Increased Serum Neopterin Levels in Women with Polycystic Ovary Syndrome

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Abstract. Polycystic ovary syndrome (PCOS) occurs in 5-10% of premenopausal women. Studies suggest that PCOS is associated with increased risk of coronary heart disease (CHD). To investigate this relationship, 15 PCOS women (group 1) and 10 healthy women (group 2) were studied. Blood leukocyte counts (white blood cells, WBC) and serum levels of total cholesterol, HDL-cholesterol, LDL-cholesterol, sensitive C-reactive protein (sCRP), and neopterin were measured in the 2 groups. There were no significant differences in serum total cholesterol, HDL-cholesterol, or LDL-cholesterol concentrations between groups 1 and 2. Blood WBC counts and serum levels of neopterin and sCRP were significantly higher in group 1 than group 2. The median (min-max) levels were: WBC, group 1: 8.05 (5.10-9.70) cells x 10^9/L, group 2: 6.25 (4.70-9.70) cells x 10^9/L (p <0.01); neopterin, group 1: 10.6 (7.5-49.5) nmol/L, group 2: 9.6 (6.5-12.9) nmol/L (p < 0.05); and sCRP, group 1: 7.0 (1.2-12.0) mg/L, group 2: 2.0 (0.1-12.0) mg/L (p <0.01). This study shows that blood WBC counts and serum sCRP and neopterin levels are significantly elevated in women with PCOS. These findings support an increased risk for early-onset cardiovascular disease in women with PCOS. This is the first report that women with PCOS have higher serum neopterin levels than healthy women with regular menstrual cycles.

Keywords: polycystic ovary syndrome, neopterin, C-reactive protein, cardiovascular disease, WBC count

Introduction

Polycystic ovary syndrome (PCOS) is seen in 5-10% of premenopausal women, as first reported in 1935 by Stein and Leventhal [1]. Women with PCOS have a number of reproductive abnormalities: chronic anovulation with oligomenorrhea, obesity, enlarged cystic ovaries, hyperandrogenism, and infertility [2]. PCOS is associated with increased risk of coronary heart disease (CHD) [3-5], including 7.4-fold increase in relative risk for myocardial infarction [3]. There is growing interest in the metabolic abnormalities that occur in women with PCOS; these resemble the characteristic findings of the metabolic syndrome. Central obesity, hyperinsulinemia, insulin resistance, glucose abnormalities predictive of type 2 diabetes mellitus, dyslipidemia, and hypertension have been associated with PCOS [6]. Low grade chronic inflammation is predictive of CHD [7] and also is involved in the development of the metabolic syndrome. Impaired glucose tolerance, dyslipidemia, hypertension, increased coagulation, impaired fibrinolysis, and inflammation have important roles in atherosclerosis [8]. Serum neopterin and sCRP concentrations are reported to be elevated in patients with CHD, insulin resistance, and other systemic inflammatory diseases [9].
Neopterin is a prerdine derivative that is produced by activated macrophages and is a marker of immune cell activation [10]. Serum neopterin levels are increased in patients with acute myocardial infarction [11] and in unstable coronary syndromes [12]. Little is known about serum neopterin levels in patients with increased cardiac risk factors but without obstructive coronary artery disease.

Inflammation plays an important role in the progression and complications of atherosclerosis. C-reactive protein (CRP), a non-specific marker of inflammation, is one of the strongest predictors of the risk of cardiovascular events even in patients without cardiovascular disease [13]. PCOS has been linked to elevated serum CRP levels and high CRP levels may explain why some PCOS women have increased risk for development of early-onset cardiovascular disease [14]. Atherogenesis represents an active, inflammatory process rather than simply passive endothelial injury with infiltration of lipids. Blood leukocytes (WBC) play a major role in these inflammatory processes [15] and elevated blood WBC count is a known risk factor for atherosclerotic vascular disease in adult women.

To evaluate PCOS as a risk factor for atherosclerosis and cardiovascular disease, this study was designed to detect chronic inflammation and cardiovascular risk by measuring blood WBC counts and serum concentrations of total cholesterol, HDL-cholesterol, LDL-cholesterol, sCRP, and neopterin in women with PCOS and in healthy control women.

Materials and Methods

Women with PCOS (n = 15), who had been diagnosed at the Department of Endocrinology of Ege University Hospital, were randomly selected from the outpatient clinic population. Informed consent was obtained from all participants. Women with body mass index (BMI) ≥25 kg/m² were excluded from the study to rule out the effects of obesity.

Diagnosis of PCOS was based on the PCOS index [16], including (1) hyperandrogenemia, (2) oligo-anovulation, and (3) exclusion of other disorders, such as Cushing syndrome and hyperprolactinemia. Hyperandrogenemia was defined as a serum free testosterone level >3.2 pg/dl (reference range 0.8-3.2 pg/dl). Oligomenorrhea was defined as <6 menstrual periods/yr. Anovulation was found in all patients with serial weekly serum progesterone levels <2.5 ng/ml, starting on the 20th day of the menstrual cycle. The women with PCOS all had clinical manifestations of hyperandrogenism, such as a hirsutism score >8 [17] and/or acne. The control group comprised 10 healthy women with regular menstrual cycles and no signs of hyperandrogenism or inflammatory diseases. Other endocrine disorders (eg, diabetes mellitus, impaired glucose tolerance, thyroid dysfunction, and hyperprolactinemia) were excluded by measurements of serum glucose, oral glucose tolerance test, serum T₃, free T₄, TSH, and prolactin levels. None of the patients or controls had a special diet or used nutritional supplements; they did not receive sex hormones, oral contraceptives, or other medications, such as statins (lipid lowering drugs) or anti-inflammatory drugs. None of the patients gave clinical evidence (medical history or physical examination) of recent or ongoing infections. Cigarette smoking and ethanol consumption were also exclusion criteria.

For hormone analyses, blood samples were drawn early in the morning (07:30-08:00 AM) during the first 5 days of the follicular phase. The subjects were fasted overnight for 12 to 14 hr prior to blood collection. Serum samples were stored at -20°C prior to analyses of neopterin and sCRP. Serum total cholesterol, HDL-cholesterol, and triglycerides were assayed with an automatic analyzer (Technicon Dax-48, Bayer Diagnostics, Toshiba, Tokyo, Japan). Serum LDL-cholesterol was calculated by Friedewald’s equation: LDL-cholesterol = total cholesterol - HDL-cholesterol - (triglyceride / 5). Body mass index was calculated according to the following equation: BMI = body weight (kg) / body height squared (m²). Serum sCRP levels were measured with an Hitachi 704 automatic analyzer (Boehringer Mannheim GmbH, Mannheim, Germany) using latex particle-enhanced turbidimetry (PET) kits (Roche Diagnostics GmbH, Mannheim, Germany). Serum neopterin was measured by reversed phase HPLC with fluorescence detection [18].

The Statistical Package for Social Sciences (SPSS version 11.0) was used for nonparametric statistical analyses. The Mann-Whitney U test was used for comparison of two independent groups and the Pearson rank correlation test was used for correlation of two independent groups. Data were expressed as median (min-max). Differences with p-values <0.05 were judged statistically significant (two-tailed test).

Results

The median (min-max) values for demographic and biochemical characteristics of the 2 groups of women are listed in Table 1. Age and BMI did not differ significantly between the women with PCOS and controls. The median levels of serum total cholesterol, HDL-cholesterol, and LDL-cholesterol were comparable. Blood WBC, serum neopterin, and serum sCRP concentrations were significantly higher in patients than controls. There were no significant correlations between blood WBC count, monocyte/macrophage count (data not shown), neopterin level, and sCRP level in the PCOS...
patients. Frequency distribution curves of serum neopterin levels in the PCOS and control groups are shown in Fig. 1.

**Discussion**

PCOS is a common disorder of premenopausal women characterized by hyperandrogenism and chronic anovulation without adrenal 21-hydroxylase deficiency, hyperprolactinemia, or an androgen secreting neoplasm [16]. In addition to the various metabolic abnormalities mentioned in the Introduction to this paper, women with PCOS have been reported to have increased risks of diabetes mellitus type 2, dyslipidemia, hypertension, and atherosclerosis [19]. The main finding of our study was that PCOS is associated with elevations of 3 inflammatory markers: blood WBC count,
serum sCRP level, and serum neopterin level. These inflammatory markers suggest a link between PCOS and cardio-vascular disease.

Obese women were excluded from this study, so the effect of body fat on insulin resistance was minimal. In this study there were no significant differences of serum total cholesterol, HDL-cholesterol, or LDL-cholesterol levels between the PCOS and control groups. Bouman et al [14] also observed that serum total cholesterol, HDL-cholesterol, and LDL-cholesterol levels were not significantly different in 116 PCOS women, compared to 91 BMI-matched controls. On the other hand, Sam et al [20] reported that serum total cholesterol and LDL-cholesterol levels were significantly higher in sisters with PCOS, compared to unaffected sisters or to control women. They associated these abnormalities with hyperandrogenism. Margolin et al [21] reported that PCOS women had dyslipidemia compared to controls, but women in their PCOS group were all postmenopausal. The PCOS group had significantly higher BMI and some PCOS women had diabetes [21].

In the present study, women with PCOS had significantly higher sCRP levels than those in the control group. Low grade chronic inflammatory processes associated with atherosclerosis may be reflected by small increases of serum CRP levels that are only detectable with highly sensitive assays [22]. CRP has been shown to be one of the strongest predictors of increased risk of cardiovascular events [23,24]. Elevated serum CRP levels reflect increased proinflammatory activity in PCOS [14,25,26]. PCOS women without any other apparent chronic disease may have an increased risk for CVD compared to women of similar age and BMI who have normal menstrual cycles.

Serum neopterin concentrations are elevated in many pathological conditions, including viral infections, renal transplant rejection, coronary artery disease, insulin resistance, severe systemic inflammatory diseases, nephrotic syndrome, autoimmune diseases, psoriasis, and certain malignancies [17]. Within atherosclerotic plaques, the local number of inflammatory cells is increased, particularly macrophages and activated lymphocytes [27]. Activated lymphocytes in atherosclerotic plaques produce interferon γ that activates macrophages and the activated macrophages synthesize neopterin [28]. In our study, there was no correlation between the blood monocyte/macrophage count and the serum neopterin level. Possibly the activated monocyte/macrophages were increased within plaques, but not in the circulation.

Serum neopterin concentration appears to be a useful tool to monitor monocyte/macrophage activation in atherosclerotic vascular disease [31]. Previous studies reported that serum neopterin levels are increased in acute myocardial infarction [11], unstable coronary syndrome [12], and chronic stable angina [9]. Avanzas et al [32] found that high serum neopterin levels predicted adverse cardiac events in patients with hypertension but without obstructive coronary artery disease; they recommended that patients with high serum neopterin levels require aggressive risk factor modification.

Neopterin (and its reduced form, 7,8-dihydro-neopterin, which is also secreted by activated macrophages) interfere with formation of oxygen free radicals; neopterin exhibits prooxidant activity and 7,8-dihydronoepterin exhibits antioxidant activity. Consequently, the serum neopterin level can be regarded as an indirect estimate of the degree of oxidative stress during cell-mediated immune responses [29,30].

Previous studies suggested that PCOS women of premenopausal age have increased risk of cardiovascular disease [2-6]. In the present study, serum neopterin levels in PCOS women were significantly higher than in healthy controls. This is the first report that women with PCOS have elevated serum neopterin levels. This finding suggests that macrophage activation occurs in women with PCOS, as has been reported in cardiovascular disease.

Elevated blood WBC count is associated with cardiovascular disease [15]. Brown et al [31] found that, after adjustments for smoking and other cardiovascular risk factors, individuals with blood WBC counts >7.6 x10⁹/L had 40% increased risk of CHD, compared to individuals with WBC count <6.1 x10⁹/L. In the present study, the blood WBC count was significantly higher in women with PCOS compared to controls. Orio et al [33] also found that WBC count was significantly higher in 150 age- and BMI-matched PCOS
women compared to 150 controls. On the other hand, Boulman et al [14] found that WBC counts were not significantly different in 116 women with PCOS, compared to 91 BMI-matched controls.

The main limitation of the present study was the small number of PCOS women and healthy control women who were investigated, which resulted in a lack of statistical power. However, the results of this study suggest that increased sCRP, neopterin, and WBC concentrations may contribute to an increased risk of cardiovascular disease in women with PCOS. In addition to well-established CHD risk factors (such as hypertension, insulin resistance, obesity, and dyslipidemia), PCOS is emerging as a new risk factor for CHD. This study shows for the first time that women with PCOS have significantly higher serum neopterin levels than healthy women with regular menstrual cycles.

References

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