Evaluation of metabolic syndrome frequency and premature carotid atherosclerosis in young women with polycystic ovary syndrome

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BACKGROUND: The aim of this study was to evaluate metabolic syndrome frequency, cardiovascular risk profile and premature carotid artery atherosclerosis in patients with polycystic ovary syndrome (PCOS) especially during early adulthood. METHODS: A case–control study was conducted on 43 young women (18–22 years of age) with PCOS and 43 age-matched volunteer controls. Anthropometrical measurements, hormone levels, lipid and glucose profile were obtained from all subjects. Two different criterias were used to assess metabolic syndrome (MS) frequency. Common carotid artery intima-media thickness (IMT) was measured and stepwise multiple linear regression analysis was used to identify the independent cardiovascular risk factors that predict IMT. RESULTS: MS was diagnosed in 11.6% (n = 5) of women with PCOS compared to no cases in the control group (P = 0.02). The mean IMT was significantly higher in PCOS subjects (0.746 ± 0.106 mm) compared to controls (0.608 ± 0.105 mm, P < 0.001). Among many cardiovascular risk factors evaluated, the diagnosis of PCOS, increased body mass index and decreased sex hormone-binding globulin were significant independent predictors of increased IMT. CONCLUSIONS: These findings indicate that adolescence may be a more appropriate time to intervene for PCOS patients, as many cardiovascular risks are already present during early adulthood. As far as IMT is concerned, mechanisms other than hyperandrogenaemia and obesity might be operating as causative factors.

Key words: cardiovascular/metabolic syndrome/PCOS/risk

Introduction

Polycystic ovarian syndrome (PCOS) is one of the most common metabolic disturbances affecting ~5% of women (Guzick, 2004). Although not a diagnostic criteria for PCOS, these women frequently carry several risk factors for future coronary heart disease, stroke or type 2 diabetes, such as obesity, insulin resistance and dyslipidaemia (Rotterdam ESHRE–ASRM-sponsored PCOS consensus workshop group, 2004).

Metabolic syndrome is another cluster of endocrine disturbances including obesity, insulin resistance, dyslipidaemia and hypertension, predisposing the individual to greater risk for developing cardiovascular disease and type-2 diabetes (Kohen-Avramoglu et al., 2003). Metabolic syndrome affects 4–8% of adolescents according to the definition criteria used (Goodman et al., 2004). In a recent study, the prevalence of metabolic syndrome among 20–29 year old PCOS patients was reported to be 8-fold greater than age-stratified population controls (Apridonidze et al., 2004).

Carotid intima-media thickness has been positively associated with the prevalence and incidence of stroke and myocardial infarction (Bots et al., 1997). Middle-aged women > 45 years of age and women ~ 30 years of age with PCOS were shown to have increased carotid intima-media thickness (IMT) when compared to controls (Talbott et al., 2000; Lakhani et al., 2004).

Despite the above studies, little is known about the pathophysiological basis and the time of onset of these clinically detectable changes. In this study, we aimed to evaluate metabolic syndrome prevalence and carotid IMT in the first 5 years of adulthood among women with PCOS.

Materials and methods

The study was designed as a prospective case–control study in Kocaeli University Department of Obstetrics and Gynecology between January 1, 2002 and January 1, 2004. The University Ethics Committee approved the study protocol and informed written consent was obtained from all the subjects. A total of 43 PCOS cases between 18 and 22 years of age and 43 age-matched control subjects were included.

The diagnosis of PCOS was made according to the criteria of the Rotterdam ESHRE–ASRM-sponsored PCOS consensus workshop group (2004) (Table I) when two out of three criteria was present:
The results are presented as numbers and percentages. Clinical hyperandrogenism was defined as total testosterone and free androgen index > 8, indicating hirsutism and/or presence of acne (Gallwey, 1962). Biochemical hyperandrogenism was defined as total testosterone and free androgen index > 95th percentile for the control group studied, which were 3.8 nmol/l and 7% respectively.

The control group consisted of regularly menstruating (26–32 day cycles) healthy, age-matched female medical students or nurses without evidence of above-mentioned endocrine disturbances based on the similar initial laboratory work-up as the PCOS patients. No restrictive diet was recommended and none of the subjects engaged in intensive aerobic activity. Women who had respiratory or cardiovascular disease, or who were taking medication such as aspirin that could influence vascular resistance, were excluded from PCOS cases and controls.

Initial physical examination included weight, height, waist and hip circumferences to calculate waist:hip ratio (WHR) and body mass index (BMI). Resting systolic (SBP) and diastolic (DBP) were measured by the same nurse with a sphygmomanometer in the morning after a 10 min rest period with the participant in a supine position. After 2 min, a second reading was taken and the mean of the two measurements was used for the analyses. All subjects were scored with Ferriman–Gallwey score for hirsutism and presence of acne was noted.

An oral glucose tolerance test (OGTT) was performed between 08:00 and 10:00 after a 3 day, 300 g carbohydrate diet and an overnight fast of 10–14 h. A 75 g oral glucose load was administered, and blood samples for glucose and insulin determinations were collected through an i.v. cannula at 0, 30, 60, 90 and 120 min. Serum glucose was measured by using gluokinase technique. Plasma insulin levels were measured by chemiluminescent enzyme immunoassay (Immulite 1000 Analyzer, Diagnostic Products Corp., Los Angeles, CA, USA) with intra- and inter-assay coefficients of variation < 6.4%. Lipid analysis in fasting serum was performed for all patients and included total cholesterol, high-density lipoprotein (HDL), low-density lipoprotein (LDL), very low-density lipoprotein (VLDL) and triglycerides. These parameters were measured by commercial enzymatic methods (Aeroset automated analyzer; Abbott laboratories, Abbott, IL, USA). OGTT results were evaluated according to the criteria of Expert Committee on the Diagnosis and Classification of Diabetes Mellitus (Lepor, 2004); impaired fasting glucose if fasting plasma glucose level is 100–125 mg/dl, impaired glucose tolerance if 120 min plasma glucose is 140–199 mg/dl. A ratio of fasting glucose to fasting insulin < 4.5 was diagnosed as insulin resistance (Legro et al., 1998). Homeostatic model assessment (HOMA) (Matthews et al., 1985) is applied by using the formula: insulin resistance (HOMA-IR) = [fasting insulin (IU/l) × fasting glucose (mmol/l)]/22.5.

All regularly menstruating women were scanned on cycle days 3–5 whereas oligo/amenorrhoeic women were scanned between days 3 and 5 after progesterin-induced withdrawal bleeding using Siemens Versa plus ultrasonography machine. The serum concentrations of estradiol ($E_2$), FSH, LH, testosterone, sex hormone-binding globulin (SHBG), prolactin, thyroid-stimulating hormone (TSH), cortisol, 17-OH-progesterone and dehydroepiandrosterone sulphate (DHEA-S) were measured by chemiluminescent enzyme immunoassay (Immulite 2000, Diagnostic Products Corp.). The intra- and inter-assay coefficients of variation were < 7% for all assays performed. Free androgen index (FAI) was calculated using the following formula: testosterone (nmol/l)/SHBG (nmol/l) × 100 (Morley et al., 2002).

An experienced sonographer (A.D.), who was unaware of the nature of the study and the diagnosis of the patients, measured the IMT of the common carotid artery. High resolution B-mode ultrasound images were obtained using a 7.5 MHz transducer (Power Vision 8000; Toshiba, Japan). The subjects rested for 15 min in supine position to allow pulse and blood pressure to stabilize. Ambient light and temperature were controlled throughout the procedure. The right and left common carotid artery, the carotid bifurcation–bulb areas were scanned from multiple planes. Images were obtained from the distal portions of both common carotid arteries, 1–2 cm proximal to the carotid bulb and immediately proximal to the origin of the bifurcation. IMT across 1 cm segments of the near and far walls of both common carotid arteries was measured as the distance between the junction of the lumen and intima and the junction of the media and adventitia and averaged. The inr-observer error was < 0.03 mm.

Metabolic syndrome (MS) was assessed both by National Cholesterol Education Program Adult Treatment Panel III (NCEP) (Goodman et al., 2004) and by revised World Health Organization (WHO) definition (Balkau and Charles, 1999). Metabolic syndrome was diagnosed if three of the five factors were present; hypertension (diastolic $\geq$ 85 mmHg, systolic $\geq$ 130 mmHg), central obesity (waist $\geq$ 88 cm), HDL $\leq$ 50 mg/dl, triglycerides $\geq$ 150 mg/dl, fasting glucose $\geq$ 110 mg/dl). For WHO-defined MS, fasting glucose $\leq$ 110 mg/dl or hyperinsulinaemia $\geq$ 20 mIU/l was required plus two of the additional parameters: hypertension parameter (diastolic $\geq$ 85 mmHg, systolic $\geq$ 130 mmHg), central obesity parameter (WHR $> 0.85$ and/or BMI $> 30$ kg/m$^2$), dyslipidaemia parameter (HDL $\leq$ 39 mg/dl and/or triglycerides $\geq$ 150 mg/dl) and presence of microalbuminuria.

**Statistical analysis**

We analysed the distribution of continuous variables with the Shapiro–Wilk normality tests. Continuous variables were expressed as mean $\pm$ SD and compared using Student’s t-test between the groups. Categorical data were expressed as numbers (percentages) and compared by using the $\chi^2$-test or Fisher’s exact test where suitable. $P < 0.05$ was accepted as statistically significant. Stepwise multiple linear regression analysis was used to identify

<table>
<thead>
<tr>
<th>Variable</th>
<th>PCOS</th>
<th>Control</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Oligo/amenorrhoea</td>
<td>43 (100)</td>
<td>0</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Clinical hyperandrogenism</td>
<td>38 (88)</td>
<td>8 (18)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Hirsutism</td>
<td>38 (88)</td>
<td>5 (11)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Acne</td>
<td>8 (18)</td>
<td>6 (14)</td>
<td>0.6</td>
</tr>
<tr>
<td>Biochemical hyperandrogenism</td>
<td>31 (72)</td>
<td>2 (4.6)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Total testosterone $&gt; 3.8$ mmol/l</td>
<td>18 (42)</td>
<td>1 (2.3)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Free androgen index $&gt; 7$%</td>
<td>29 (68)</td>
<td>1 (2.3)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Polycystic ovaries on ultrasound</td>
<td>41 (95)</td>
<td>4 (9)</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

The results are presented as numbers and percentages.
Age at menarche (years) 13.4
Ferriman–Gallwey score 13.4
Age (years) 21.4
LH (mIU/ml) 7.2
Waist:hip ratio 0.77

DHEA-S = dehydroepiandrosterone sulphate; SHBG = sex hormone-binding globulin; NS = not significant.

Variable PCOS (n = 43) Control (n = 43) P

<table>
<thead>
<tr>
<th>Variable</th>
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<th>Control</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>21.4 ± 1.8</td>
<td>20.8 ± 2.2</td>
<td>NS</td>
</tr>
<tr>
<td>Age at menarche (years)</td>
<td>13.4 ± 1.4</td>
<td>12.3 ± 1</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Married</td>
<td>7 (16.3)</td>
<td>9 (20.9)</td>
<td>NS</td>
</tr>
<tr>
<td>Smoking</td>
<td>6 (14)</td>
<td>8 (18.6)</td>
<td>NS</td>
</tr>
<tr>
<td>Body mass index (kg/m²)</td>
<td>23.4 ± 4.7</td>
<td>21.5 ± 3</td>
<td>0.02</td>
</tr>
<tr>
<td>Waist:hip ratio</td>
<td>0.77 ± 0.05</td>
<td>0.73 ± 0.05</td>
<td>0.008</td>
</tr>
<tr>
<td>Ferriman–Gallwey score</td>
<td>13.4 ± 5</td>
<td>6.8 ± 1</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>FSH (mIU/ml)</td>
<td>5.6 ± 1.7</td>
<td>6.1 ± 2</td>
<td>NS</td>
</tr>
<tr>
<td>LH (mIU/ml)</td>
<td>7.2 ± 3.7</td>
<td>5.1 ± 2.7</td>
<td>0.005</td>
</tr>
<tr>
<td>DHEA-S (µg/dl)</td>
<td>260 ± 118</td>
<td>179 ± 61</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Testosterone (ng/dl)</td>
<td>94.4 ± 52.8</td>
<td>66.6 ± 21.7</td>
<td>0.002</td>
</tr>
<tr>
<td>Estradiol (pg/dl)</td>
<td>31 ± 12</td>
<td>38 ± 14</td>
<td>0.01</td>
</tr>
<tr>
<td>SHBG (nmol/l)</td>
<td>33.4 ± 17</td>
<td>54.3 ± 19</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

Values are means ± SD or numbers and percentages.

DHEA-S = dehydroepiandrosterone sulphate; SHBG = sex hormone-binding globulin; NS = not significant.

The fasting insulin, fasting glucose and lipid levels and response to 75 g OGTT in women with and without PCOS are presented in Table III. Mean fasting insulin level and HOMA-IR level were significantly higher in PCOS patients while the fasting glucose:fasting insulin ratio was significantly lower than the control group. The criteria of insulin resistance or decreased insulin sensitivity such as fasting glucose:fasting insulin < 4.5 was significantly more frequent in the PCOS group when compared to the controls.

We found significantly lower mean HDL level and higher mean VLDL level in PCOS patients (Table III). Although the mean total cholesterol level was similar in the two groups, the frequency of women with total cholesterol: HDL ratio > 5, which is a risk factor for coronary heart disease, was significantly higher in the PCOS group when compared to the control group.

Metabolic syndrome frequency according to NCEP criteria was very low among PCOS patients (2.3%), which was nil among the controls (Table IV). WHO criteria more frequently labelled women as the metabolic syndrome among PCOS patients (11.6%) compared to no cases among the control group. Carotid artery ultrasound measurements in women with and without PCOS revealed that the mean carotid artery IMT was significantly higher while the pulsatility index, resistance index and systole:diastole ratios were significantly lower in the PCOS group when compared to the control group (Table IV).

In the multivariate linear regression model, increased BMI (β = 0.47, P < 0.001), the diagnosis of PCOS (β = 0.29, P = 0.01) and decreased SHBG (β = −0.28, P = 0.01) were independent predictors of increased IMT (adjusted R² = 0.57, standard error of the estimate = 0.08).

Discussion
Our findings suggest that young women with PCOS have more frequent cardiovascular risk factors such as increased...
IMT and MS frequency compared to the controls. Although there is a clear association between MS and PCOS, which can be defined as intertwined insulin resistance syndromes, an underlying common and predictive vascular risk parameter both in PCOS and MS is unknown.

Studies on the association of PCOS and vascular diseases yielded conflicting results. In one study, deaths due to cardiovascular disease were found not to increase among women with PCOS (Pierpoint et al., 1998). In a follow-up study on PCOS patients, the same investigators found an insignificant increase in coronary heart disease but a significant increase in cerebrovascular disease (Wild et al., 2000). In a larger study, using menstrual irregularity as a marker for PCOS, relative risk for coronary heart disease was 1.5-fold higher than in the controls (Solomon et al., 2002).

We found that higher frequency of MS and increased carotid artery intima-media thickness are already present in early adulthood in PCOS patients. This is not surprising as both PCOS and MS can be detected during adolescence (Kent and Legro, 2002; Goodman et al., 2003; Yildiz, 2004). In a recent study, young, normal weight, non-dyslipidaemic, non-hypertensive women with PCOS were shown to have higher endothelin-I levels and common carotid artery intima-media thickness (Orio, 2004b). Furthermore, a study conducted on women with PCOS aged <35 years stated that after adjusting for blood pressure, BMI, cholesterol and insulin, PCOS remains an independent risk for arterial disease (Lakhani et al., 2004). Our findings further support this hypothesis and suggest that PCOS may cause increased IMT due to high C-reactive protein and endothelin levels. Similar inflammatory mechanisms have been suggested to explain the relationship between MS and premature atherosclerosis respectively (Tracy, 2003; Ridker et al., 2004).

We have found a higher mean IMT in both controls and PCOS group than reported by other small sample-sized studies on 12, 20 and 30 young women (Lakhani et al., 2002, 2004; Orio et al., 2004b). As our sample size (n = 43 in both groups) is also relatively small, it is always prone to some selection errors. Also, our centre is a tertiary referral centre where mostly severe cases of PCOS with clinical hyperandrogenaemia are referred which might explain the differences in populations. As all cases were initial diagnosis of PCOS, none had any treatment up to study date. In addition to these, our diagnosis of PCOS was made according to recently formulated Rotterdam ESHRE–ASRM criteria, which was not the case in other studies.

### Table IV. Metabolic syndrome according to NCEP and WHO guidelines and carotid artery ultrasound measurements in PCOS patients and controls

<table>
<thead>
<tr>
<th>Variable</th>
<th>PCOS (n = 43)</th>
<th>Control (n = 43)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Metabolic syndrome (NCEP)</td>
<td>1 (2.3)</td>
<td>0</td>
<td>0.3</td>
</tr>
<tr>
<td>Mean arterial pressure</td>
<td>90.0 ± 10.0</td>
<td>88.0 ± 6.0</td>
<td>&lt;0.001^b</td>
</tr>
<tr>
<td>Intima-media wall thickness</td>
<td>0.76 ± 0.106</td>
<td>0.68 ± 0.105</td>
<td>&lt;0.001^b</td>
</tr>
<tr>
<td>(range in mm)</td>
<td>(5.0–1.0)</td>
<td>(0.45–0.80)</td>
<td></td>
</tr>
<tr>
<td>Pulsatility index</td>
<td>1.06 ± 0.44</td>
<td>1.73 ± 0.44</td>
<td>&lt;0.001^b</td>
</tr>
<tr>
<td>Resistance index</td>
<td>0.54 ± 0.1</td>
<td>0.71 ± 0.11</td>
<td>&lt;0.001^b</td>
</tr>
<tr>
<td>Systole/diastole</td>
<td>2.3 ± 0.3</td>
<td>3.7 ± 1.1</td>
<td>&lt;0.001^b</td>
</tr>
</tbody>
</table>

Values are mean ± SD and numbers and percentages.  
^aCompared to control group, Student’s t-test.  
^bCompared to control group, Fisher’s test.  
NCEP = National Cholesterol Education Program Adult Treatment Panel III;  
WHO = World Health Organization;  
PCOS = polycystic ovarian syndrome.
On the other hand, our measurements are not out of line. In a cross-sectional study, Stein et al. (2004) found that carotid IMT increased by 0.005 mm/year (CI: 0.002–0.008) in white females; 50th percentile was 0.61 mm in a 25 year old white female. Our control group is an average of 5 years younger and the 50th percentile can be expected to be ~0.60 mm, which was 0.61 mm in our study. The 90th percentile of carotid IMT in 25 year old white females was 0.759 (Stein et al., 2004), which can be estimated to be ~0.73 in a group composed of women 5 years younger. Our finding of 0.746 mm average carotid IMT indicates that almost half of the young women with PCOS have IMT above the 90th percentile.

We conclude that cardiovascular risk factors such as metabolic syndrome and increased common carotid artery intima-media thickness are already present as early as the first few years after adolescence. Screening during adolescence and controlling the BMI and PCOS with exercise or medical therapies may alter and modify the risk factors for future cardiovascular and cerebrovascular disease.

References


Legro RS, Finegood D and Dunaif A (1998) A fasting glucose to insulin ratio is a useful measure of insulin sensitivity in women with polycystic ovary syndrome. J Clin Endocrinol Metab 83,2694–2698.


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