European Review for Medical and Pharmacological Sciences 2016; 20: 1185-1191

Statin ameliorates endothelial dysfunction and insulin resistance in Tibet women with polycystic ovary syndrome

B. YANG[1,2], Z.-J. SUN[1], B. CHEN[2], J. ZHANG[1], H. ZHAO[1], C.-W. LI[1], Y.-K. CAO[1], F. CAO[1]

[1]Department of Cardiology, Chinese PLA General Hospital, Beijing, China
[2]Department of Cardiology, Tibet General Hospital, Lhasa, Tibet, China

Bo Yang, Zhi-Jun Sun and *Bin Chen* contributed equally to this article

Abstract. – OBJECTIVE: Polycystic ovary syndrome (PCOS) is a disorder characterized by hyperandrogenism, ovulatory dysfunction, and polycystic ovaries. In Tibet population, little is known about the correlation between PCOS and endothelial function and insulin resistance. In this study, we investigated the efficacy of simvastatin on ameliorating endothelial dysfunction and insulin resistance in Tibet patients with PCOS.

PATIENTS AND METHODS: A group of 21 PCOS women was compared with 21 age-paired controls for blood pressure, body mass index (BMI), lipids, glucose and insulin levels. Plasma ET-1 was quantitated using a commercially available ELISA kits. The invasive vascular endothelial function was evaluated through the measurement of brachial artery flow-mediated dilation (FMD) in the fasting state using high-resolution B-mode ultrasound and ankle-brachial index (ABI) by using a blood pressure cuff and a Doppler instrument.

RESULTS: PCOS women had higher BMI ($p < 0.05$), blood pressure ($p < 0.05$), cholesterol ($p < 0.05$), triglyceride ($p < 0.05$), LDL cholesterol ($p < 0.05$) and lower HDL cholesterol ($p < 0.05$). Fasting insulin ($p < 0.001$) and 2-hour plasma glucose ($p < 0.001$) of PCOS were also found to be higher than that of controls. HOMA-IR of PCOS was calculated to be much higher than that of the control group ($p < 0.001$). ET-1 levels were significantly higher in the PCOS women compared with the control group ($p < 0.001$). FMD and ABI were impaired in the PCOS group when compared with the controls ($p < 0.001$). After simvastatin intervention for 6 months, FMD, ABI, HOMA-IR, lipid profile, BMI were ameliorated in PCOS women. Also, FMD, ABI, HOMA-IR, lipid profile were nearly normalized in PCOS women after statin treatment compared with the control women. Tibet PCOS patients present a clustering of atherosclerosis risk factors (obesity, adverse lipid profile, hypertension and hyperglycemia) and more prevalent in insulin resistance and endothelial dysfunction than non-PCOS women.

CONCLUSIONS: The endothelial function and insulin resistance of PCOS patients were ameliorated after statin administration.

Key Words:
Endothelial function, Insulin resistance syndrome, Polycystic ovary syndrome, Tibet, Statin.

Introduction

Polycystic ovary syndrome (PCOS) is a frustrating experience for women, often complex for managing clinicians and is a scientific challenge for researchers. PCOS is the most common endocrine disturbance in women of reproductive age, affecting 4% to 8% from studies performed in Greece, Spain and the USA[1]. PCOS affects 6-10% of women during their reproductive life[2]. The prevalence of PCOS has increased with the use of different diagnostic criteria and has recently been shown to be 18% (17.8 ± 2.8%) in the first community-based prevalence study based on current Rotterdam diagnostic criteria[3]. Insulin resistance is a pathophysiological contributor in around 50% to 80% of women with PCOS[4], especially in those with more severe PCOS diagnosed on National Institutes of Health (NIH) criteria and in women who are overweight[5]. The association of PCOS with metabolic disorders was investigated which indicated that insulin resistance and impaired insulin secretion are key components of PCOS[6]. The prevalence of IRS (insulin resistance syndrome), IGT (impaired glucose tolerance) and DM (diabetes mellitus) has been clearly shown to be race-related[7]. To the best of our knowledge, the associ-

Corresponding Author: Bo Yang, MD; and Zhi Jun Sun, MD; e-mail: dryangb@aliyun.com

ation of PCOS with IRS was not fully understood in Tibet population. Endothelial dysfunction was characterized by the elevated concentration of serum ET-1 produced by endothelium[8]. Insulin resistance may induce to endothelial dysfunction by a cluster of risk factors disturbance, especially lipid profile, ET-1, glucose metabolic disorders. The aim of this study was to compare the prevalence of cardiovascular risk factors in the Tibet PCOS patients and a control group selected from a random population sample. The efficacy of statin in ameliorating endothelial dysfunction and insulin resistance was focused on in the study.

Patients and Methods

Patients

The Human Ethics Committee of our institute approved the research protocols in accordance with international agreements (Helsinki Declaration[9,10] of 1975, revised 2008). All participants obtained written informed consent. The participants were recruited through PCOS patients registered in Tibetan General Hospital. All subjects were native Tibetan, who were born in and grown up in Tibet with Tibetan nationality, which is one of the minorities in the southwest of China. A group of 21 women aged 25-42 [mean (± SD) age 31.5 ± 5.2] years meeting the menstrual, laboratory and ultrasound criteria proposed by the 2006 Androgen Excess PCOS Society guideline were included[11]. Menstrual criteria included oligo- or amenorrhea (cycle length irregular, > 45 days or < 6 periods per year). Biochemical criteria included an elevated menstrual LH: FSH ratio (> 2.5), or elevated (>2.8 nmol/L) total serum testosterone. Ultrasound criteria included an enlarged ovary with ≥10 peripherally arranged small follicular cysts and a hyperechogenic central stroma. These criteria are widely accepted to diagnose PCOS.

Controls

A control group of 21 age-paired women aged 25-42 [mean (± SD) age 31.5 ± 5.2] years was randomly selected from women who were registered in the gynecology department of Tibetan General Hospital. These control subjects had regular menstrual periods, normal ovulatory cycles, had no ultrasound of clinical signs of PCOS. The control subjects were also native Tibetan residents with Tibetan nationality.

Serum prolactin, thyroid hormone and 17-alpha-hydroxy-progesterone were done on all subjects and controls and were within normal limits.

Physical Examination

A standardized questionnaire was used to record the general features of the subjects. All subjects underwent the physical examination, and the medical history was taken. Weight (to the nearest 0.5 kg) and height (to the nearest 0.5 cm) were measured while the subjects were fasting and wearing only underwear. Body mass index (BMI) was calculated as weight (kilograms) divided by height (meters) squared. Blood pressure was measured with a standard mercury sphygmomanometer on the left arm in the supine position.

75 g Oral Glucose Tolerance Test (OGTT)

OGTT is the most sensitive test to detect a mild disturbance of glucose metabolism. In our study, OGTT is required for all subjects. It is useful to clarify the diagnosis. In order to obtain accurate results, the following procedures are strongly recommended. Recipients should take meals including more than 150 g carbohydrate for 3 days. A 250-350 ml solution of 75 g anhydrous glucose is administered orally and blood is drawn before and at certain time intervals after glucose loading. The test should be done after an overnight fast for 10-14 hours. No food or drink except water is allowed during the test. Smoking is prohibited also. Blood should be centrifuged immediately to separate plasma or kept cold, preferably with addition of NaF to prevent glycolysis until centrifugation. Fasting and 2h blood samples should be taken. The plasma glucose values are classified according to 1999 WHO criteria[12]. Subjects were subdivided into diabetes (FPG ≥ 7.0 mmol/L or 2hPG > 11.1 mmol/L), impaired glucose tolerance (FPG < 7.0 mmol/L and 7.8 mmol/L ≤ 2hPG ≤ 11.1 mmol/L and normal glucose tolerance (FPG < 6.1 mmol/L and 2hPG < 7.8 mmol/L).

Biochemistry Assay

The biochemical parameters were obtained after an overnight fast for 12 hours. Venous blood was sampled for the measure of fasting insulin (FINS), total cholesterol (CH), triglyceride (TG), high-density lipoprotein cholesterol (HDL-C) and low-density lipoprotein cholesterol (LDL-C). All the blood samples were evaluated with Hitachi 7600 biochemical analyst (Hitachi, Tokyo,

Japan). The estimate of insulin resistance by the homeostasis model assessment (HOMA-IR) was calculated by the formula: FINS (mυ/L)*FPG (mmol/L)/22.5[13,14].

Quantitation of Plasma ET-1

For plasma ET-1, 10 ml of venous blood was collected into an EDTA tube and centrifuged immediately at 2500 g for 20 min at 4°C. ET-1 was quantitated using a commercially available enzyme-linked immunosorbent assay (ELISA) kit (Morinaga and R&D System, Minneapolis, MN, USA). Standards, reagents, and test samples were prepared and assayed according to the instructions of the manufacturer[8].

FMD

Left brachial artery diameter was measured from B-mode ultrasound images at rest and during reactive hyperemia. Briefly, a resting scan was performed and arterial flow velocity was measured using a Doppler signal. The method has been described previously[8].

ABI

ABI was measured using a blood pressure cuff and a Doppler instrument (UltraTech® PD1v with a 5 MHz vascular probe, Medisave UK Ltd, Weymouth, Dorset, UK) with the subject in a supine position. We measured the systolic blood pressure (SBP) of the left and right brachial arteries in the upper arms. In the lower limbs, both the dorsalis pedis arterial pressure and posterior tibial arterial pressure were measured if available. The cuff was placed just above the level of the malleoli. A trained technician performed all measurements. The ABI was the higher SBP of the lower limbs divided by the higher of the brachial SBPs. ABI >1.00-1.40 was considered normal[8].

Statin Protocol

The PCOS women were oral administrated simvastatin 20 mg daily for 6 months (MSD Pharmaceuticals Private Limited). No severe side effects were reported during the study. After 6 months of treatment, the lipid profile, ET-1, HOMA-IR, FMD, ABI measurements were repeated.

Statistical Analysis

All analyses were carried out with Stata 7.0 software system. Data were expressed as mean ± standard deviation (SD). Analysis of variance (ANOVA) and Student's *t*-test was used to evaluate the measurement data. Chi square statistic was used to compare count data. Statistical significance was inferred at $p < 0.05$.

Results

Comparison of Hormone Levels

When comparing PCOS women with controls, we found that PCOS subjects had significantly higher LH ($p < 0.001$) and testosterone levels ($p < 0.001$). The FSH, prolactin and estradiol levels did not differ significantly between groups (Table I).

Characteristics of all subjects are shown in Table II. There was no significant difference in age between groups. PCOS subjects were found to be more obese than controls. BMI of PCOS subjects was much higher than that of controls ($p < 0.05$). The systolic and diastolic blood pressures of PCOS subjects were significantly higher than those of controls ($p < 0.05$). OGTT was performed in all participants. There was no statistical significance in fasting plasma glucose between groups ($p > 0.05$). But the 2-hour plasma glucose of PCOS was much higher than that of control group ($p < 0.001$). Fasting insulin of PCOS was also found to be higher than that of the controls ($p < 0.001$).

Table I. Comparison of hormone levels (21 subjects in each group).

	PCOS	Control	*t*-value	*p*-value
LH (IU/L)	10.6 ± 5.9	4.7 ± 3.4	3.9705	0.0003
FSH (IU/L)	6.1 ± 2.2	5.9 ± 2.9	0.2518	0.8025
Testosterone (nmol/L)	2.6 ± 0.8	1.7 ± 0.7	3.8798	0.0004
Prolactin (mIU/L)	245.5 ± 111.9	251.2 ± 108.9	0.1673	0.8680
Estradiol (pmol/L)	248.7 ± 149.8	218.7 ± 163.1	0.6208	0.5383

Values are mean ± SD, unless otherwise indicated.

Table II. Comparison of baseline characteristics.

	PCOS	Control	*t*-value	*p*-value
Age (years)	31.5 ± 5.2	31.5 ± 5.2	NS	NS
BMI (Kg/m^2)	29.8 ± 6.8	25.1 ± 5.3	2.4982	0.0167
SBP (mmHg)	130.6 ± 13.2	115.2 ± 12.8	3.8381	0.0004
DBP (mmHg)	82.7 ± 12.8	74.9 ± 8.8	2.3011	0.0267
FPG (mmol/L)	5.6 ± 0.7	5.4 ± 0.9	0.8038	0.4262
2hPG (mmol/L)	8.4 ± 0.7	6.6 ± 0.6	8.9469	< 0.001
FINS (mu/L)	3.8 ± 0.6	2.6 ± 0.7	5.9646	< 0.001

NS: no significant.

Comparison of Lipids and Insulin Resistance

The PCOS group had higher cholesterol ($p < 0.05$), triglyceride ($p < 0.05$), LDL cholesterol ($p < 0.05$) and lower HDL cholesterol ($p < 0.05$) than controls. HOMA-IR of PCOS was calculated to be much higher than that of the control group ($p < 0.01$), which indicated that PCOS group had more prevalence in insulin resistance. The lipid profile and HOMA-IR were ameliorated after statin administration (Table III).

Comparison of Endothelial Function and Plasma ET-1 Concentration

PCOS women suffered deteriorated FMD and ABI values ($p < 0.001$) and increased plasma ET-1 concentration ($p < 0.001$), which indicated that PCOS women have endothelial dysfunction (Table IV). After statin treatment for 6 months, the endothelial function and ET-1 concentration were significantly ameliorated ($p < 0.001$).

Discussion

A large numbers of studies have shown that PCOS is not only a gynecological condition affecting reproductive age, but also a comprehensive syndrome with a variety of metabolic disorders[15]. The endocrine, reproductive and metabolic consequences of PCOS include increased circulating LH levels, with normal-to-low FSH secretion leading to increased ovarian and adrenal androgen production resulting in acne and hirsutism, insulin resistance, hyperinsulinemia and dyslipidemia[16]. Although metabolic disorders

Table III. Comparison of lipids and insulin resistance.

	Pre-statin	Post-statin	Control	*p*1	*p*2	*p*3
CH (mmol/L)	5.2 ± 0.9	4.2 ± 0.8	4.6 ± 0.7	0.0206	0.0005	0.0924
TG (mmol/L)	1.6 ± 0.6	1.3 ± 0.4	1.2 ± 0.5	0.0240	0.0638	0.4783
HDL (mmol/L)	1.1 ± 0.7	1.4 ± 0.7	1.6 ± 0.6	0.0172	0.1726	0.3261
LDL (mmol/L)	3.1 ± 0.6	2.2 ± 0.5	2.6 ± 0.8	0.0273	< 0.001	0.0591
HOMA-IR	1.3 ± 0.6	1.0 ± 0.3	0.8 ± 0.5	0.0055	0.0470	0.1239

*p*1: compared between Pre-statin Group and control Group; *p*2: compared between Pre-statin Group and Post-statin Group; *p*3: compared between Post-statin Group and control Group.

Table IV. Comparison of FMD, ABI and plasma ET-1.

	Pre-statin	Post-statin	Control	*p*1	*p*2	*p*3
FMD (%)	5.73 ± 1.21	8.93 ± 0.95	9.18 ± 0.77	< 0.001	< 0.001	0.3545
ABI	0.87 ± 0.09	1.12 ± 0.07	1.15 ± 0.06	< 0.001	< 0.001	0.1438
ET-1 (pg/ml)	6.13 ± 1.01	4.62 ± 0.99	4.78 ± 1.08	0.0002	< 0.001	0.6195

were found in western countries, little has been known in Tibet PCOS females. This study was designed to reveal the association of PCOS with IRS in Tibet population and the efficacy of statin on ameliorating of endothelial function.

Results from this study indicated that women with PCOS have more prevalence of obese, higher blood pressure, higher blood glucose, insulin, cholesterol, triglyceride, LDL level and lower HDL level. All of them are traditional risk factors of atherosclerosis[17,18]. The definition of metabolic syndrome includes dyslipidemia, specifically elevated triglyceride and low HDL cholesterol, elevated glucose and hypertension[19]. HOMA-IR is a surrogate marker to evaluate insulin resistance[20]. In this study, HOMA-IR of PCOS subjects were significantly higher than that of controls, which showed evidence that PCOS subjects are more prevalence in IRS among Tibetan population. Our data are consistent with others, which indicate that insulin resistance is more prevalence in PCOS subjects among American whites[21], blacks[22], Indian[23], Turkish[24], Chinese Han women[25]. Chinese Han population is the majority in China, more than 90% of the total Chinese population. Tibet population is a minority in the southwest of China, about 0.2% of the total Chinese population. Although Chinese Han and Tibet are both yellow race, a lot of differences exist in disease profiles. To the best of our knowledge, few studies were performed on the correlation between PCOS and insulin resistance and endothelial function among Tibet population. The study demonstrated impaired endothelial function, assessed by FMD, ABI and plasma ET-1 in PCOS women, which were nearly normalized to control levels after statin intervention. Statins ameliorate lipid profile and play its anti-inflammation role by the inhibition of HMG-COA reductase, through which pathway to improve endothelial function[26].

Tibet is a developing high-altitude area in the southwest of China. We concerned about the public health of Tibetan population because of the poor environmental situation including nutrient and health education[27]. We concerned about the situation, that's why we recruited Tibetan subjects into the study. In line with other population, we found, in Tibet PCOS patients, an adverse profile characterized by low HDL cholesterol, an increase in LDL cholesterol, total cholesterol and triglyceride. Our data showed higher systolic and diastolic blood pressure values in PCOS patients. We also found a significantly higher BMI in PCOS subjects than in controls. All the findings put PCOS women at greater risk for developing diabetes and cardiovascular disease.

Obesity and excess weight are major chronic diseases in Western world countries. Obesity increases hyperandrogenism, hirsutism, infertility and pregnancy complications both independently and by exacerbating PCOS. In general populations, obesity and insulin resistance further increase type 2 diabetes (DM2) and cardiovascular disease (CVD). Likewise, in PCOS obesity worsens insulin resistance and exacerbates reproductive and metabolic features. Furthermore, women with PCOS have increased risk factors for DM2 and CVD, increased impaired glucose tolerance (IGT), DM2 and potentially increased CVD. As obesity rates rise, the public health significance of PCOS will increase. Treatment of obesity through lifestyle intervention is a key treatment strategy in PCOS and improves insulin resistance, reproductive and metabolic features[28]. Thus, an early prevention and comprehensive management may have the potential to reduce cardiac complications and to improve survival[29].

In summary, Tibet PCOS patients present, even in their twenties to thirties, a clustering of atherosclerosis risk factors (obesity, adverse lipid profile, hypertension and hyperglycemia). Based on our data, we propose that all women, when diagnosed with PCOS, should have their lipid, glucose, blood pressure values determined. All patients should have a regular metabolic follow-up as a group at potential risk for early development of CVD.

Conclusions

These findings indicate that Tibet PCOS patients present a clustering of atherosclerosis risk factors (obesity, adverse lipid profile, hypertension and hyperglycemia) and more prevalent in insulin resistance than non-PCOS women. Statin administration can improve the endothelial function and insulin resistance in Tibet PCOS women.

Acknowledgements

This work was supported by Beijing Natural Science Foundation (No.7132227, Bo Yang), National Natural Science Foundation of China (81570272, Bo Yang), Beijing Nova Program (No. Z141107001814113-XXHZ201401, Bo Yang) from Beijing Municipal Science & Technology Commission, and Discovery Foundation from the Chinese Medical Doctor Association (DFCMDA201311, Bo Yang).

Conflict of Interest

The Authors declare that there are no conflicts of interest.

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