Chronic Inflammation in Women with Polycystic Ovarian Syndrome

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OBJECTIVE: Our aim was to assess whether C-reactive protein (CRP) which is not only a marker of inflammation but a strong predictor of atherosclerosis and coronary heart disease was increased among women with polycystic ovary syndrome (PCOS).
STUDY DESIGN This cross-sectional study was carried out on 18 women with PCOS and 20 healthy subjects matched for body mass index (BMI) and age. Androgenic hormones, anthropometric measurements, metabolic parameters and serum CRP levels were assessed. Further analysis whether there was a correlation between CRP and other parameters was carried out in the PCOS group as well.
RESULTS: The androgenic hormones total testosterone (54.51±13.58 vs. 25.0±14.82 ng/dL, p<0.001), and androstenedione (3.7±1.1 vs. 2.78±1.19 ng/dL, p=0.049) were higher in the study group than in control subjects. Dehydroepiandrostenedione sulfate (DHEAS) (191.66±68.79 vs. 179.85±86.32 mg/dL), fasting insulin (15.1±4.1 vs. 14.5±1.7µlu/mL) and postprandial glucose (105±12.7 mg/dL vs. 97.4±10.8 mg/dL) were similar. Fasting glucose was higher in the study group compared to controls (96.1±7.9 vs. 86.6±8.9 mg/dL, p=0.007). Mean CRP levels were higher in patients with PCOS than in healthy controls (1.40±0.98 mg/dL and 0.88±0.39 mg/dL respectively, p<0.001). Regression analysis revealed no correlation between CRP and other factors studied, except for a positive relationship that existed with BMI (r=0.286, p=0.045).
CONCLUSION: PCOS patients have increased levels of inflamation marker CRP which may also signal the tendency to develop cardiovascular disease in addition to established risk factors among PCOS patients.
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Key Words: Chronic inflammation, C-reactive protein, Polycystic ovary syndrome

The polycystic ovary syndrome (PCOS) is among one of the most common reproductive endocrine disorders, affecting approximately 5-10% of the female population.[1] It is a heterogeneous clinical condition, characterized by hirsutism, irregular menstrual cycles, infertility, and endocrine abnormalities such as hyperandrogenism and inappropriate luteinizing hormone (LH) secretion. Moreover, a considerable percentage of women with PCOS have insulin resistance and compensatory hyperinsulinaemia.[2] PCOS is associated not only with primary dysfunction of ovarian steroidogenesis, but also insulin postreceptor binding defects.[3,4]

Women with PCOS have multiple risk factors for diabetes such as obesity, a family history of type 2 diabetes, and abnormalities in insulin action including insulin resistance and beta-cell dysfunction. There is now clear evidence that women with PCOS are also at increased risk for developing type 2 diabetes.[5] Insulin-resistant states are associated with greater susceptibility to coronary heart disease, and women with PCOS have evidence of dyslipidemia and abnormal vascular function.[6,7] There are several lines of evidence suggesting that women with PCOS are at increased risk of cardiovascular disease, and may have increased risk for myocardial infarction up to 7.4-folds.[8]

Inflammatory process appears to play a crucial role in the pathogenesis of atherosclerotic process and cardiovascular disease. Inflammation is an important contributor to atherothrombosis. It mediates the initial stages of atheroma development including leukocyte recruitment and eventual rupture of the unstable atherosclerotic plaque.[9,10] Low-grade chronic inflammation is associated with insulin resistance and type II diabetes mellitus[11] and is a risk factor for the development of coronary heart disease.[12,13] C-reactive protein (CRP), a hepatically-derived acute phase reactant protein, is a marker for inflammation.[14] Chronic low-grade systemic inflammation, usually a persistent but more subtle than acute phase inflammatory response, can be measured by circulating CRP which may also have direct pro-inflammatory actions. CRP may reflect the response of the body to inflammatory reactions in atherosclerotic vessels or myocardium. CRP may have a direct role in atherogenesis via adhesion molecule expression, complement activation, and mediation of low density lipoprotein (LDL) uptake by macrophages.[14] Recent data suggest that CRP is also produced in the atherosclerotic lesions[15] and it has been shown to predict myocardial infarction, death due to coronary artery disease, stroke, peripheral arterial disease and sudden death.[16]

A recent interest is focused on PCOS regarding the markers of inflammation and their contribution to the increased risk of cardiovascular disease however current data is scarce on this subject. In the present study we aimed to assess the level of CRP concentrations in patients with PCOS in order

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to show whether CRP a marker of inflammation is increased in patients with PCOS or not.

Materials and methods

This cross-sectional study was carried out in the Department of Obstetrics and Gynecology of the Ankara University. Eighteen women diagnosed with PCOS (study group), and 20 healthy volunteer women matched for age and body mass index (BMI) (control group) were enrolled in this study. The diagnosis of PCOS was based on the positive findings of hyperandrogenism, anovulation, elevated serum luteinizing hormone (LH) concentrations or LH/FSH ratio, increased concentrations of serum testosterone, androstenedione and dehydroepiandrostenedione sulfate (DHEAS), ultrasound evidence of bilateral enlarged polycystic ovaries and the absence of ovarian or adrenal neoplasm or Cushing's syndrome. Although patient recruitment began before the establishment of Rotterdam criteria, in the final analysis each patient with PCOS in the study met diagnostic criteria for PCOS based on the Rotterdam criteria.[17] Anovulation was defined as the presence of amenorrhea or oligomenorrhea (cycle length >35 days) and the absence of ovarian follicular activity on serial ultrasound scans. Patients who had used oral contraceptives, any hormonal, antidiabetic or antiobesity drugs within the last 3 months, those with hyperprolactinaemia (serum prolactin concentrations >20 µg/L on two different occasions), thyroid dysfunction, or hypertension and current smokers were excluded. None of the patients had evidence of acute or chronic infection at the time of recruitment, which was assessed by history, physical examination and white blood cell count. All of the women in the control group had a regular menstrual cycle, ranging between 26 and 30 days. The normal ovulatory state of healthy subjects was confirmed by measuring serum progesterone concentration in the luteal phase. A luteal progesterone level >10ng/ml was considered the criteria for ovulation to have occurred.

Anthropometric measurements included waist/hip ratio (WHR) (waist circumference recorded at the narrowest point divided by hip circumference at the level of the greater trochanter), and BMI (kilograms per meter squared). The score for hirsutism was determined according to the original method described by Ferriman and Gallwey.[18]

A 300 g carbohydrate diet of 3-day was given to all patients before blood samples were taken. The hormonal and metabolic assessments were made between days 2 and 5 of the menstrual cycle and if the patients were amenorrheic, uterine bleeding were induced by the intake of medroxyprogesterone acetate (Farlutal 5 mg; Pharmacia, Istanbul, Turkey) (10 mg×10 days). After an overnight fast, blood samples were drawn from the antecubital vein for determination of blood glucose and insulin. Serum was stored at –20°C until analyzed. Blood samples were also drawn for luteinizing hormone (LH), follicle stimulating hormone (FSH), estradiol (E2), dehydroepiandrosterone sulphate (DHEA-S), total testosterone, androstenedione and C-reactive protein (CRP). All samples were obtained between 08 A.M. and 10 A.M. After 2 hour of a 75 g oral glucose tolerance test (OGTT), postprandial serum glucose concentration was measured. The study was performed with the approval of the Institutional Ethical Committee of the School of Medicine. Written consent for participation was obtained after the design and aim of the study was explained to all participants.

Assays

FSH, LH, estradiol, androstenedione, total testosterone, DHEA-S and insulin levels were measured using chemilumiescent enzyme immunoassay (Immulite Automated Analyzer and commercial kits, DPC-Diagnostic Products Corporation, Los Angeles, USA). Blood glucose was measured by hexokinase method (Olympus AU 600 autoanalyzer, NY, USA) Serum concentrations of CRP were measured by rate immunonephelometry using automated instrumentation (Automated Immunochemistry System, Beckman Instruments Inc, Fullerton, CA). The level of detection was 0.4 mg/dL.

Statistical analysis

Statistical analysis was performed using Statistical Package for Social Sciences (Windows version 11.0, SPSS Inc, Chicago, IL) program. Differences between patients with PCOS and controls were analyzed using Student's t-test and Mann-Whitney U test, where applicable. Correlation between CRP and age, BMI, WHR, fasting glucose, fasting insulin, total testosterone, DHEAS, and androstenedione were assessed by linear regression analysis. $P<0.05$ was considered statistically significant.

Results

The clinical and hormonal profile of PCOS and control groups are shown in Table 1. The mean age (29.7±5.8 vs. 29.9±4.75), mean BMI (25.68±4.12 vs. 24.42±2.48) and WHR (0.81±0.06 vs. 0.76±0.06) were similar between the study and control group. The clinical hirsutism score assessed by Ferriman and Gallwey was significantly higher in patients with PCOS compared to control patients (11.2±2.2 vs. 6.9±1.52, $p<0.001$). LH/FSH ratio (1.82±0.43 vs. 0.89±0.57, $p=0.001$), mean serum levels of total testosterone (54.51±13.58 vs. 25.0±14.82 ng/dL, $p<0.001$), and androstenedione (3.7±1.1 vs. 2.78±1.19 ng/dL, $p=0.049$) were higher in the study group than in the control group. No difference was found in the mean levels of DHEAS between the study and control group (191.66±68.79 vs. 179.85±86.32) (Table 1).

Mean fasting glucose was significantly higher in patients with PCOS compared to controls (96.1±7.9 mg/dL and 86.6±8.9 mg/dL respectively, $p=0.007$), but no difference was observed in fasting insulin (15.1±4.1 vs.14.5±1.7 µIu/mL) and postprandial blood glucose levels (105±12.7 vs. 97.4±10.8 mg/dL) between the groups. Mean C-reactive

Table 1. Anthropometric measurements, endocrine profile, metabolic parameters and CRP levels in patients with PCOS and healthy subjects.

Variable	PCOS (n=18)	Control group (n=20)	P value
Age (yr)	29.7±5.8	29.90±4.75	NS
BMI (kg/m^2)	25.68±4.12	24.42±2.48	NS
WHR	0.81±0.06	0.76±0.06	NS
Hirsutism score	11.2±2.2	6.9±1.52	<0.001
LH (mIU/mL)	10.98±2.68	4.28±1.54	0.001
FSH (mIU/mL)	6.08±0.9	5.41±1.44	NS
LH/FSH ratio	1.82±0.43	0.89±0.57	0.001
Estradiol (pg/mL)	51.6±22.6	60.8±29.8	NS
Total testosterone (ng/dL)	54.51±13.58	25.0±14.82	<0.001
DHEAS (µg/dL)	191.66±68.79	179.85±86.32	NS
Androstenedione (ng/mL)	3.7±1.1	2.78±1.19	0.049
Fasting insulin (µIu/mL)	15.1±4.1	14.5±1.7	NS
Fasting glucose (mg/dL)	96.1±7.9	86.6±8.9	0.007
Postprandial glucose (mg/dL)	105±12.7	97.4±10.8	NS
CRP (mg/dL)	1.40±0.98	0.88±0.39	<0.001

All values are given as mean ± SD
NS = not significant
BMI: Body mass index, WHR: waist/hip ratio, LH: luteinizing hormone, FSH: follicle stimulating hormone, DHEAS: dehydroepiandrosterone sulphate, CRP: C-reactive protein, PCOS: polycystic ovarian syndrome.

protein level was found to be significantly higher in patients with PCOS compared to control subjects (1.40±0.98 and 0.88±0.39 mg/dL respectively, p<0.001).

In the PCOS group, we further analyzed whether a correlation exists between CRP and age, BMI, WHR, fasting glucose and insulin, postprandial glucose and endocrine parameters including androstenedione, DHEAS, and total testosterone. No correlation was found between CRP and any of the parameters studied, apart from a positive relationship that existed with BMI (r=0.286, p=0.045).

Discussion

Women with PCOS have a wide range of cardiovascular risk factors such as obesity, lipid abnormalities, impaired glucose tolerance, and hypertension. More recently increased CRP level has also added to this list.[19-21]

Data suggests that atherothrombosis is not only a simple lipid accumulation but associated with chronic inflammatory process as well. Elevated CRP levels have been generally considered non-specific but sensitive markers of the acute inflammatory response.[9] Rather than being simply a marker of inflammation, involvement with the atherogenic process by promoting endothelial dysfunction and complement activation is also suggested.[22,23] A number of epidemiological studies have shown that the acute-phase reactant CRP is an important risk factor for atherosclerosis and coronary heart disease.[24,25] Among many inflammation markers such as serum amyloid A, interleukin-6, soluble intercellular adhesion molecule type 1, and homocysteine, high-sensitivity C-reactive protein (hs-CRP) was demonstrated as the strongest univariant predictor of death from coronary heart disease, nonfatal myocardial infarction or stroke, or the need for coronary-revascularization procedures.[12] Patients with PCOS are also at increased risk for developing type 2 diabetes.[19] Recently, it has been repeatedly demonstrated that increased CRP level is an independent risk factor predicting development of type II diabetes. Consistently elevated CRP levels in PCOS might in part be responsible for the increase in the risk of diabetes through an inflammatory process.[25,26]

Women with PCOS are also at increased risk for subclinical atherosclerotic disease as demonstrated by thicker carotid intima media thickness (IMT) and higher levels of coronary calcifications.[7,27,28] Talbott and associates evaluated subclinical atherosclerosis among women with PCOS and age-matched control subjects, and demonstrated that PCOS remained an independent risk factor of IMT even after adjustment for BMI.[28] In another study, the same authors found that PCOS was associated with IMT independent of insulin and visceral fat.[29] In their study, CRP levels were significantly higher in PCOS than in control subjects, however the association between PCOS and IMT was independent of CRP, which was primarily driven by BMI.

Kelly et al. conducted a prospective study on 17 women with PCOS and 15 healthy women matched as a group for body mass index to evaluate low grade chronic inflammation in PCOS.[27] Women with PCOS had significantly elevated CRP concentrations relative to controls (2.12 and 0.67 mg/L, respectively; P=0.016). Similarly Boulman et al. demonstra-

ted higher CRP levels in a large group of PCOS patients (n=116) than in a similar control group (n=94).[30] None of the patients had any sign of chronic inflammation, and they suggested that the presence of low grade chronic subclinical inflammatory process might explain the mechanism of atherosclerosis in some of the patients with PCOS. In another study Tarkun et al. showed low-grade chronic inflammation in PCOS is indicated by the presence of several elevated markers such as CRP levels.[31] Üstün et al conducted a study on 30 women with PCOS, 30 women with PCO (women in this group had regular, spontaneous ovulatory menstrual cycles, no clinical or biochemical evidence of PCOS, and a baseline transvaginal ultrasound scan showing polycystic ovaries), and 30 healthy women matched as a group for body mass index to evaluate low grade chronic inflammation in PCOS.[32] Serum CRP levels were significantly higher in the women with PCOS and PCO than in controls, whereas no difference was found between PCOS and PCO women with regard to CRP.

Conflicting with these studies Möhlig et al. concluded that, plasma CRP levels were not increased in patients with PCOS when compared with age- and BMI-matched controls.[33] They found BMI as the parameter most strongly related to CRP concentrations in PCOS and control subjects. Moreover they demonstrated a close correlation between CRP and insulin resistance, but not with any of the endocrine factors in these patients, and suggested that obesity and metabolic alterations were the main factors that significantly impact chronic inflammation. In line with this, a decrease in BMI in obese women was shown linked to decreased CRP levels.[34] Bickerton et al. found no differences in endothelial function between women with polycystic ovary syndrome (n=11) and age and weight matched controls (n=12) by standard venous occlusion plethysmography technique to measure reactive hyperaemic forearm blood flow.[35] Also they revealed no significant differences in glucose, lipid, or lipoprotein concentrations between the two groups and none of the novel biochemical markers (CRP, sialic acid, fibrinogen, homocysteine) of cardiovascular risk was raised in PCOS group.

Two previous studies have examined endothelial function in PCOS. Mather et al, using brachial artery ultrasound, found no evidence of endothelial dysfunction in healthy women with PCOS compared with age but not weight matched controls.[36] Conversely, Paradisi et al, using leg blood flow responses to the vasodilator methacholine chloride, compared age and weight matched controls with obese patients with PCOS.[37] They were able to demonstrate endothelial dysfunction in the patients with PCOS compared with the control group. Nasiek et al demonstrated statistically significantly higher CRP concentration in females with PCOS compared to healthy individuals in BMI≤25 and BMI>25 subgroups.[38]

In the present study, we found a significant difference with respect to CRP levels between the study and control groups matched for age and BMI. We suggest that the presence of low grade chronic inflammation as reflected by increased CRP concentration may contribute to the increased risk of atherosclerosis and cardiovascular disease in women with PCOS, in addition to known risk factors such as insulin resistance, hypertension, central obesity, and dyslipidemia. Even though CRP is an acute phase reactant we believe the ongoing but subdued acute inflammmation associated with PCOS causes the elevation.

Importantly, the study and control groups were similar with respect to anthropometrical measurements including BMI, WHR and age. As consistently demonstrated we found a positive correlation between CRP and BMI, however no correlation observed between CRP and any of the other parameters including WHR, androgens, fasting insulin and glucose, and postprandial glucose.

The small numbers of the study may indicate lack of power and thus possible associations of CRP with other hormonal parameters could not have become evident. This is a limitation of the study.

Another limitation may be the use of the routine automated procedure to measure CPR instead of high sensitive hs-CRP method. However we believe this does not change the results of our study because even with a relatively non sensitive system like routine automated CRP measurement we found a significant difference in CRP levels of PCOS patients as compared control group.

In summary, CRP is a marker of inflammation that shows a significant correlation with BMI. PCOS might confer a state of low grade chronic inflammation. CRP may be an ideal marker for screening of apparently healthy young PCOS patients to predict cardiovascular morbidity and mortality. We need large scale prospective randomized studies to make a clearer conclusion regarding the association between PCOS and chronic inflammation.

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