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Factors Associated With Increased Carotid Intima-Media Thickness and Being Nondipper in Nonobese and Normotensive Young Patients Affected by PCOS

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Abstract

Polycystic ovary syndrome (PCOS) is characterized by chronic unovulation, hyperandrogenism, and insulin resistance. We evaluated factors that affect "nondipper" status during 24-hour ambulatory blood pressure monitoring (ABPM) and carotid intima-media thickness (cIMT) in PCOS. Forty-two nonobese women newly diagnosed as PCOS and 32 healthy women were included. After biochemical and hormonal measurements, the ovaries were imaged by pelvic ultrasonography and cIMT was measured by B-mode ultrasonography. A 24-hour ABPM was performed thereafter. Carotid IMT and the ratio of nondippers were elevated compared with controls. Homeostasis model assessment insulin resistance index (HOMA-IR) and low-density lipoprotein cholesterol (LDL-C) were found to be related with being a nondipper in PCOS. None of the parameters evaluated were found to correlate with cIMT. In conclusion, patients with PCOS had increased nondipping ratios and cIMT when compared with controls. Insulin resistance and LDL cholesterol are factors that are related to diurnal variation in normotensive and young patients with PCOS.

Keywords

homeostasis model assessment (HOMA), insulin resistance, nondipper, polycystic ovary syndrome, ambulatory blood pressure monitoring

Introduction

Polycystic ovary syndrome (PCOS) is a reproductive disorder with complex metabolic abnormalities and a risk for the development of glucose intolerance and cardiovascular disease.[1] In fact, the incidence of insulin resistance and hyperinsulinism in patients with PCOS is 50% to 70% and metabolic syndrome prevalence is significantly higher than age and weight-matched controls.[2,3] Not only obesity[4] but also insulin resistance, hyperandrogenism, low-grade chronic inflammation,[5] dyslipidemia,[6] and altered fibrinolytic system[7,8] contribute to the increased cardiovascular disease risk together with endothelial[9] and left ventricular diastolic dysfunction.[10] Since PCOS is one of the most common endocrinologic disorders affecting 6% to 7% of women in their reproductive age,[11] it is important to consider the long-term metabolic and cardiovascular aspects of this syndrome together with fertility.

The correlation between carotid and coronary atherosclerosis is known.[12] Increased carotid intima-media thickness (cIMT) ultrasonographic measurement is a noninvasive and reproducible method to detect subclinical atherosclerosis and it is correlated with cardiovascular events.[13] Talbott et al showed the association between early carotid atherosclerosis

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and PCOS in middle-aged women.[14] The comparative study for the factors related with cIMT in young patients with classical PCOS who do not have metabolic syndrome is still lacking in the literature.

Mean blood pressure (BP) in this group of patients tend to be correlated with aldosterone[15] and androgen levels.[16]

Blood pressure has a reproducible circadian pattern characterized by a low period during sleep; an early morning, postawakening rise and a high plateau period while the participant is awake.[17] When hypertensives have this typical circadian pattern of BP, they are referred to as ''dippers.'' When the normal nocturnal fall of BP is diminished or blunted, the term ''nondipper'' is used to characterize these patients. Nowadays, nondippers have been identified as participants whose nocturnal fall in BP is smaller than 10% of the daytime mean BP in 24-hour ambulatory blood pressure monitoring (ABPM).[18]

In normotensive nondiabetic patients, nondipper status may have a predominant effect on cardiac damage and nondipping of nocturnal BP seems to be a determinant of cardiac hypertrophy and remodelling, and may result in a cardiovascular risk independent of ABPM levels in normotensives.[19] The nondippers also show greater left ventricular mass which is regarded as an independent predictor of cardiac mortality in hypertensive patients.[20] To the best of our knowledge, there has not been any study about diurnal changes in BP and the related factors in this group of patients in medical literature.

In this context, the main aim of our study was to evaluate the clinical and hormonal factors correlated with cIMT and being ''nondipper'' on 24-hour ABPM in nonobese, normotensive, and normoglycemic patients having PCOS without metabolic syndrome and to compare the results with age-matched controls.

Materials and Methods

Patients and Procedure

We consecutively enrolled 42 patients newly diagnosed with classical PCOS according to the 2003 Rotterdam European Society for Human Reproduction/American Society of Reproductive Medicine (ESHRE/ASRM) criteria, in our hospital's Endocrinology and Metabolic Diseases outpatient clinic.[21] Patients who have diagnosis of metabolic syndrome, diabetes, hypertension, hyperlipidemia, hypo- or hyperthyroidism, and known cardiovascular disorders were excluded from the study.

An age-matched 32 healthy and nonobese women were recruited as the control group. Besides height and weight measurements, fasting blood glucose, glycohemoglobin (HbA1c), total cholesterol, high-density lipoprotein cholesterol (HDL-C), low-density lipoprotein cholesterol (LDL-C), triglycerides, total and free testosterone levels, high-sensitive C-reactive protein (hsCRP), fibrinogen, and eryhtrocyte sedimentation rate (ESR) were measured. For each control participant, cIMT measurement and 24-hour ABPM were also performed.

In the PCOS group, we measured height and weight on first physical examination and calculated the body mass index (BMI; kg/m^2). Follicular-stimulating hormone (FSH) and luteinizing hormone (LH) levels were measured on the 3rd day of menstrual cycle during follicular phase, and on the 21st day of the cycle progesterone peak was evaluated to determine ovulation. All patients in the PCOS group had 75 g oral glucose tolerance test (OGTT) together with fasting and postprandial 120-minute insulin measurements. Total cholesterol, LDL-C, HDL-C cholesterol, triglycerides, fibrinogen, hsCRP, ESR, total and free testosterone levels, and HbA1c were also measured. Homeostasis model assessment insulin resistance index (HOMA-IR) was calculated as the product of fasting plasma glucose (mg/dL) by plasma insulin (mIU/L), divided by a constant (405), as a measure of insulin resistance.[22] Presence of acne vulgaris was recorded, and hirsutism was scored according to the modified Ferryman Gallwey scores.[23] A 24-hour ABPM and cIMT measurement were also performed for each participant in the PCOS group.

The study was approved by local Ethics Committee and written informed consent was obtained from each participant.

Biochemical and Hormonal Measurements

All laboratory measurements were carried out with standardized methods in the central laboratories of our university hospital.

Follicular stimulating hormone, LH, total testosterone, and insulin were measured by Elecsys Analyzer (Roche, Germany) through electrochemiluminescense immunoassay (ECLIA) method and free testosterone was measured by radioimmunoassay (RIA) method. Fibrinogen and hsCRP (immunoturbidometric assay) were measured by Cobas Integra 400 autoanalyzer (Roche, Germany).

Fasting blood glucose, total cholesterol, HDL-C, LDL-C, and triglyceride measurements were carried out by enzymatic colorimetric methods. Glycohemoglobin was measured by high-performance liquid chromatography (HPLC) method with an interassay coefficient of variation of 2.3% with Cobas Integra 400 Autoanalyzer. Electroluminometric immunoassay (ECLIA) was used for insulin measurements, with an interassay coefficient of variation of 2.6%.

Ambulatory Blood Pressure Monitoring

A 24-hour ABPM was carried out on the nondominant arm by means of automatic Meditech 04 ABPM equipment which records BP each 15 minutes during the day (6:00 AM to 9:00 PM) and 30 minutes during the night (9:00 PM to 6:00 AM). The time of application was early in the morning for all patients and the control group. The recording was then analyzed by the same cardiologist who was blinded to the diagnosis of the patients. Nondippers were defined as those participants whose nocturnal systolic BP fall was less than 10% of daytime systolic BP mean as stated before.[18]

Ultrasonographic Measurements

Ultrasonographic analysis of the carotid artery was performed with a high-resolution ultrasound scanner, Logic 7 (General

Electric, Indiana) with a 7.5 mHz linear transducer after 15 minutes of rest to allow for pulse and BP stabilization. All the participants were examined in the supine position. Electrocardiogram (ECG) leads were placed appropriately on the chest wall and each scan of the common carotid artery began just above the clavicle, and the transducer was moved cephalad through the bifurcation and along the internal carotid artery. Three segments were identified on each side: 1 cm distal to the common carotid artery, proximal to the bifurcation; the bifurcation itself; and 1 cm proximal to the internal carotid artery using electrocardiogram gating. At each of the 3 segments, for distant walls in the left and right carotid arteries, IMT was defined as the distance between the leading edge of the lumen-intima interface and the leading edge of the media-adventitia interface. Maximum thickness of the wall was calculated at each side. The reported cIMT for each participant is the average of 5 measurements of distant walls from the right and left common carotid arteries. Then the mean value of right and left common carotid artery measurements was used for the statistical analysis. Carotid IMT measurements were done by the same operator blinded to the diagnosis of the patients. Intraobserver variation was 5%. Evaluation of the ovaries was performed for only patients with PCOS by the same ultrasonography equipment using a 3.5 mHz convex transducer from the suprapubic area by a different operator on a different day.

Statistical Analyses

Patients with PCOS and controls were compared by Mann-Whitney *U* test by using SPSS 15.0 program (SPSS, Inc, Chicago, Illinois) for differences in 2 groups regarding age, BMI, fasting blood glucose, testosterone levels, ESR, hsCRP, fibrinogen, mean systolic and diastolic BP, HbA1c, and lipid parameters. Factors affecting cIMT were evaluated by regression analysis in PCOS group, and factors assumed to be related with being nondipper were evaluated by logistic regression analysis. In both of the groups, comparison of cIMT between dippers and nondippers were tested by independent samples *t* test after testing normal distribution by Kolmogorov-Smirnov test. A difference was considered significant at a $P < .05$ (2-tailed).

Results

Polycystic ovary syndrome and control groups were properly matched according to the age and BMI (Table 1). Patients in the PCOS group had significantly higher fasting blood glucose ($P = .02$), total and free testosterone levels ($P = .002$ and .001, respectively), ESR ($P = .008$), hsCRP ($P = .036$), and fibrinogen levels ($P = .001$) when compared with the controls. The mean cIMT was significantly increased in the PCOS group compared with the controls ($P = .001$). According to the 24-hour ABPM results, although mean systolic and diastolic BP did not differ between the groups, the number of nondippers was significantly higher in the PCOS group ($P = .001$).

Table 1. Comparison of Patients With Polycystic Ovary Syndrome (PCOS) and Control Group Regarding Anthropometric and Laboratory Measurements, Carotid Intima-Media Thickness (cIMT), and Nondipping Ratio on 24-hour Ambulatory Blood Pressure Monitoring

	PCOS (n = 42)	Control (n = 32)	*P*
Age (years)	26.9 ± 8.0	27.5 ± 8.7	.156
BMI (kg/m^2)	23.8 ± 6.1	22.2 ± 1.8	.312
Fasting blood glucose (mg/dL)	89 ± 7	83 ± 8	.02[a]
HbA1c (%)	4.9 ± 0.5	4.7 ± 0.5	.104
BUN (mg/dL)	28 ± 6	27 ± 7	.407
Creatinine (mg/dL)	0.87 ± 0.15	0.84 ± 0.13	.215
Uric acid (mg/dL)	4.6 ± 1.3	4.5 ± 0.9	.412
HDL-C (mg/dL)	52 ± 12	53 ± 6	.373
LDL-C (mg/dL)	105 ± 23	106 ± 13	.747
Triglycerides (mg/dL)	95 ± 38	99 ± 20	.250
Total testosterone (ng/mL)	0.69 ± 0.67	0.32 ± 0.15	.002[a]
Free testosterone (pg/mL)	2.61 ± 0.99	1.24 ± 0.48	.001[a]
ESR (mm/h)	10 ± 5	7 ± 3	.008[a]
hs-C-reactive protein (mg/L)	3.7 ± 3.1	1.2 ± 0.3	.036[a]
Fibrinogen (mg/dL)	341 ± 77	233 ± 49	.001[a]
Mean cIMT (mm)	6.3 ± 0.7	5.0 ± 0.7	.001[a]
Mean SBP (mm Hg)	113.1 ± 10.6	111.0 ± 9.1	.245
Mean DBP (mm Hg)	71.0 ± 7.9	70.8 ± 7.3	.935
Nondippers (%)	50.0	12.5	.001[a]

Abbreviations: BMI, body mass index; BUN, blood urea nitrogen; cIMT, carotid intima-media thickness; DBP, diastolic blood pressure; ESR, erythrocyte sedimentation rate; HbA1c, glycohemoglobin; HDL-C, high density lipoprotein cholesterol; LDL-C, low density lipoprotein cholesterol; SBP, systolic blood pressure.
[a] $P < .05$.

A total of 51.4% of the patients with PCOS presented with hirsutism and 81% had acne vulgaris on physical examination. Oligomenorrhea was reported in 45.9% of the patients with PCOS. Pelvic ultrasonography revealed polycysts in both ovaries in 56.9% of the patients with PCOS and 85.7% of them were anovulatory according to the hormonal parameters. Mean HOMA-IR was 2.17 ± 1.19 in PCOS group.

Regression analysis was performed to test the relationship between HOMA, LDL-C, HDL-C, triglyceride, HbA1c, and hsCRP and being a nondipper on 24-hour ABPM. Among these parameters, only HOMA ($P = .040$, $\exp(\beta) = 2.268$, 95% CI: 1.037-4.960) and LDL-C ($P = .039$, $\exp(\beta) = .958$, 95% CI = .921-.998) were related with nondipping (Table 2).

Factors related with mean cIMT were also tested by regression analysis. Likewise, HOMA, HbA1c, LDL-C, HDL-C, triglycerides, hsCRP, and mean systolic and diastolic BP were the tested parameters. None of them showed significance in relation to mean cIMT. In order to test the relationship between being a nondipper and cIMT; independent samples *t* test was used to compare mean cIMT values of dipper and nondippers in PCOS group. Mean cIMT was found to be significantly higher in the nondipper group ($P = .006$).

Table 2. Parameters That Were Analyzed for Their Correlation With Being Nondipper in Patients With Polycystic Ovary Syndrome (PCOS)

Parameter	β	*P*	Exp(β) odds ratio (OR)	95% CI for exp(β)	
				Lower	Upper
HOMA	.819	.040[a]	2.268	1.037	4.960
HbA1c	1.052	.154	2.862	0.674	12.149
HDL-C	.050	.107	1.051	0.989	1.118
LDL-C	.042	.039[a]	.958	0.921	0.998
Triglycerides	.010	.364	1.010	0.988	1.033
hs CRP	.026	.692	1.026	0.904	1.165

Abbreviations: HbA1c, glycohemoglobin; HDL-C, high density lipoprotein cholesterol; HOMA, homeostasis model assessment; hs CRP, high-sensitive C-reactive protein; LDL-C, low density lipoprotein cholesterol.
[a] $P < .05$.

Discussion

Polycystic ovary syndrome is a reproductive endocrine disorder characterized by chronic unovulation, hyperandrogenism, and insulin resistance. Polycystic ovary syndrome has been linked to an increased risk of metabolic cardiovascular syndrome. Talbott et al demonstrated that women with PCOS have adverse lipid profiles, including elevated LDL-C and triglycerides and decreased HDL-C, compared with controls.[24] More recently, Dejager et al demonstrated that atherogenic modifications of LDL-C, specifically a shift toward smaller more dense particles, were evident among 31 women with PCOS compared with 27 controls, suggesting a more atherogenic lipid profile and, potentially, a higher risk of coronary heart disease among women with PCOS.[25] In our study, however, we did not find any difference between the 2 groups according to the lipid parameters. Inflammatory markers have been postulated to have a more significant role in atherosclerosis than lipid levels and Kelly et al[26] found significantly elevated hsCRP levels in patients with PCOS and proposed low-grade chronic inflammation as a novel mechanism contributing to increased coronary heart disease and type 2 diabetes risks in women with PCOS. In accordance with the results of Kelly et al, we found increased levels of hsCRP and reflecting chronic low-grade inflammation in PCOS, fibrinogen, and ESR were also found to be significantly increased when compared with controls. More recent studies have suggested that PCOS cases have increased subclinical atherosclerosis as evidenced by increased cIMT. In a follow-up study of the total Pittsburgh PCOS cohort, Talbott et al[14] evaluated cIMT in 125 Caucasian women with PCOS and 142 age-matched controls. Among women 45 years of age and older, PCOS cases had a significantly greater mean cIMT than control women. As a group of lean and young women with PCOS who are normotensive and normolipidemic, we demostrated increased mean cIMT in PCOS group when compared to controls. That is why subclinical atherosclerosis may be considered to begin at an earlier stage in the disease process even in the absence of risk factors. We evaluated HOMA, HbA1c, LDL-C, HDL-C, triglycerides, hsCRP, and mean systolic and diastolic BP as factors that may affect mean cIMT in PCOS group; but we found that none of these parameters was related to mean cIMT. The factors that promote this early atherosclerotic process in PCOS need to be determined by further research.

Insulin resistance is found in 50% to 70% of women with PCOS and is now generally accepted as an important risk factor for the development of the metabolic syndrome in such women.[27] Hyperandrogenism correlates positively with insulin resistance in obese and lean women with PCOS.[28] As a result, insulin resistance and compensatory hyperinsulinemia are consistently documented in lean and obese women with PCOS compared with weight-matched controls.[29] The hyperinsulinism caused by insulin resistance may also be responsible both for an increased androgenic production and for greater values of free androgens (testosterone) via reduced hepatic synthesis of the sex hormone–binding globulin.[30,31] The mean HOMA-IR was 2.17 ± 1.19 in our patients with PCOS. Although the upper limit of HOMA-IR is still not clear in medical literature, this value is still high for lean patients with PCOS in our study group,[32] showing the presence of insulin resistance independent from obesity, in concordance with the study of Dunaif and Finegood.[33] There is also evidence that women with PCOS may have pancreatic beta cell dysfunction together with postreceptor level defect in insulin action as occurs in type 2 diabetics; causing inadequate amount of insulin secretion for the degree of peripheral insulin resistance that they experience during the disease process.[27,34] In patients with PCOS, there is a vicious cycle; excess insulin enhances androgen production in ovarian theca cells in response to LH stimulation, resulting in hirsutism and acne.[35] On the other hand, hyperinsulinemia suppresses hepatic production of sex hormone–binding globulin synthesis and causes hyperandrogenemia.[36] In our analyses, HOMA and LDL-C levels were found to be related with nondipping on 24-hour ABPM. Since patients with PCOS in our study group did not have diabetes and hypertension, we can only postulate effects of HOMA and LDL-C levels—even in normolipidemic individuals—may have effect on nondipping profile in these patients. Relationship between insulin resistance and diurnal BP variation is well known in essential hypertension and type 2 diabetics. On the other hand, there is no known relationship between LDL-C levels and nondipping. It is known that endothelial dysfunction underlies the nondipping state as shown in newly diagnosed type 1 diabetics.[37] Therefore, subclinical endothelial dysfunction may be related with LDL-C levels in these patients. But since we did not find any difference between the groups according to the lipid parameters, this relationship should be investigated further.

Obesity, diabetes, and prediabetes are risk factors for abnormal diurnal BP variation. But, there are few studies that assessed diurnal BP variation in patients with PCOS. In the study of Zimmermann et al, 14 patients with PCOS were compared with 18 normal controls and despite hyperinsulinemia in the PCOS group, there was no increased BP or left ventricular hypertrophy.[38] In the study of Kaya et al, HOMA index and

testosterone levels were found to be related to nondipping profile on 24-hour ABPM.[39] Holte et al determined a relation between elevated systolic BP and insulin resistance in both obese and lean patients with PCOS.[40] To the best of our knowledge there has not been any study that assessed diurnal changes and factors related to being a nondipper in patients with PCOS. Our study also revealed that the frequency of nondipping was higher among normotensive and lean patients with PCOS and mean cIMT was higher among nondippers when compared with dippers in the PCOS group.

The relationship between BP variation and cIMT has been recently studied in normo- and hypertensive participants and their relationship was superior to central hemodynamics and small variations in BP may cause medial hypertrophy in large arteries.[41,42] We also evaluated the relationship between cIMT and nondipping by comparing cIMT of dippers and nondippers in patients with PCOS; we found that nondippers have significantly higher cIMT ($P = .006$). Since our patients were normoglycemic, normolipidemic, normotensive, and lean, low-grade chronic inflammation caused primarily by insulin resistance may be the important underlying factor. But since we did not find any relationship between cIMT and HOMA-IR, hsCRP, mean BP, perhaps other methods of insulin resistance measurement and other inflammatory markers or cytokines should be tested to further define the early atherosclerotic process in PCOS.

Our study has some limitations. These include the small number of patients and the controls as well as the lack of evaluation of nondippers in the control group. Inflammatory markers other than fibrinogen and hsCRP could also be tested in future studies. Insulin resistance was calculated by HOMA-IR, but other methods to evaluate insulin resistance could also be used. Lastly, since we did not perform an OGTT and insulin measurements in controls, we are not able to compare HOMA between patients with PCOS and controls. These results could also be compared with classical PCOS patients with and without metabolic syndrome.

In conclusion, we found that young and lean patients with PCOS have greater cIMT and high number of nondippers compared with controls and being a nondipper was related with HOMA-IR and LDL-C level. Since our study was cross-sectional, we cannot extrapolate the results long term, but we can suggest a relationship between being nondipper and insulin resistance and LDL-C levels even in nonobese and normolipidemic patients. Although cIMT was higher in the PCOS group and in nondippers when compared with the dipper group, none of the tested parameters were significantly related to cIMT. Thus, our study emphasizes the presence of abnormal BP variation, insulin resistance, and high cIMT in this patient group even in the absence of obesity, hypertension, and hyperlipidemia.

Declaration of Conflicting Interests

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