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Serum asymmetric dimethylarginine, apelin, and tumor necrosis factor-α levels in non-obese women with polycystic ovary syndrome

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ABSTRACT

Polycystic ovary syndrome (PCOS) is associated with multiple risk factors for cardiovascular disease (CVD), including insulin resistance, type 2 diabetes mellitus, obesity, hypertension, and dyslipidemia. In addition, hyperandrogenism may contribute to the pathogenesis of CVD, independent of obesity and insulin resistance. We investigated serum levels of asymmetric dimethylarginine (ADMA), apelin, and tumor necrosis factor (TNF)-α as CVD risk markers and their relationship with hyperandrogenism in non-obese women with PCOS. In this study were included 82 non-obese women with PCOS and 33 controls. Women with PCOS were further divided into two groups: women with hyperandrogenism (HA-PCOS, $n = 37$) and those without hyperandrogenism (NA-PCOS, $n = 45$). Serum ADMA, apelin, and TNF-α levels were compared among the three groups and their relationship with hyperandrogenism was evaluated. Serum ADMA levels were significantly higher in the HA-PCOS group than in the NA-PCOS and control groups (0.45 ± 0.09 *vs.* 0.38 ± 0.08 *vs.* 0.40 ± 0.07; $P < 0.0005$). Serum TNF-α levels were significantly higher among women with PCOS compared with controls (2.91 ± 1.25 *vs.* 1.74 ± 0.77; $P < 0.001$) and in the HA-PCOS group compared with the NA-PCOS group (3.21 ± 1.24 *vs.* 2.60 ± 1.24; $P < 0.0001$). Both PCOS groups had significantly lower serum apelin levels compared with controls (1.31 ± 0.54 *vs.* 1.16 ± 0.34 *vs.* 2.78 ± 1.10; $P < 0.0001$). ADMA and TNF-α were positively correlated with total testosterone ($r = 0.219$, $P = 0.022$; $r = 0.332$, $P < 0.001$, respectively) and free androgen index ($r = 0.287$, $P = 0.002$; $r = 0.289$, $P = 0.002$, respectively), whereas apelin was negatively correlated with these parameters ($r = -0.362$, $P < 0.001$; $r = -0.251$, $P = 0.008$). These findings may indicate that non-obese women with PCOS are at an increased risk for CVD, which is further aggravated by hyperandrogenism.

1. Introduction

Polycystic ovary syndrome (PCOS) is the most frequent endocrine disorder among women of reproductive age, affecting 5–10% of all women in their life span [1]. Multiple risk factors for cardiovascular disease (CVD) may be found in young women with PCOS, including metabolic syndrome, type 2 diabetes mellitus (T2DM), dyslipidemia, abdominal obesity, and hypertension [2–4]. Although evidence is limited for cardiovascular events among reproductive-aged women who are affected by PCOS, available data suggest that CVD more frequently occurs in women with classic PCOS, which is characterized by chronic anovulation and hyperandrogenism [2,5]. Moreover, women with classic PCOS have greater menstrual irregularity, hyperandrogenism, total and abdominal obesity, and insulin resistance. These patients also have more severe risk factors for T2DM and CVD than PCOS patients diagnosed using criteria not established by the National Institutes of Health [6].

Insulin resistance plays a pivotal role in the pathophysiology of PCOS by inducing endothelial dysfunction, which is reflected by impaired nitric oxide (NO)-mediated vasodilation. Consequently, subclinical atherosclerosis and chronic low-grade vascular inflammation may occur more frequently than among women without PCOS [7–10]. However, it should be noted that the impaired endothelial function has also been observed independent of insulin

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resistance in women with PCOS [2–4]. Therefore, the increased CVD in women with PCOS might be partially attributed to hyperandrogenism [8,9].

Asymmetric dimethylarginine (ADMA), an endogenous competitive inhibitor of NO synthase, acts as a regulator of the ι-arginine/NO pathway, and increased levels of ADMA are involved in endothelial dysfunction [11]. Furthermore, the concentrations of ADMA are a potential independent predictor of CVD [12], and levels of ADMA are associated with the incidence of cardiovascular events [13,14]. Along these lines, the putative atherogenic potential of ADMA has been investigated in young patients with PCOS [14,15]. A recent study suggested that women with PCOS have elevated plasma ADMA levels, which mainly depended on the degree of insulin resistance, and to a lesser extent, on hyperandrogenemia. In this study, however, metformin treatment contributed to improved hyperandrogenism and decreased ADMA levels, which was independent of a change in insulin sensitivity [13].

Tumor necrosis factor-α (TNF-α), the proinflammatory cytokine implicated in insulin resistance, adversely influences endothelial function and contributes to atherosclerosis [16]. Additionally, TNF-α has been widely considered as a marker of underlying inflammatory processes in PCOS. Furthermore, Gonzalez et al. suggested that women with PCOS have unique proinflammatory and proatherogenic risk profiles independent of obesity, and that hyperandrogenism might be capable of promoting the inflammation that leads to atherosclerosis [17].

Apelin is expressed in a variety of tissues involved in cardiovascular homeostasis. In particular, it is expressed in the vascular endothelium where its vasoactive effects occur through an NO-dependent mechanism [18]. In cultured endothelial cells, for instance, apelin stimulates the phosphorylation of endogenous NO synthase and increases the secretion of NO metabolites [19]. Changes in plasma apelin levels have also been reported in patients with cardiometabolic disorders, including T2DM, dyslipidemia, and cardiopulmonary diseases [20–22]. Recently, it has been suggested that serum apelin levels were lower in women with PCOS and positively correlated with levels of apolipoprotein A [23].

To our knowledge, there have been few reports on these circulating markers for CVD which are focused on hyperandrogenism in women with PCOS. This study aimed to compare serum ADMA, apelin, and TNF-α levels between non-obese women with PCOS and healthy body mass index (BMI)-matched controls, and to evaluate their relationship with hyperandrogenism.

2. Experimental

2.1. Subjects

A total of 115 Korean women with PCOS were included in this prospective observational study. All subjects were non-obese with a BMI < 25 kg/m^2. The women with PCOS were outpatients of the Department of Obstetrics and Gynecology of Severance Hospital. Women with PCOS were further divided into two groups: hyperandrogenic PCOS (HA-PCOS, $n = 37$) and normoandrogenic PCOS (NA-PCOS, $n = 45$). For the controls, 33 BMI-matched premenopausal healthy volunteers were recruited. They visited our hospital for annual comprehensive medical examinations without specific health problems. The diagnosis of PCOS was based on the revised criteria of the Rotterdam consensus conference by at least two of the following three features: (i) oligo- or anovulation; (ii) clinical and/or biochemical signs of hyperandrogenism; and (iii) polycystic ovaries after excluding other etiologies including hyperprolactinemia, non-classical congenital adrenal hyperplasia, Cushing's syndrome, and androgen-secreting tumor [24]. Oligomenorrhea and amenorrhea were defined as <8 spontaneous menstrual cycles per year and the absence of a menstrual period for three consecutive months. Hyperandrogenemia was defined as a total serum testosterone level >68 ng/dL or a free androgen index (FAI) >5.36 [25], and clinical hyperandrogenism was defined as a modified Ferriman-Gallwey (FG) score ⩾8 [26]. The criteria for polycystic ovaries required visualization of 12 or more follicles in each ovary measuring 2–9 mm in a diameter, and/or increased ovarian volume (>10 cm^3) by transvaginal ultrasonography.

The healthy controls had neither signs of hyperandrogenism nor menstrual irregularities. They also had no polycystic ovaries on ultrasound scan. All participants were non-smokers and none of them was currently taking drugs, nor had they taken hormonal contraceptives or steroids for at least 3 months before participation in this study.

The study was approved by the institutional review board of Severance Hospital, and informed consent was obtained from all subjects.

2.2. Clinical characteristics and biochemical assays

Anthropometric data including height, body weight, waist and hip circumference, and blood pressure were assessed on the initial visit. BMI was calculated as weight (kg) divided by the square of the height (m^2). The waist-to-hip ratio (WHR) was calculated as waist circumference divided by hip circumference. Blood pressure was measured in the sitting position after a rest period of at least 5 min. The average of three measurements was obtained.

Blood samples were collected between 08:00 and 11:00 h after an overnight fasting on the third day after a spontaneous or progesterone withdrawal bleeding. Basal serum hormones, such as luteinizing hormone (LH), follicle stimulating hormone (FSH), total testosterone, and sex hormone binding-globulin (SHBG) were measured using standard enzymatic methods with a fully automated random access chemiluminescence-enhanced enzyme immunoassay system (Roche Laboratory Systems, Mannheim, Germany). The FAI was calculated according to the following equation: FAI (%) = 3.47 × total testosterone (ng/dL)/SHBG (nmol/L).

Fasting serum glucose and insulin concentrations were measured with a fully automated analyzer (Roche Diagnostics GmBH, Mannheim) by the glucose oxidase technique and electrochemiluminescence immunoassay, respectively. To estimate insulin sensitivity, the fasting glucose/insulin ratio, the homeostasis model assessment for insulin resistance (HOMA-IR), and the quantitative insulin sensitivity check index (QUICKI) were calculated using the following formulas: HOMA-IR = fasting glucose (mg/dL) × fasting insulin (μIU/mL)/405 and QUICKI = 1/[log(fasting insulin) + log(fasting glucose)].

An oral glucose tolerance test was also performed in women with PCOS. Serum glucose and insulin levels were measured 2 h after a 75 g oral glucose load. The Matsuda index was calculated by the following formula: Matsuda index = 10,000/square root of [(fasting glucose × fasting insulin) × (mean glucose × mean insulin during OGTT)] [27].

The blood samples were centrifuged to separate the serum and stored at −80 °C until used for the apelin, ADMA, and TNF-α assays. Serum apelin-36, ADMA, and TNF-α levels were measured using commercial enzyme-linked immunosorbent assay kits (Phoenix Pharmaceuticals, Belmont, CA; Immundiagnostik AG, Bensheim, Germany; Quantikine human TNF-α/TNFSF1A, R&D systems, Minneapolis, MN, respectively). The intra- and inter-assay coefficients of variation were of 8.2% and 6.5% for apelin, 6.8% and 8.0% for ADMA, and 5.6% and 6.2% for TNF-α.

2.3. Statistical analyses

The sample size was calculated to compare differences regarding serum ADMA and TNF-α levels among each group. Power