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RAPID COMMUNICATION

The Increase of Leukocytes as a New Putative Marker of Low-Grade Chronic Inflammation and Early Cardiovascular Risk in Polycystic Ovary Syndrome

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White blood cell (WBC) count is a known risk factor for atherosclerotic vascular disease in adult women. Polycystic ovary syndrome (PCOS) is potentially a risk factor for atherosclerosis and cardiovascular disease. The aim of the present study was to investigate leukocyte count in PCOS. One hundred and fifty PCOS women matched for age and body mass index with 150 healthy women were enrolled. WBC count, C-reactive protein, and a complete anthropometrical, metabolic, and hormonal evaluation were performed in both groups. Serum insulin, glucose level, and lipid profile were also measured in each subject. WBC count was significantly higher ($P < 0.0001$) in PCOS with (interquartile range in *parentheses*) 7260 (393) cells/mm^3, compared with controls with 5220 (210) cells/mm^3. C-reactive protein levels were significantly increased ($P < 0.0001$) in PCOS with 2 (1) mg/liter compared with healthy women with 0.7 (0.8) mg/liter. In both groups, there was a significant ($P < 0.0001$) linear correlation between WBC count and homeostasis model assessment score (PCOS, r = 0.94; controls, r = 0.91). Multiple linear regression analysis showed that other hormone levels are not predictors of leukocyte count both in PCOS and control women. In conclusion, our data demonstrate that PCOS women have an increased WBC count that correlates with homeostasis model assessment values. (*J Clin Endocrinol Metab* 90: 2–5, 2005)

POLYCYSTIC OVARY SYNDROME (PCOS) is a common endocrine-metabolic disorder associated with long-term health risks, including diabetes mellitus (1–4) and coronary artery disease (5, 6). In particular, insulin resistance, hyperandrogenemia, and dyslipidemia are likely to be the major risk factors for the occurrence of cardiovascular disease in PCOS (6, 7).

Inflammation is now thought to play a key role in the pathophysiological mechanism of atherosclerosis (7, 8) and cardiovascular disease (9). Several markers of inflammation, such as C-reactive protein (CRP), IL-6, soluble intercellular adhesion molecule type 1, and white blood cell (WBC) count, are found to be significant predictors of the risk of coronary heart disease (10) and future cardiovascular events (10). In particular, an elevated WBC count is a risk factor for atherosclerotic vascular disease (11) and is present in adult women with genetic predisposition to type 2 diabetes (12). Inflammation may also be associated with the metabolic syndrome (13, 14) and a raised WBC count (14). Therefore the association of leukocyte count with cardiovascular risk factors may represent a manifestation of subclinical disease, or alternatively leukocyte count could be part of a chain leading to atherosclerosis (15).

Recently, low-grade chronic inflammation has been linked to the insulin-resistance syndrome (16), and it was reported that women with PCOS have significantly increased CRP concentrations (13). The present study was performed to investigate the relationship between leukocyte count and hormonal-metabolic features in women with PCOS.

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Abbreviations: A, Androstenedione; BMI, body mass index; CRP, C-reactive protein; DHEAS, dehydroepiandrosterone sulfate; E2, 17β-estradiol; HOMA, homeostasis model assessment; 17-OHP, 17-hydroxyprogesterone; P, progesterone; PCOS, polycystic ovary syndrome; PRL, prolactin; T, testosterone; TV-USG, transvaginal ultrasonography; WBC, white blood cell; WHR, waist/hip ratio.

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Subjects and Methods

The procedures used were in accordance with the guidelines of the Helsinki Declaration on human experimentation. The study was approved by the Institutional Review Board of the University of Naples, Italy. The purpose of the protocol was explained both to the patients and control women, and written consent was obtained at the beginning of the study.

Subjects

One hundred and fifty women with PCOS were enrolled in this study. The diagnosis of PCOS was made according to the National Institutes of Health criteria (17). One hundred and fifty women were enrolled and considered as the control group. PCOS and control groups were matched for age and body mass index (BMI). The controls were defined as age and BMI matched with PCOS cases when the differences between the cases and controls were less than 2 yr and 1 kg/m^2 for age and BMI, respectively. The subjects were selected in Naples (Department of Endocrinology) and in Catanzaro (Department of Obstetrics and Gynecology). Specifically, 78% of the PCOS were enrolled in Naples, and 100% of controls were enrolled in Catanzaro. To match adequately the cases and the controls, we screened 354 healthy women who attended a cervical cancer prevention clinic (University of Catanzaro) for a routine Pap test. The healthy state of the women in the control group was determined by medical history, physical and pelvic examination, and complete blood chemistry. Their normal ovulatory state was confirmed by transvaginal ultrasonography (TV-USG) and plasma progesterone (P) assay. Both procedures were performed during the luteal phase of the menstrual cycle (7 d before the expected menses). The presence of fluid in the cul-de-sac at TV-USG and a plasma P assay greater than 31.8 nmol/liter (>10 ng/ml, metric units) were considered criteria for ovulation having occurred (18).

For all subjects, exclusion criteria included pregnancy, hypothyroidism, hyperprolactinemia, Cushing's syndrome, congenital adrenal hyperplasia, and current or previous (within the last 6 months) use of oral contraceptives, glucocorticoids, antiandrogens, ovulation induction agents, antidiabetic and antiobesity drugs, or other hormonal drugs. None of the patients were affected by neoplastic, metabolic, or cardiovascular disorder or other concurrent medical illness (including diabetes or kidney, liver, thyroid, autoimmune, cerebrovascular, and ischemic heart disease). All subjects were nonsmokers and had normal glucose tolerance (19) and normal physical activity, and none drank alcoholic beverages. Moreover, acute and chronic inflammation was excluded on the basis of medical history, physical examination, and routine laboratory tests, including measurement of oral temperature, WBC count, and urinalysis. In each woman, weight and height were measured to calculate the BMI. The waist/hip ratio (WHR) was also calculated as previously described (20).

Protocol

At study entry, all subjects underwent venous blood samples for complete hormonal assays, WBC count, lipid profile, oral glucose tolerance test, and measurement of insulin values. All blood samples were obtained in the morning between 0800 and 0900 h after an overnight fast and resting in bed during the early follicular phase (d 2–5) of a spontaneous or P-induced menstrual cycle.

During the same visit, all subjects underwent TV-USG and anthropometric measurements, including BMI and WHR.

Biochemical and hormonal analysis

Basal blood samples were obtained to evaluate complete hormonal assays. The following hormonal serum levels were measured: LH, FSH, 17β-estradiol (E2), 17-hydroxyprogesterone (17-OHP), testosterone (T), androstenedione (A), dehydroepiandrosterone sulfate (DHEAS), prolactin (PRL), and sex-hormone-binding globulin (SHBG). All blood samples for each woman were assayed in duplicate and immediately centrifuged, and the serum was then stored at −80 C until analysis.

In each woman, the free androgen index and the homeostasis model assessment (HOMA) score were calculated as previously described (20).

Leukocyte count was determined within 2 h after venipuncture with an automatic workstation cell counter, Technicon H3 (Bayer Diagnostics, Munich, Germany). The instrument was calibrated with the Setpoint hematology calibrator (Bayer Diagnostics) with mean values for each parameter of five replicates counted. The calibration obtained was successfully verified with Testpoint hematology control normal (Bayer Diagnostics) and with three levels of Emacheck quality hematology reference controls (Hematronix, Concord, CA): low abnormal level, normal level, and high abnormal level. The coefficient of variation (CV) determined on five replicates of each control ranged from 0.9–1.0% for leukocyte parameters. CRP was measured by latex immunoturbidometric methodology on an automated clinical analyzer system, Synchron CX4 (Beckman Diagnostics, Fullerton CA). The lower detection limit was 2 mg/liter, and the intraassay CV was 5%.

Plasma glucose levels were determined by the glucose oxidase method on a Beckman glucose analyzer (Fullerton, CA). Plasma PRL, LH, FSH, E2, P, T, A, and DHEAS were all measured by specific RIA, as previously described (21, 22). The mean of two hormonal results was calculated. Serum 17-OHP levels were determined using a RIA (Diagnostic Systems Laboratories 5000, Webster, TX) with a sensitivity of 0.5 nmol/liter and an intra- and interassay CV of 8.9 and 9.0%, respectively. SHBG levels were measured using an immunoradiometric assay (Radim, Pomezia, Rome, Italy) with a sensitivity of 2.5 nmol/liter and an intra- and interassay CV, respectively, of 5.1 and 5.2%. Serum insulin was measured by a solid-phase chemiluminescent enzyme immunoassay using commercially available kits (Immunolite Diagnostic Products Co., Los Angeles, CA). The intra- and interassay CVs were less than 5.5%.

Statistical analysis

The Kolmogorov-Smirnov statistic with a Lilliefors significance level was used for testing normality. Because of the non-Gaussian distribution of the variables, the differences between continuous variables of the two groups were analyzed using the Mann-Whitney U test. Bivariate correlations were performed calculating the Spearman coefficient. In addition, simple and multiple regression analysis was performed to determine which variables predicted WBC or CRP. In assessing the suitability of the data for a linear regression model, the collinearity diagnostics were evaluated. Data are presented as median and interquartile range, and $P < 0.05$ was considered statistically significant. All analyses were run using SPSS 12.0.1 (SPSS Inc., Chicago, IL).

Results

The demographic, hormonal, and biochemical data of the PCOS and control groups are reported in Table 1.

In the PCOS group, LH, 17-OHP, T, A, E2, DHEAS, SHBG, serum insulin, and insulin sensitivity (HOMA index) were significantly different in comparison with control women. The leukocyte count was significantly higher in PCOS women compared with the control group, even though no case of leukocytosis was found in either group. Analyzing the leukocyte formula, we observed a significant ($P < 0.0001$) increase of lymphocytes and monocytes in PCOS women *vs.* controls. Also, CRP levels were significantly increased in PCOS compared with the controls.

In both groups, there was a significant association between leukocyte count and HOMA index (PCOS, r = 0.94 with $P < 0.0001$; controls, r = 0.91 with $P < 0.0001$).

Simple linear regression analysis showed that for the entire population the measure of HOMA was useful to predict WBC count as expected (linear regression coefficient $\beta = 0.97$; $P < 0.0001$). A multiple linear regression analysis was performed by adding to the previous model a second independent variable (PCOS status no/yes = 0/1). In this model, predictors of WBC were HOMA ($\beta = 0.35$; $P < 0.0001$) and PCOS status ($\beta = 0.66$; $P < 0.0001$). Moreover, simple linear regression analysis showed that for the entire population, the measure of HOMA was useful to predict CRP levels ($\beta = 0.68$; $P < 0.0001$). A multiple linear regression analysis was performed by adding to the previous model a second independent variable (PCOS status no/yes = 0/1). The predictor of CRP levels was PCOS status ($\beta = 0.62$; $P < 0.0001$) and not HOMA ($\beta = 0.10$; $P = 0.43$). Finally, multiple linear regression analysis showed that other hormone levels are not predictors of leukocyte count both in PCOS and control women.

TABLE 1. Clinical, hormonal, and metabolic features of women with PCOS and controls

	PCOS (n = 150)	Controls (n = 150)	*P*
Age (yr)	25 (11)	25 (11)	1.00
BMI (kg/m^2)	26 (4)	24 (7)	0.11
WHR	0.9 (0.4)	0.8 (0.3)	<0.0001
Ferriman-Gallwey score	12 (5)	4 (2)	<0.0001
FSH (IU/liter)	9.1 (1.3)	9.1 (1.1)	0.41
LH (IU/liter)	22.1 (0.9)	8.5 (0.9)	<0.0001
PRL (ng/ml)	12.2 (2.5)	12 (2.1)	0.31
E2 (pmol/liter)	58.6 (4.5)	46.4 (2.7)	<0.0001
P (nmol/liter)	1.2 (0.7)	1.9 (0.2)	<0.0001
17-OHP (nmol/liter)	2.2 (0.6)	0.8 (0.3)	<0.0001
T (nmol/liter)	2.5 (1.0)	0.5 (0.4)	<0.0001
A (nmol/liter)	4.8 (0.7)	1.2 (0.4)	<0.0001
DHEAS (μmol/liter)	4.3 (0.5)	3.2 (0.4)	<0.0001
SHBG (nmol/liter)	29.3 (2.2)	53.5 (2.3)	<0.0001
WBC count (cell/mm^3)	7260 (393)	5220 (210)	<0.0001
Neutrophiles ($\times$1000 μl)	4.4 (1.8)	4.1 (1.5)	0.11
Lymphocytes ($\times$1000 μl)	2.3 (0.6)	2.1 (0.4)	<0.0001
Monocytes ($\times$1000 μl)	0.4 (0.2)	0.3 (0.1)	<0.0001
Basophiles ($\times$1000 μl)	0.2 (0.1)	0.2 (0.1)	1.00
CRP (mg/liter)	2 (1)	0.7 (0.8)	<0.0001
Glucose (mmol/liter)	6.50 (0.7)	5.1 (1.3)	<0.0001
Insulin (pmol/liter)	75.3 (13.1)	45.2 (10)	<0.0001
HOMA score	3.7 (0.2)	1.7 (0.1)	<0.0001
FAI	8.8 (1.8)	0.9 (0.4)	<0.0001

Values are given as median (interquartile range). Conversion factors for metric units: E2, 3.671; A, 0.0349; T, 3.467; DHEAS, 2.714; 17OHP, 0.03026; glucose, 0.055; insulin, 6.0. FAI, Free androgen index.

Discussion

To the best of our knowledge, this is the first study in the literature to demonstrate an increased leukocyte count in PCOS. The results of this study show that insulin resistance is probably the main factor responsible for the increase of leukocytes in patients with PCOS.

Multiple regression analysis did not demonstrate that hormone levels are predictors of leukocyte count both in PCOS and control women, suggesting that leukocyte increase is not affected by elevated circulating concentrations of T, A, DHEAS, LH, 17-OHP, or E2. In comparison with matched controls, PCOS women had greater serum insulin concentration and HOMA index, confirming previous data (2). In our study, leukocyte count significantly correlates with either fasting insulin concentrations or estimates of insulin sensitivity, suggesting that increased insulin production affects perhaps WBC count. Furthermore, a significant linear correlation between WBC count and HOMA index, both in PCOS and controls, could explain the increase of leukocyte count in subjects with lower insulin sensitivity. A large body of evidence shows a correlative and causative relationship between inflammation and insulin resistance (23, 24) and a linear correlation between HOMA index and WBC count (12, 25, 26). Indeed, inflammation has been recognized to play a central role in both initiation and progression of the atherosclerotic process (7); therefore, elevated leukocyte count is directly associated with increased incidence of coronary heart disease, ischemic stroke, and mortality from cardiovascular disease in African-American and white men and women (9). In agreement with Kelly *et al.* (13), we also found increased CRP concentrations in women with PCOS, hence suggesting CRP as a predictor of cardiovascular events and independently related to insulin insensitivity (16). In fact, Festa *et al.* (16) reported, in a larger study, the relationship between CRP, fibrinogen, WBC count, and components of insulin resistance in a nondiabetic population. The association of leukocyte count with cardiovascular risk factors could be a manifestation of subclinical disease.

In conclusion, we suggest that women with PCOS appear to show higher levels of leukocyte count, a marker of low-grade inflammation and cardiovascular risk, than age- and BMI-matched controls.

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