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Autonomic dysfunction and flow-mediated dilation in polycystic ovary syndrome (PCOS): a case-control study.
Dysautonomia in polycistic ovary syndrome

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ABSTRACT

Aim: a case-control study was conducted on women with polycystic ovary syndrome (PCOS) with a view to identifying endothelial and autonomic nervous system dysfunctions capable of explaining the higher cardiovascular risk associated with PCOS.

Material and methods: 35 women (mean age 26.51 ±3; BMI 24.25 ±1.65) with documented PCOS and 35 controls matched for age, BMI and cardiovascular risk factors were studied during a solar year.

Endothelial dysfunction was assessed using flow-mediated dilation (FMD) and early atherosclerosis from the intima media thickness (IMT) of the carotid district measured using eco-color Doppler. Autonomic dysfunction was assessed with the classic tests (tilt, lying-to-standing, deep breath, Valsalva, Stroop).

Results: by comparison with controls, patients with PCOS had an altered response in some of the autonomic tests, i.e. lying-to-standing (mean values 3.25 ±4 mmHg vs -3.4 ±2.04, p<0.01); deep breath (RR max/RR min 1.03 ±0.04 vs 1.35 ± 0.05, p<0.01) and Valsalva (RRmax/RRmin 1.10 ± 0.05 vs 1.35 ± 0.05, p<0.01), but no relevant differences in the tilt, hand grip and Stroop tests. A diminished FMD (0.56 ± 0.8mm vs 0.74 ± 0.06mm, p<0.01) and a greater IMT (0.72 ± 0.03 vs 0.67 ± 0.03mm, p=0.031) were found in cases than in controls.

Conclusions: PCOS coincides with an endothelial dysfunction and a greater IMT, and autonomic assessment reveals sympathetic hyperactivity. These two findings may explain the link between PCOS and cardiovascular disease. Further studies are needed to shed light on this apparent link and demonstrate whether it is real and quantifiable.

Keywords: polycystic ovary syndrome, autonomic disease, intima media thickness, flow-mediated dilation.

SOMMARIO

Scopo: studio caso-controllo su donne affette da sindrome dell'ovaio policistico (PCOS) al fine di evidenziare alterazioni endoteliali e/o autonomiche in grado di spiegare l'alto rischio cardiovascolare riscontrato in queste pazienti.

Materiali e Metodi: sono state esaminate 35 donne (età media 26.51± 3 anni; BMI 24.25 ± 1.65) affette da PCOS e 35 controlli appaiati per età, BMI e fattori di rischio cardiovascolari durante un intero anno solare.

La disfunzione endoteliale è stata valutata mediante la flow-mediated dilation (FMD) e l'arterioscleorosi precoce mediante lo studio dello dell'intima-media thickness (IMT) mediante eco-color-Doppler delle arterie carotidi. La disfunzione autonomica (AD) è stata valutata mediante i seguenti test: tilt, lying to standing, deep breath, Valsalva, Stroop.

Risultati: nel confronto con i controlli, le pazienti con PCOS hanno presentato un'alterata risposta nei test autonomici lying to standing (valori medi 3.25±4 mmHg vs 3.4±2.04, p<0.01); deep breath (RR max/RR min 1.03±0.04 vs 1.35±0.05, p<0.01) e Valsalva (RRmax/RR min 1.10±0.05 vs 1.35±0.05, p< 0.01) ma non nel tilt, hand grip e Stroop test. Riguardo l'aterosclerosi precoce, i casi hanno dimostrato una ridotta FMD (0.56±0.8 mm vs 0.74±0.06 mm, p<0.01) ed un aumentato IMT (0.72±0.03 vs 0.67±0.03).

Conclusioni: PCOS presenta una disfunzione endoteliale ed un incremento dell'IMT e la valutazione autonomica rivela un'aumentata attività del sistema simpatico. Questi due risultati possono spiegare il link fra PCOS e rischio cardiovascolare. Ulteriori studi sono necessari per rafforzare questi dati visto i risvolti clinici e terapeutici che potrebbero implicare.

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INTRODUCTION

Polycystic ovary syndrome (PCOS) is a metabolic syndrome of uncertain etiology that affects young women. It is characterized by anovulation, hyperandrogenism and insulin resistance, and is often associated with obesity, hypertension, hypercholesterolemia, diabetes and metabolic syndrome (33-47%)[1]. Women with PCOS carry a higher risk of cardiovascular disease than healthy controls. The question is whether PCOS represents a cardiovascular risk per se or whether this increased cardiovascular risk in PCOS patients is associated with other factors.
Some authors have suggested that the greater risk of cardiovascular disease in PCOS patients is unrelated to any obesity, hypertension or diabetes[2], and another study found that this higher risk (55% of increased risk) persisted after correcting results for age and obesity[3]. Diastolic dysfunction, ejection fraction abnormalities and inflammatory states are generally considered responsible for the extra cardiovascular risk.

An autonomic response to sympathetic stimulation has been demonstrated in PCOS patients[4,5]. This sympathetic hyperactivity can be explained in the light of the following considerations: 1. the ovary has a large quantity of catecholaminergic fibers[6]; 2. nerve growth factor (NGF), a marker of sympathetic activity, is over-expressed in PCOS[7]; 3. mouse models with high NGF levels have an increase in plasma levels of luteinizing hormone (LH)[8]; 4. PCOS patients have an altered heart rate reserve (HRR) and heart rate variability (HRV), which are autonomic alterations[9]; and 5. muscle sympathetic nerve activity (MSNA) is higher in PCOS patients than in controls matched for BMI and age[10,11]. All these observational findings need to be confirmed by new research, however, and correlated with biohumoral parameters.

Endothelial dysfunction has also been identified in PCOS using biochemical markers (endothelin, ADMA) and imaging technologies (flow-mediated dilation [FMD] and intima media thickness [IMT])[12,13].

The results of the above-mentioned studies indicate that PCOS involves endothelial changes (particularly in terms of a reduced FMD), and variably altered IMT and biochemical marker levels[14,15,16]. According to some authors, the reduction in FMD only occurs in patients with abdominal obesity[17].

Another issue clouding the picture is that PCOS is not always classified in the same way: while some studies used the NH criteria, others adopted the Rotterdam criteria[1,18,19].

AIM OF THE STUDY

To identify any endothelial dysfunction and autonomic variations in a sample of PCOS patients paired with healthy controls, and to see if this syndrome poses a cardiovascular risk per se, and whether PCOS cardiovascular risk correlates with the patient's autonomic dysfunction.

SUBJECTS AND METHODS

Thirty-five women with PCOS according to the Rotterdam criteria[18] referring to the Endocrinology Clinic at Padua University were paired with 35 controls (nnPCOS) recruited from the II Medical Clinic at Padua University during the course of a solar year 2012. Any women with cardiovascular, immunological, or other endocrinological disorders, neoplasms, liver disease or pregnancy were ruled out.

The control group was paired for age, sex, BMI and cardiovascular risk factors (**Table 1**).

We considered women with a BMI >25<30 as overweight, and a BMI >30<39.9 as obese. For all the individuals involved in the study, we obtained a medical history and completed a physical examination, biohumoral tests, a standard electrocardiogram, and autonomic, FMD and IMT assessments. Regard therapy,noone of PCOS or controls patients takes drugs even oral contraceptives.

Institutional review board approval this study was obtained and all patients provided written informed consent.

Table 1.
Characteristics of cases (PCOS) and controls (nnPCOS).

	Mean Cases	Median Cases	Mean Controls	Median Controls
Age	26.51 ± 3	25	27.42 ± 2,72	26
BMI	26.25 ± 1.65	23.5	24 ± 1.6	23.43
Systolic blood pressure (mmHg)	119.7 ± 5.5	115	116.8 ± 3.2	115
Diastolic blood pressure (mmHg)	71.98 ± 3.36	70	73 ± 1.8	71.5
Heart rate (b/min)	72.75 ± 3	73	69.75 ± 2.3	68
Smoking (>5 cigarettes/die)	9 (25%)		9 (25%)	
Hypertension (BP>140/90 mmHg)	6 (17%)		6 (17%)	
Diabetes mellitus	3 (8%)		3 (8%)	
Hypercholesterolemia (Cholesterol>200 mg/dl)	7 (20%)		7 (20%)	

AUTONOMIC ASSESSMENT

Participants underwent cardiovascular autonomic nerve function tests[20] in the following order: deep breath, Valsalva, isometric hand grip, lying-to-standing, tilt and Stroop.

The deep breath test measures vagal heart rate control[21]. The duration of the expiratory and inspiratory breaths was 5 seconds each, for a total of 40 seconds (4 breathing cycles). The ratio of the longest to the shortest respiratory rate (RR) interval was measured from the ECG for each breathing cycle, and the mean of all four ratios was taken as the expiratory/inspiratory (E/I) ratio. The RR was measured using a chronograph.

The Valsalva test measures both parasympathetic and sympathetic function. Participants blew into a manometer to maintain an intrathoracic pressure of 40 mmHg for 15 seconds. The ratio between the shortest RR interval during the expiratory effort and the longest RR interval during the subsequent 20 seconds (the Valsalva ratio) was calculated. The RR interval was measured on the ECG printout, while blood pressure was assessed using an automated sphygmomanometer identical to the one used at our Critical Care Unit.

In the isometric hand grip test, participants squeezed a dynamometer in their dominant hand for 3 minutes using a force corresponding to 30% of their maximal squeezing force. Heart rate and blood pressure were measured at rest, and again before and after squeezing the dynamometer.

In the lying-to-standing test, participants stood up suddenly after lying quietly in a supine position for 5 minutes. Heart rate and blood pressure were measured at rest and then 1, 3, 5 and 7 minutes after standing up[22].

In the tilt test, participants lay supine on an electric tilting table. A restraining strap across the upper abdomen secured them to the table during the test, applying a negligible pressure to the surface of the body. Blood pressure recordings and ECGs were obtained continuously throughout the test, which lasted 30 minutes and the result was judged to be positive for autonomic dysfunction only in the event of symptoms fully reproducing the patient's original pre-syncopal or syncopal symptoms accompanied by arterial hypotension or bradycardia, or both[23].

In the Stroop test, the women were asked to read the name of 7 colors written on a sheet of paper as fast as they could while lying on a couch. The names of the colors were written using different colors from those they named (e.g. the word "green" was written in yellow, the word "white" was written in grey, and so on). This test is a mental stressor and blood pressure and heart rate should rise in normal subjects at the end of the test[24].

FLOW-MEDIATED DILATION AND INTIMA MEDIA THICKNESS

Cases and controls meeting the inclusion/exclusion criteria underwent

ultrasound assessment of the arterial system (carotid and brachial arteries). The test was performed with an Esaote Technos instrument (Tokyo, Japan) and a 7.5 MHZ probe for B-mode echography, and a 6 MHz probe for pulsed Doppler.

The carotid arteries were assessed first, with patients lying on the couch with their neck rotated through 45°. The examiner sat by the patient's shoulder. All the arteries examined (common, bifurcation, internal and external) were divided into three segments (inferior, medial and superior) with an angle of incidence of 60°.

The brachial artery was studied in the antecubital fossa.

An ultrasound technician completed the tests, which were all videotaped and reassessed by another ultrasound technician for validation purposes. In cases of discrepancy, the videotapes were further examined blindly by a senior expert.

Atherosclerotic plaque was defined as a protrusion into the vessel lumen of at least 2 mm, as measured from the border between the adventitial and medial layers[25]. Stenosis due to atherosclerotic plaque was classified according to the NASCET study[26] as follows: class 1 (an obstruction from 0 to 30%), class 2 (from 31 to 50%), class 3 (from 51 to 69%), class 4 (from 70 to 99%) and class 5 (complete obstruction). IMT was measured at preset levels and expressed in millimeters. The method for measuring IMT was based on the principle of "two parallel echogenic lines"[27]. Only images with the characteristic double-line arterial pattern were transferred to the computer by the sonographer.

After digitalization to obtain 640 x 580 peak cells with 256 gray levels, images were stored in a memory mass system and analyzed off-line. The highest of the values for each arterial system investigated (carotid and brachial) was recorded.

FLOW-MEDIATED DILATION

We studied endothelial function by measuring independent endothelial vasodilation in the

forearm; then we focused on the IMT of the carotid district.

Endothelial dysfunction was assessed from brachial artery FMD measurements. We used the procedures described in the Guidelines of the International Brachial Artery Reactivity Task Force[27]. We chose to test the brachial artery of the non-dominant arm at rest to avoid any influence of daily activity on the vessel's diameter and capacity to dilate. Vascular reactivity is influenced by many factors, such as temperature, food intake, drug use and sympathetic stimuli. After fasting for 12 hours, participants were assessed at rest in a quiet room at a controlled ambient temperature. Any vasoactive medication was withheld for at least four half-lives, wherever possible. Participants lay supine with their arms in a comfortable position for brachial artery imaging in the longitudinal plane 3-5 cm above the antecubital fossa. We only considered the images with a clear anterior and posterior intimal interface between the lumen and the vessel wall (near and far walls, respectively). The skin surface was marked and the arm was kept in the same position throughout the study. First, a sphygmomanometer cuff was placed around the forearm distally to the target artery. A baseline image was acquired to assess brachial artery diameter at rest. Then the cuff was inflated to a pressure of 200 mmHg, which was maintained for 5 minutes, during which time the operator constantly monitored the vessel on the screen.

A reactive hyperemia was then induced by suddenly deflating the cuff, causing a rise in shear stress and a consequent NO-mediated vasodilation. Longitudinal scans of the brachial artery were taken continuously from 60 seconds before deflating the cuff until 120 seconds afterwards . After inducing the reactive hyperemia, arterial diameter was measured between 45 and 60 seconds after deflating the cuff, as this is considered the period of maximal response[29]. Fifteen minutes later, another resting scan was obtained to confirm vessel recovery. The variation in vessel diameter due to shear stress was expressed as the percentage increase in diameter over the vessel diameter measured at rest (%FMD). All patients completed the procedure without any complications; most of them reported a sense of warmth and "pins and needles" in their hand and forearm after the sudden cuff deflation; all such symptoms disappeared within a few minutes.

STATISTICAL ANALYSIS

We examined the differences between PCOS and nnPCOS patients as regard autonomic test results, IMT, and FMD. The PCOS patients who smoked or had hypercholesterolemia, diabetes mellitus, or hypertension, were paired with controls with the same cardiovascular risk factors. The statistical analysis was performed using Student's t-test. A p value <0.05 was considered statistically significant.

RESULTS

PCOS patients revealed autonomic alterations in 3 tests (the lying-to-standing, Valsalva and deep breath test) by comparison with the nnPCOS controls (**Table 2**).

Table 2.
Autonomic tests in cases and controls.

	Mean Cases	Mean Controls	P
Lying to Standing (mmHg)	3.25 ± 4	-3.4 ± 2.04	0.0037
Hand Grip (increase diastolic blood pressure mmHg)	5.37 ± 3.15	7.71 ± 1.86	0.19 (n.s.)
Stroop Test (mmHg)	1.05 ± 3.2	0.25 ± 0.63	0.6 (n.s.)
Tilt Table Test (RR intervals ECG)	1.11 ± 0.05	1.176 ± 0.03	0.08 (n.s.)
Valsalva (Valsalva ratio)	1.109 ± 0.05	1.35 ± 0.05	<0.0001
Deep Breath (E/I ratio)	1.03 ± 0.04	1.35 ± 0.05	<0.0001

In the light of these results, we tested whether common cardiovascular risk factors could influence the autonomic tests. We would have preferred to calculate the correlation coefficient probability, but the homogeneous samples (75% of the PCOS patients were 15-35 years old and 91% of the controls were 15-40 years old) and the few cases with cardiovascular risk factors obliged us to opt for an exam of risk group.

We divided the samples into three groups by BMI:

-1. normal-weight (BMI<25): 61% of the study population as a whole (20 PCOS and 23 controls);

-2. overweight (BMI 25-30): 21% (9 PCOS and 6 controls); and

-3. obese (BMI>30): 18% (6 PCOS and 6 controls).

The 3 women in the overweight PCOS group had a BMI of 25.5.

Table 3 shows the autonomic test results by BMI and confirms the autonomic changes, despite the small size of the sample. The Valsalva and deep breath test results were significantly reduced in normal-weight PCOS and overweight PCOS patients. The variability emerging for the obese PCOS patients is due to the small size of the sample involved. The small sample size prevented any useful conclusions from being drawn for the other risk factors too.

Table 3.
Autonomic tests in participants grouped by BMI.

	Normal Weight (BMI<25)			Over Weight (BMI 25-30)			Obese (BMI>30)		
	Mean cases	**Menu controls**	**P**	**Mean cases**	**Menu controls**	**P**	**Mean cases**	**Menu controls**	**P**
LYING TO STANDING mmHg	2.65 ± 3.96	-3.00 ± 2.3	0.05	7.33 ± 4.17	-2.00 ± 0.87	0.08	-0.83 ± 4.07	-6.5 ± 1.47	0.29
HAND GRIP mmHg	5.85 ± 2.79	6.04 ± 1.78	0.9	7.44 ± 2.95	9.00 ± 1.53	0.69	0.67 ± 12.83	12.83 ± 1.31	0.05
STROOP mmHg	1.10 ± 2.5	0.26 ± 0.73	0.4	3.78 ± 3.63	0.17 ± 0.34	0.4	4.17 ± 3.36	0.33 ± 0.47	0.36
TILT TEST R/R	1.1 ± 0.06	1.16 ± 0.04	0.13	1.18 ± 0.07	1.2 ± 0.04	0.79	1.09 ± 0.04	1.2 ± 0.04	0.11
VALSALVA Ratio	1.11 ± 0.06	1.37 ± 0.06	<0.01	1.15 ± 0.06	1.33 ± 0.03	0.04	1.06 ± 0.04	1.32 ± 0.03	<0.01
DEEP BREATH E/I Ratio	1.05 ± 0.06	1.35 ± 0.06	<0.01	1.05 ± 0.04	1.33 ± 0.06	<0.01	0.98 ± 0.01	1.38 ± 0.04	<0.01

As for the correlations between IMT and FMD in the cases and controls (**Table 4**), PCOS coincided with an increased IMT and a reduced FMD by comparison with the controls, but with no difference in brachial artery diameter at rest (3.17 mm vs 3.16 mm in cases and controls, respectively).

Table 4.
Endothelial function in cases and controls: intima media thickness (IMT) and flow-mediated dilation (FMD).

Endothelian function			
	Cases	**Controls**	**P**
IMT (mm)	0.72 ± 0.03 mm	0.67 ± 0.03 mm	0.031
FMD (mm)	0.56 ± 0.8 mm	0.74 ± 0.06 mm	0.0006

Concerning the risk factors,about FMD variations in relation to age we evidenced that FMD declines with increasing age, but the PCOS patients had a greater reduction than controls, and PCOS seemed to influence FMD irrespective of age. About the correlation between IMT and age the study pointed out that the older PCOS patients seemed to be protected from precocious atherosclerosis, but this result is affected by the two women over 45 years of age.

As regards FMD and BMI (**Table 5**), the normal-weight and overweight PCOS patients had a lower FMD than controls, while this was not true of the obese patients. This finding is important because other studies have reported that FMD variations in PCOS depended on BMI (28), but in our sample FMD was altered in normal-weight PCOS patients too (**Table 5a**). IMT was greater in the normal-weight PCOS patients than in controls, but not in the overweight or obese patients (**Table 5b**). The small size of our sample prevents us from drawing any conclusions concerning the other cardiovascular risk factors such as type 2 diabetes,hypertension or hyperlipidemia and FMD or IMT.

Table 5.
(a) Flow-mediated dilation (FMD) and (b) Intima media thickness (IMT) in cases (PCOS) and controls (nnPCOS) by BMI.

a)

FMD	**PCOS Cases**	**Nn PCOS Controls**	**P**
BMI < 25	0.55 ± 0.09	0.76 ± 0.09	0.001
BMI 25-30	0.46 ± 0.04	0.75 ± 0.06	0.005
BMI > 30	0.71 ± 0.13	0.65 ± 0.05	0.71 (N.S.)

b)

IMT (mm)	**PCOS Cases**	**Nn PCOS Controls**	**P**
BMI < 25	0.72 ± 0.03	0.64 ± 0.02	0.006
BMI 25-30	0.74 ± 0.05	0.76 ± 0.05	0.96 (N.S.)
BMI > 30	0.71 ± 0.02	0.68 ± 0.03	0.69 (N.S.)

DISCUSSION AND CONCLUSIONS

PCOS is a complex syndrome and women with this disorder are at greater cardiovascular risk. In this study, we examined two aspects of the syndrome: autonomic dysfunction and endothelial dysfunction. PCOS patients have endothelial alterations irrespective of their age or BMI, and other cardiovascular risk factors are probably likewise uninvolved. FMD was found lower in PCOS patients than in controls, even after excluding obese patients perhaps with a small sample . While some studies on obese women with PCOS demonstrated a correlation between obesity and a lower FMD in their patients[30], we found no differences in FMD between obese PCOS patients and controls, which would confirm other reports indicating that PCOS per se is also important in endothelial dysfunction [30]. Further cohort studies are needed to assess the influence of obesity and PCOS on cardiovascular risk factors. IMT was found greater in normal-weight, young PCOS patients than in controls, but this was not true of the obese or older women with PCOS. These findings confirm that PCOS is an independent cardiovascular risk factor.

As for autonomic dysfunction, our study identified a sympathetic hyperactivity. The tests

that we conducted are very simple and have a good reproducibility[4]. The lying-to-standing, Valsalva and deep breath tests revealed an increased sympathetic activity in the patients' cardiovascular response, which can impair cardiac function. These results confirm the findings of other studies conducted using different methods[5,11] and may contribute to explaining the cardiovascular risk in PCOS patients. Hypersympathetic activity is known to represent a cardiovascular risk, particularly relating to sudden death[31], and sympathoexcitation may be involved in the pathogenesis of PCOS[31]. While we can say nothing on the link between diabetes or hypertension and autonomic dysfunction due to the small size of our sample, we did find evidence of smoking and hypercholesterolemia exacerbating the PCOS patients' worse FMD and autonomic dysfunction. FMD and IMT may be affected by cardiovascular risk factors[31], but in our study - and particularly in the younger PCOS patients who were not obese - the influence of cardiovascular risk factors was not important (confirming other reports of FMD being unassociated with BMI or other factors[32]). The same can be said of IMT[32]. The link between PCOS and cardiovascular events is unclear from the literature[34,35], but our data suggest a rule of endothelial dysfunction in the cardiac outcome of the polycystic disease. Other factors, such as inflammation, may contribute to cardiovascular disease in PCOS patients[34]. The importance of quantifying endothelial dysfunction in PCOS stems from the fact that it is a simple method for stratifying patients by cardiovascular risk and monitoring the efficacy of therapy.

We might be accused of failing to assess the effect of insulin on all the functions examined in this study. In 2009 Pieracciante considered insulin as a possible explanation for the hyperactive sympathetic function seen in PCOS patients[36] and recently, Goodman stressed that insuline resistance is belived to play an intrinsic role in the pathogenesis of PCOS .It is implicated in the ovulatory dysfunction of PCOS by distrupting the hypothalamic-pituitary-ovarian axis[37]. We restricted ourselves to assessing only the classic risk factors because of the small size of our sample. Further studies will be necessary to ascertain the link between the autonomic system, PCOS, insulin and FMD.

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