger's regression intercept, –0.79; 95% CI, –3.85 to 2.26; P=0.598) [24]. The meta-analysis also showed that elevated circulating CRP in PCOS is independent of obesity, because the finding persisted after excluding all the studies with mismatches in frequency of obesity or body mass between groups. This result is important because obesity is a well documented proinflammatory state that is independently associated with elevations in all three of these markers (IL-6, TNF-α and CRP) [22,34], and adipose tissue is a known source of IL-6 and TNF-α [8], the former of which stimulates CRP synthesis in the liver [35].

Concordant with our results, the same meta-analysis demonstrated that the degree of elevation in circulating levels of CRP (but also IL-6) in PCOS is much higher when obesity is also present [24].

In fact, several studies show that serum CRP elevations in women with PCOS are no longer statistically significant when controlling for indices of obesity such as body mass index [36,37] or that obesity alone is responsible for the CRP increase [38-40]. Moreover, Oh et al [41] and Mohlig et al [42] published 2 studies that showed that hsCRP levels were increased in women with PCOS,

but the levels were correlated with BMI and adipose tissue and not with the presence of PCOS *per se*. Also, the results of Boulman et al. [43] and Tarkun et al. [44], showed that the CRP elevation in normal weight women with PCOS (<3.0 mg/L) is much lower compared to obese women (>3.0 mg/L) regardless of whether or not they have PCOS. Thus, CRP elevations attributable to PCOS are obscured in the presence of obesity, and are below the range to predict metabolic or cardiovascular risk [13].

The possible limitations of our study, beside the relatively small number of subjects included, are the fact that independent variables, such as smoking, which has been associated with increased hsCRP levels, physical exercise and alcohol consumption (which are shown to reduce inflammation [9,45]), and other as yet unidentified variables were not taken into consideration in the statistical analysis.

Our study has also several strengths. One is the fact that we studied a drug-naïve population. Another is that there were no dietary restrictions in both groups, so that the hsCRP levels were not influenced by the dietary factors [46].

Conclusion

Our study showed that hsCRP levels, a circulating marker of the chronic proinflammatory state, are increased in patients with PCOS, but are correlated with obesity and fat accumulation and not with the presence of PCOS *per se*.

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