No Phenotypic Differences for Polycystic Ovary Syndrome (PCOS) Between Women With and Without Type 1 Diabetes Mellitus

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Context: Women with type 1 diabetes mellitus (DM1) have a higher prevalence of polycystic ovary syndrome (PCOS) than the general population.

Objective: The aim of this study was to clarify, in DM1 women with PCOS (PCOS-DM1), the influence of insulin therapy and glycemic control and evaluate the hormonal and phenotypic differences with age-matched and body mass index (BMI)-matched women with PCOS without diabetes.

Design, Setting, and Patients: We evaluated 103 DM1 women with and without PCOS treated with intensive insulin therapy; 38 age-matched and BMI-matched women with PCOS without diabetes were compared in a cross-sectional study.

Outcome Measurements: Clinical, anthropometric, and metabolic parameters were evaluated. Hormonal evaluation and ovary ultrasound were performed during the follicular phase of the menstrual cycle.

Results: Applying the diagnostic criteria of the Androgen Excess Society, 38 (36.89%) women with DM1 showed PCOS. The 38 PCOS-DM1 women showed no differences in treatment and glycemic control compared with DM1 women without PCOS. The only difference was a higher visceral adiposity index in PCOS-DM1 (1.21 $\pm$ 0.70 vs 0.90 $\pm$ 0.32; $P = .002$). PCOS-DM1 showed no phenotypic differences with age-matched and BMI-matched PCOS without diabetes. The hormonal pattern was similar except that higher levels of Δ4androstenedione were found in PCOS-DM1 (12.89 $\pm$ 3.49 vs 2.79 $\pm$ 1.75 nmol/L; $P = .010$).

Conclusions: The women with PCOS-DM1 do not exhibit particular phenotypic characteristics compared with nondiabetic women with PCOS. However, this pathological disorder must not be underestimated because it could be an additional cardiovascular risk factor in women with DM1. **(*J Clin Endocrinol Metab* 99: 203–211, 2014)**

Polycystic ovary syndrome (PCOS) is the commonest endocrine disorder among women of reproductive age, with a reported prevalence in the general population of 5% to 10% (1, 2); recently, however, the variability in the prevalence data (3–5) was found to be related mainly to the diagnostic criteria used and to the body mass index (BMI) of the study population, in some cases even reaching about 20% using the Rotterdam criteria (6). Many studies have demonstrated a significantly high prevalence of clinical hyperandrogenism, menstrual cycle abnormalities, or overt PCOS in women with diabetes mellitus type 1 (DM1), but the data are discordant due to the different diagnostic criteria adopted and the difference in the ethnicity of the population studied (7–14). In 2000 Escobar-Morreale et al (7) reported a prevalence of PCOS in 85 Spanish patients with a DM1 of about 18.8% according to

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Abbreviations: AES, Androgen Excess Society; BMI, body mass index; CSII, continuous subcutaneous insulin infusion; DHEA-S, dehydroepiandrosterone sulfate; DM1, type 1 diabetes mellitus; E2, 17-b-estradiol; FAI, free androgen index; FG, Ferriman–Gallwey; HbA_{1c}, glycosylated hemoglobin; HDL, high-density lipoprotein; 17OH-Pg, 17OH-progesterone; PCOM, polycystic ovary morphology; PCOS, polycystic ovary syndrome; VAI, visceral adiposity index.

the National Institutes of Health criteria (15); this prevalence would be higher using the Rotterdam criteria or those of the Androgen Excess Society (AES) (16). Indeed, subsequently Codner et al (12) studied 42 Chilean patients with DM1 and reported a PCOS prevalence of 40.5% according to the Rotterdam criteria and 31% according to the AES criteria.

Nevertheless, there are studies that have reported prevalence similar to that given in the general population (prevalence of 7.4% in 54 Caucasian patients with DM1, according to the Rotterdam criteria) (17). In all patients with DM1 and PCOS a high frequency of laboratory hyperandrogenism was shown.

Although the pathophysiological bases of PCOS in DM1 are not clear, it has been suggested that exogenous insulin therapy administered in a nonphysiological fashion could stimulate synthesis of ovarian androgens (18). In DM1 women, through glucotoxicity, chronic hyperglycemia can induce a reduction of peripheral insulin sensitivity, which sometimes leads to metabolic syndrome and ovarian hyperandrogenism (19–21).

Exogenous hyperinsulinism at the onset of ovarian function during puberty could reprogram the ovarian function toward increased androgen secretion, leading to hyperandrogenism and PCOS later in life (19).

In this regard Codner et al (12) reported that 75% of DM1 women on intensive insulin therapy had either PCOS or asymptomatic polycystic ovarian morphology on ultrasound scans, as compared with only 33% of patients on a more conservative conventional therapy using two daily insulin injections. However, this study and others have never shown a real major daily insulin requirement (units per kilogram daily) in DM1 women with PCOS.

The aims of our study were the following:

To clarify, in DM1 women, the influence of insulin therapy and glycemic control on the prevalence and phenotype of PCOS

To evaluate the phenotypic differences with age-matched and BMI-matched women with PCOS without diabetes.

Materials and Methods

Subjects

This study was approved by the Institutional Review Board at the Faculty of Medicine of the University of Palermo. At the time of observation all patients regularly signed an informed consent for the scientific use of their data.

Women with DM1

One hundred twenty-eight consecutive Caucasian postmenarcheal women with DM1 of reproductive age, followed up in our dedicated Outpatients Clinic (from January 1, 2011 to December 31, 2012), were cross-sectionally studied. Inclusion criteria were a 2-year or longer history of DM1 (glutamic acid decarboxylase antibody positivity at onset of disease and low C-peptide levels [<0.05 ng/mL] during mixed-meal tolerance test or glucagon stimulation test) at study entry, treated with insulin from the time of diagnosis. All patients received basal/bolus insulin therapy (at least four daily injections) or continuous subcutaneous insulin infusion (CSII).

Fifteen patients were excluded because they refused to enter the study, as well as three pregnant patients, five patients with autoimmune polyglandular syndrome, and two patients with hyperprolactinemia. Consequently, 103 women with DM1 (mean: 26.83 y; SD: 6.84; range: 14–42) were studied.

A detailed clinical history was obtained, including age at the time of the study, age at diagnosis of DM1, age of menarche, type of insulin treatment used, daily insulin requirement (units per kilogram per day), and indexes of metabolic control.

By applying the diagnostic criteria of the AES (16), 38/103 (36.89%) women with DM1 showed a PCOS.

Age- and BMI-matched PCOS women without DM1

A control group of 38 nondiabetic women (matched for age and BMI) with PCOS (according to the AES criteria) was retrospectively selected from our clinical database at the Outpatients Clinic dedicated to PCOS. The patients were matched as follows: for age (16 patients based on exact age, 10 [±1 y], 2 [±2 y], 6 [±3 y], 4 [±4 y] and 1 [+5 y], and for BMI 28 patients based on [±1 kg/m^2], 5 [±2 kg/m^2], 2 [±3 kg/m^2], and 3 [±4 kg/m^2]).

Also, from the two groups the following were excluded: all women treated with clomiphene citrate, oral contraceptives, antiandrogens, drugs to control their appetite, or insulin-sensitizing drugs (metformin and pioglitazone) during the 6 months before the first examination; women with 17OH-progesterone (17OH-Pg) levels >6.05 nmol/L who, after 250 mg Synacthen (synthetic analog of adrenocorticotrophic hormone) showed 17OH-Pg >30.26 nmol/L at 60 minutes; women with dehydroepiandrosterone sulfate (DHEA-S) >16.32 mmol/L, who, when screened with a computerized axial tomography scan, presented adrenal hyperplasia or adenoma or virilizing androgen-secreting neoplasias; women whose clinical and hormone evaluation (phenotype, increased 24-h free urinary cortisol, high cortisol levels after 1 mg of overnight dexamethasone) suggested Cushing's syndrome.

Among all patients with DM1, eight were taking angiotensin-converting-enzyme inhibitors due to microalbuminuria and four were taking angiotensin-converting-enzyme (ACE) inhibitors due to high blood pressure. Among patients with PCOS without DM1 only three were taking antihypertensive drugs; 10 patients (six with DM1 and four control PCOS without DM1) were using statins due to hypercholesterolemia.

The following relevant data were obtained from our databases: possible family history of diabetes, infertility, and hirsutism; age of menarche, history of prepubertal hypertrichosis, premature pubarche, and eating disorders; weight, BMI, waist circumference, blood pressure, and Ferriman–Gallwey (FG) score. The degree of hirsutism was evaluated using the FG map scoring system, which divides the body up into 11 domains.

Hirsutism was defined as FG score >8 (22). Both groups of women, according to outpatient practice routine, were trained to compile a calendar of menstrual cycles. The mean duration of menstrual cycles (days) of the last 6 months was obtained for each woman. All patients were tested for FSH, LH, 17-b-estradiol (E2), 17OH-Pg, basal prolactin, total testosterone, DHEA-S, Δ4androstenedione, and SHBG, during the follicular phase (on the seventh day of the spontaneous menstrual cycle or 7 days after withdrawal of bleeding, administering a single 100-mg im dose of medroxyprogesterone acetate in cases of secondary amenorrhea [four women with DM1 and PCOS and three women with PCOS without DM1]). On the same day, we also tested for total cholesterol, high-density lipoprotein (HDL) cholesterol, low-density lipoprotein cholesterol, triglycerides, and glycosylated hemoglobin (HbA_{1c}). Serum progesterone level was determined between days 20 and 24 of the menstrual cycle and chronic oligoanovulation was established if two consecutive cycles were anovulatory (Pg level <3 ng/mL [International System: <9.54 nmol/L]) (23). Biochemical hyperandrogenism was diagnosed when androgen levels were as follows: total testosterone >2.84 nmol/L, DHEA-S >12.14 mmol/L, Δ4androstenedione >10.72 nmol/L (calculated on the basis of the 95th percentile upper limits of basal serum androgen concentrations in 144 healthy normal Sicilian eumenorrheal women without hirsutism and with no family history of PCOS [used as a control group in a previous study]) (24). Transvaginal ovarian ultrasound scanning was performed between days 5 and 10 after the beginning of the last period using a 7.5-MHz vaginal probe transducer (General Electric LOGIQ 400MD). Both ovaries were measured in the sagittal, transverse, and coronal planes. Ovaries were classified as polycystic if 12 or more follicles measuring 2 to 8 mm in diameter were present in each ovary, and/or there was an increase in ovarian volume (<10 mL) (6, 25, 26).

Assays

All hormones were measured in our laboratory using commercial kits. These included ELISA (DRG Diagnostics; DRG Instruments GmbH) for FSH (mUI/mL), LH (mUI/mL), 17-β-E2 (pg/mL), 17OH-Pg (ng/mL), Pg (ng/mL), PRL (ng/mL), total testosterone (ng/mL), Δ4androstenedione (ng/mL; Arnika), and insulin (mUI/L; the intra- and interassay coefficient of variance were ≤4% and ≤3.6%, respectively). Chemiluminescence assays were used for DHEA-S (μg/dL; Immulite, Diagnostic Products) and serum SHBG (nmol/L; Immulite; Diagnostic Products). Blood glucose levels (mg/dL) were measured using an electrochemical system (Glucocard; Menarini Diagnostics). Total cholesterol, HDL, and triglycerides were measured in our laboratory using standard assays. HbA_{1c} was determined by HPLC with ion-exchange resin (HA8121; Hi-AutoA1c). HDL cholesterol levels were calculated with Friedewald's formula. The conversion factors for the International System were the following: glucose (mg/dL vs mmol/L: 0.0555), insulin (mUI/L vs pmol/L: 6.945), total cholesterol (mg/dL vs mmol/L: 0.0259), total testosterone (ng/mL vs nmol/L: 3.467), free testosterone (pg/mL vs pmol/L: 3.47), DHEA-S (μg/dL vs mmol/L: 0.0272), Δ4androstenedione (ng/mL vs nmol/L: 3.492), 17-β-E2 (pg/mL vs pmol/L: 3.671), 17OH-Pg (ng/mL vs nmol/L: 3.026), Pg (ng/mL vs nmol/L: 3.180), PRL (ng/mL vs μg/L: 1), FSH (mUI/mL vs IU/L: 1), and LH (mUI/mL vs IU/L: 1).

Free androgen index (FAI) was calculated as the ratio of total testosterone levels in nmol/L to SHBG levels in nmol/Lt × 100 (%) (27).

Visceral adiposity index (VAI) was calculated as described (28, 29) using the following sex-specific equation, where TG is triglyceride levels expressed in mmol/L and HDL is HDL cholesterol levels expressed in mmol/L:

$$\text{Females: } VAI = \left(\frac{WC}{36.58 + (1.89 \times BMI)}\right) \times \left(\frac{TG}{0.81}\right) \times \left(\frac{1.52}{HDL}\right)$$

Statistical methods

The SPSS version 17 and MedCalc version 11.3 were used for data analysis. Baseline characteristics were presented as mean ± SD for continuous variables; rates and proportions were calculated for categorical data. Normality of distribution for quantitative data was assessed by the Shapiro-Wilk test. The differences between the two groups were detected by the unpaired Student's *t* test for continuous variables (after testing for equality of variance: Levene test) and by the χ^2 test and Fisher's exact test (when appropriate) for categorical variables. To evaluate the independent metabolic variables influencing several hormonal parameters, in all DM1 women linear regression models were performed.

The ANOVA was used for comparison of the same hormonal variables between groups (DM1 without PCOS, DM1 with PCOS, and PCOS without DM1) after testing for equality of variance. The Fisher least significant difference post-hoc correction was applied if the variables had equal variances and the Dunnett post-hoc correction was applied if the variables did not have equal variances.

For comparison between women with DM1 (with and without PCOS), the group sizes gave 78.44% power to detect a moderate effect size (Cohen's d = 0.5) using *t* test, with α at 0.05.

For comparison between women with PCOS (with and without DM1), the group sizes gave 69.65% power to detect a moderate effect size (Cohen's d = 0.5) using *t* test, with α at 0.05. Post-hoc power analysis was performed using G*Power Version 3.1.6 software (Franz Faul, University of Kiel, Germany).

A *P* value of <.05 was considered statistically significant.

Results

Women with DM1 show a PCOS prevalence of 36.89% (38/103). Regarding age at onset of diabetes, no differences were found between diabetic women with and without PCOS and the prevalence of patients with prepubertal onset of diabetes was similar in the two groups (6/38 [15.8%] vs 14/65 [21.5%]; $P = .477$).

DM1 women with PCOS showed a higher prevalence of family history for hirsutism (10.0% vs 0%; $P = .017$), premature pubarche (13.2% vs 1.5%; $P = .025$), polycystic ovary morphology (PCOM) (73.7% vs 7.7%; $P < .001$), oligo/amenorrhea (81.6% vs 7.7%; $P < .001$), hirsutism (97.4% vs 4.6%; $P < .001$), acne/seborrhea (50% vs 12.3%; $P < .001$), and hyperandrogenemia (71.1% vs 3.1%; $P < .001$). DM1 women without PCOS showed a

Table 1. Clinical, Anthropometric, and Biochemical Characteristics of 103 Women With DM1 With and Without PCOS[a]

	Subjects (%)	Subjects (%)	DM1 Without PCOS (N = 65) Mean ± SD	DM1 With PCOS (N = 38) Mean ± SD	*P*
Age, y			27.57 ± 7.07	25.55 ± 5.01	.126
BMI, kg/m^2			21.60 ± 2.98	22.79 ± 3.80	.102
Waist circumference, cm			77.89 ± 7.90	80.57 ± 10.63	.181
Birth weight, kg			3.31 ± 0.60	3.08 ± 0.59	.102
Age of menarche, y			12.65 ± 1.16	12.44 ± 1.40	.457
FG score			2.47 ± 2.68	10.13 ± 5.30	<.001
Age at onset of diabetes, y			19.27 ± 8.61	16.68 ± 5.36	.097
Mean duration of menstrual cycles, d			28.61 ± 3.81	46.10 ± 15.06	<.001
Successful pregnancies			0.55 ± 0.84	0.18 ± 0.45	.015
Miscarriages			0.18 ± 0.46	0.27 ± 0.60	.460
Premenarchal onset of diabetes	14 (21.5)	6 (15.8)			.477
Prepubertal hypertrichosis	0	1 (2.6)			.369
Premature pubarche	1 (1.5)	5 (13.2)			.025
Eating disorders	1 (1.5)	1 (2.6)			.317
Current or former smokers	12 (19.1)	10 (27.7)			.490
Family history					
Family history for diabetes	54 (83.1)	32 (84.2)			.881
Family history for infertility	0	1 (2.6)			.369
Family history for hirsutism	0	4 (10.5)			.017
Hormonal profiles					
FSH, IU/L			7.14 ± 2.14	6.55 ± 2.28	.388
LH, IU/L			4.61 ± 1.42	8.05 ± 2.76	<.001
LH/FSH			0.69 ± 0.28	1.35 ± 0.55	<.001
17-β-E2, pmol/L			363.03 ± 101.43	246.03 ± 68.15	<.001
17OH-Pg, nmol/L			2.39 ± 1.00	3.30 ± 1.09	.017
Pg, nmol/L			25.57 ± 17.20	3.88 ± 6.36	<.001
Total testosterone, nmol/L			1.49 ± 0.62	2.95 ± 0.94	<.001
SHBG, nmol/L			90.72 ± 37.80	101.47 ± 40.53	.387
FAI (100 × [total testosterone/SHBG]), %			0.73 ± 0.62	0.95 ± 0.69	.383
DHEA-S, μmol/L			6.16 ± 1.86	7.96 ± 4.97	.129
Δ4androstenedione, nmol/L			7.26 ± 1.92	12.89 ± 3.49	<.001
Glycemic compensation					
HbA_{1c}, %			8.42 ± 1.40	8.63 ± 1.67	.508
Insulin therapy					
Rapid-acting analogs IR, U/kg			0.43 ± 0.15	0.41 ± 0.12	.528
Basal analogs IR, U/kg			0.28 ± 0.11	0.29 ± 0.09	.680
Total IR, U/kg			0.71 ± 0.21	0.71 ± 0.16	.856
Basal insulin therapy					
Glargine	36 (55.4)	18 (47.4)			.635
Detemir	22 (33.8)	13 (34.2)			
Neutral protamine lyspro	4 (6.2)	4 (10.5)			
CSII	3 (4.6)	3 (7.9)			

Abbreviation: IR, insulin requirement.

[a] According to Consensus Statement by the Androgen Excess and PCOS (AE-PCOS) Society (2010), univariate analysis: qualitative variables were analyzed through the χ^2 test or Fisher exact test; quantitative variables were analyzed through Student's *t* test after testing for equality of variance (Levene test).

higher mean number of successful pregnancies than DM1 women with PCOS (0.55 ± 0.84 vs 0.18 ± 0.45; P = .015). In addition, diabetic women with PCOS had a significantly higher LH/FSH ratio (1.35 ± 0.55 vs 0.69 ± 0.28; $P < .001$), higher levels of 17OH-Pg (1.35 ± 0.55 vs 0.69 ± 0.28 nmol/L; $P < .001$), total testosterone (2.95 ± 0.94 vs 1.49 ± 0.62 nmol/L; $P < .001$), Δ4androstenedione (12.89 ± 3.49 vs 7.26 ± 1.92 nmol/L; $P < .001$), and significantly lower levels of 17-β-E2 (246.03 ± 68.15 vs 363.03 ± 101.43 pmol/L; $P < .001$) and Pg in the luteal phases (3.88 ± 6.36 vs 25.57 ± 17.20 nmol/L; $P < .001$); no significant difference was found for all other hormonal parameters investigated (Table 1). Regarding metabolic parameters, only VAI (1.21 ± 0.70 vs 0.90 ± 0.32; P = .002) and triglycerides (1.0 ± 0.52 vs 0.75 ± 0.44 mmol/L; P = .013) were significantly higher in patients with PCOS (Figure 1); no differences were found for glycemic compensation (HbA_{1c}), daily insulin requirement, and type of basal insulin used (Table 1). In all DM1 women, the hormonal parameters that showed significant differences between the two groups (LH, 17-β-E2, 17OH-Pg, total testosterone, Δ4androstenedione, and Pg) were analyzed in

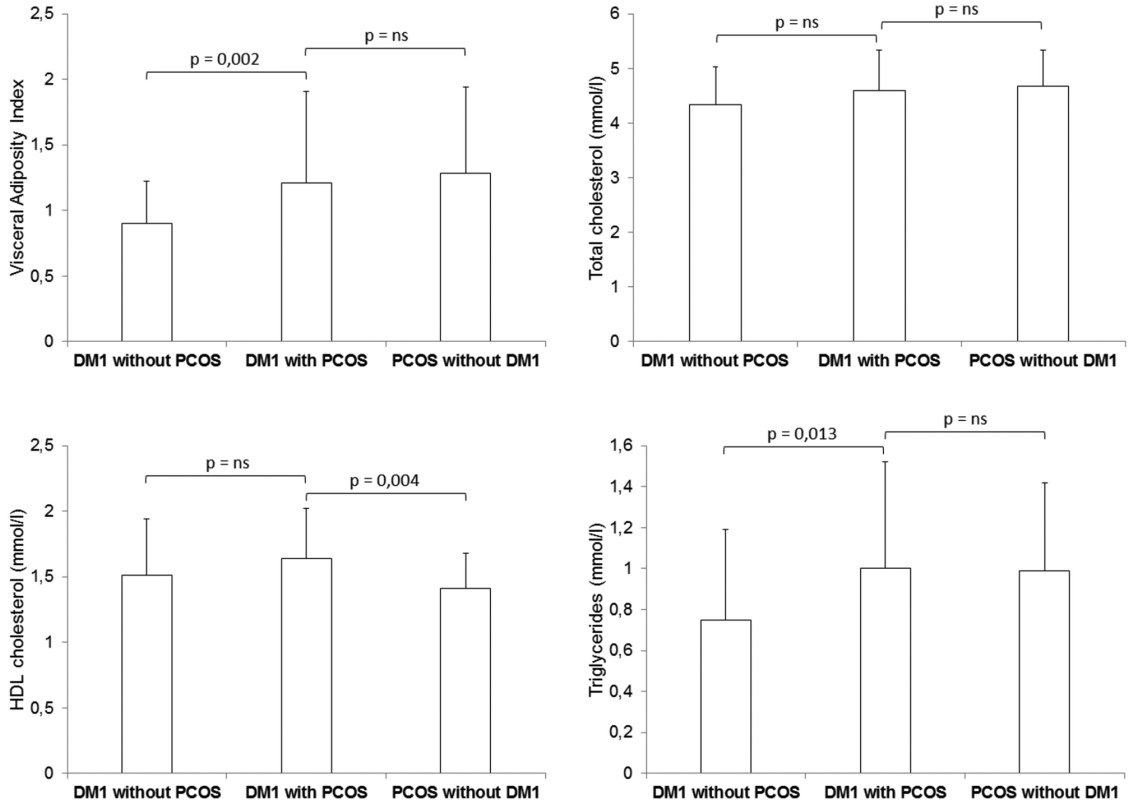

Figure 1. VAI and lipid profile in women with DM1 (with and without PCOS) and in women with PCOS without diabetes.

several multiple linear regression models, in which the dependent variables were represented by the metabolic parameters HbA_{1c}, VAI, age at onset of diabetes, and insulin requirement. In this multivariate analysis, VAI was the only metabolic variable independently associated with LH ($\beta = 0.46$; $P = .003$) and age of onset of diabetes was the only variable independently associated with Pg in the luteal phases ($\beta = 0.34$; $P = .028$) (Supplemental Table 1, published on The Endocrine Society's Journals Online web site at http://jcem.endojournals.org).

PCOS women with DM1 (PCOS-DM1) vs PCOS women without DM1 (PCOS-NO-DM1)

No significant differences in PCOS phenotype (using the AES diagnostic criteria) were found between PCOS-DM1 and PCOS-NO-DM1 (Tables 2 and 3). Instead, a significantly higher prevalence of prepubertal hypertrichosis (36.8% vs 2.6%; $P < .001$), family history for infertility (37.5 vs 2.6; $P = .013$), and family history for hirsutism (45.2% vs 10.5%; $P = .002$) was found in PCOS-NO-DM1. PCOS-DM1 women showed a higher

Table 2. Prevalence of All Possible Phenotypes Based on the Presence or Absence of Oligo-Anovulation, Hyperandrogenemia, Hirsutism, and PCOM, Using AES Criteria, in PCOS Women Without DM1 and in PCOS Women With DM1

	Potential Phenotypes								
	A	B	C	D	E	F	G	H	I
Hyperandrogenemia	X	X	X	X			X		X
Hirsutism	X	X			X	X	X	X	
Oligo-anovulation	X	X	X	X	X	X			
PCOM	X		X		X		X	X	X
PCOS women with DM1[a]	13 (34.2)	9 (23.7)	1 (2.6)		9 (23.7)	1 (2.6)	4 (10.5)	1 (2.6)	
PCOS women without DM1[a]	9 (23.7)	5 (13.2)	4 (10.5)	2 (5.3)	4 (10.5)	5 (13.2)	1 (2.6)	4 (10.5)	4 (10.5)
P[b]	.448	.374	.357	.493	.222	.199	.357	.357	.115

[a] Values are expressed as number (%).

[b] Univariate analysis: χ^2 test or Fisher exact test.

Table 3. Clinical, Anthropometric, and Biochemical Differences Between 38 PCOS Women With DM1 and 38 Age-Matched and BMI-Matched PCOS Women Without DM1

	Subjects (%)	Subjects (%)	PCOS Women With DM1 (N = 38) Mean ± SD	PCOS Women Without DM1 (N = 38) Mean ± SD	*P*
Age, y			25.55 ± 5.01	24 ± 2.68	.096
BMI, kg/m^2			22.79 ± 3.80	23.15 ± 1.75	.598
Waist circumference, cm			80.57 ± 10.63	72.86 ± 8.03	.001
Birth weight, kg			3.08 ± 0.59	2.83 ± 0.73	.094
Age of menarche, y			12.44 ± 1.40	12.00 ± 1.09	.126
FG score			10.13 ± 5.30	11.76 ± 6.29	.226
Mean duration of menstrual cycles, d			46.10 ± 15.06	47.39 ± 21.04	.760
Successful pregnancies			0.18 ± 0.45	0.18 ± 0.51	1
Miscarriages			0.27 ± 0.60	0.10 ± 0.38	.164
Prepubertal hypertrichosis	1 (2.6)	14 (36.8)			<.001
Premature pubarche	5 (13.2)	5 (13.2)			1
Eating disorders	1 (2.6)				1
Current or former smokers	10 (27.7)	7 (18.4)			.582
Family history					
Family history for diabetes	32 (84.2)	26 (68.42)			.177
Family history for infertility	1 (2.6)	3 (37.5)			.013
Family history for hirsutism	4 (10.5)	14 (45.2)			.002
Hormonal profiles					
FSH, IU/L			6.55 ± 2.28	6.50 ± 2.41	.941
LH, IU/L			8.05 ± 2.76	8.50 ± 4.19	.606
LH/FSH			1.35 ± 0.55	1.40 ± 0.75	.769
17-β-E2, pmol/L			199.48 ± 74.19	173.01 ± 81.17	.142
17OH-Pg, nmol/L			3.30 ± 1.09	3.54 ± 1.76	.523
Pg, nmol/L			3.88 ± 6.36	8.14 ± 9.95	.054
Total testosterone, nmol/L			2.95 ± 0.94	2.57 ± 1.73	.243
SHBG, nmol/L			101.47 ± 40.53	91 ± 51.80	.441
FAI (100 × [total testosterone/SHBG]), %			0.95 ± 0.69	1.37 ± 1.58	.203
DHEA-S, μmol/L			7.96 ± 4.97	8.51 ± 4.25	.635
Δ4androstenedione, nmol/L			12.89 ± 3.49	9.74 ± 6.11	.010

[a] According to Consensus Statement by the Androgen Excess and PCOS (AE-PCOS) Society (2010); Univariate analysis: qualitative variables were analyzed through χ^2 test or Fisher exact test; quantitative variables were analyzed through Student's *t* test after testing for equality of variance (Levene test).

waist circumference than PCOS-NO-DM1 women (80.57 ± 10.63 vs 72.86 ± 8.03 cm; $P = .001$).

Regarding the hormonal data, PCOS-DM1 only showed significantly higher levels of Δ4androstenedione than PCOS-NO-DM1 women (12.89 ± 3.49 vs 9.74 ± 6.11 nmol/L; $P = .010$). No differences were found for the metabolic profile, except for significantly lower levels of HDL cholesterol in PCOS-NO-DM1 (1.41 ± 0.27 vs 1.64 ± 0.38 mmol/L; $P = .004$) (Figure 1).

To confirm what was found in the two separate statistical approaches, a univariate ANOVA with post-hoc test was performed between the three groups (DM1 without PCOS, PCOS-DM1, and PCOS-NO-DM1) for the hormonal parameters that were significantly higher in PCOS-DM1 compared with DM1 without PCOS (Supplemental Table 2).

Discussion

Our study confirms the well-known higher prevalence of PCOS in women with DM1 than in the general population (7–14). However, in some previous studies, a precise diagnostic criterion for PCOS has not always been applied; in some cases only an increased prevalence of menstrual disturbances, hirsutism, and polycystic ovarian morphology has been reported in women with DM1.

In our opinion, the application of a criterion such as that proposed by the National Institutes of Health or by the AES (which requires the necessary presence of a condition of hyperandrogenism) is a very important point for the diagnosis of PCOS in women with DM1. In this connection, applying the Rotterdam criteria, we could mistakenly make a diagnosis of PCOS in women with DM1 that due to a long period of glycemic decompensation may develop a transient ovulatory phenotype without hyperandrogenism.

Unfortunately, our study, like most studies on the subject, was not able to identify the precise cause of this increased prevalence compared with the general population.

Our main finding was the observation of a similar insulin requirement in DM1 women with and without

PCOS. The link to the insulin requirement was not found that was observed in other studies, in which it was suggested that exogenous hyperinsulinemia may contribute to PCOS development by enhancing androgen production (12, 18–21). This could be due to the fact that some studies have not quantified the total insulin requirement, limiting the distinction between conventional and intensive insulin therapy (12). The link between PCOS and DM1 could involve supraphysiological concentrations of insulin within the systemic circulation, which does not necessarily correlate with insulin requirement or with type of insulin treatment. In women with DM1, subcutaneously administered insulin leaves out the first hepatic passage and leads to abnormally high insulinemia, which can interfere with ovarian function (30). Our study also showed no differences in the distribution of the different modes of insulin administration (multiple daily injections, CSII) and in the various types of insulin analogs used in diabetic women with PCOS and without PCOS. This finding is confirmed by a systematic review that reported no significant differences between multiple daily injection and CSII in the peripheral effects of hyperinsulinemia (31). Furthermore, the fact that our DM1 women with PCOS did not present a higher HbA_{1c} cannot exclude the influence of glucose toxicity in the pathogenesis of PCOS suggested by some studies (19, 30, 32); in this connection, rather than the existing glycemic control, it would be interesting to know the status of glucose toxicity during puberty (20). Furthermore, in the case of onset of diabetes before puberty, strong exposure to exogenous insulin during pubertal development may unmask a PCOS in genetically predisposed girls. In this regard, in our study, although the mean age of menarche was lower in women with DM1 and PCOS, this datum showed borderline statistical significance, probably due to the small sample size. These issues should be further investigated through prospective studies involving girls with onset of diabetes before menarche.

The only aspects that characterize the presence of PCOS in our population of women with DM1 are a greater number of cases of family history for hirsutism, lower levels of 17-β-E2 in the follicular phase, lower levels of Pg in the luteal phase, an increase in the LH/FSH ratio, and hyperandrogenemia.

An unexpected finding is the lack of an increase in FAI in women with DM1 and PCOS, despite the significantly higher levels of total testosterone. This is attributable to the absence of significant differences in the SHBG levels, which could be explained by a condition of exogenous hyperinsulinization that may involve all women with DM1 (with or without PCOS). In fact, there is evidence to suggest that hyperinsulinism may inhibit the hepatic synthesis of SHBG (33).

It would seem that in genetically predisposed women with DM1, exogenous insulin therapy and insulin resistance caused by glucose toxicity could increase the activity of several steroidogenic enzymes (CYP17, p450scc, 3-β-HSD) (34), which may lead to the hyperandrogenism observed in women with T1D and PCOS (12).

An interesting datum emerging from our study is the increased cardiometabolic risk in women with DM1 and PCOS, indirectly expressed by high VAI and triglyceride levels. VAI being an index of impaired fat distribution and function, in addition to indirectly expressing altered production, release, and/or function of adipocytokines and inflammatory factors, involved in the genesis of PCOS (35), also expresses a condition of cardiometabolic risk (24, 28, 36). Recently, in a population of young women with PCOS, VAI proved to be able to replace visceral computed tomographic scanning as a marker for visceral adiposity and to predict insulin resistance (37). These findings have led to the proposal of a precise VAI cutoff to differentiate metabolically healthy PCOS from metabolically unhealthy PCOS (38). However, in our study, DM1 women with PCOS, while presenting a higher VAI, did not show an increased insulin requirement. This datum would seem to conflict with the alleged lower insulin sensitivity of DM1 women with PCOS. In fact, insulin requirement, in type 1 persons with diabetes, is not a good surrogate of insulin sensitivity, because in addition to reflecting the degree of insulin sensitivity, it is also influenced by residual pancreatic function and by variability in absorption of insulin in subcutaneous adipose tissue. In the future, a study using the hyperinsulinemic euglycemic clamp could clarify this aspect.

Regarding comparison with nondiabetic women with PCOS, applying the AES criteria, no substantial phenotypic differences were observed except for an increased production of Δ4androstenedione in diabetic women with PCOS. Moreover, the impact of genetics (family history for infertility and hirsutism) was more striking in nondiabetic women with PCOS. The hormone profiles of DM1 patients with PCOS seem comparable to that of nondiabetic women with PCOS. In the former, the higher levels of Δ4androstenedione, also observed in other studies (12, 39), suggest that in DM1 women with PCOS the androgen excess appears to be mostly of ovarian origin (40, 41).

A limit of the study is, however, the small sample of women with PCOS and DM1; this made it impossible to perform a valid statistical analysis to compare DM1 women without PCOS with the various phenotypes of PCOS.

We conclude that in DM1, PCOS presents no particular phenotypic characteristics compared with PCOS in nondiabetic women. However, the diagnostic framing re-

mains of fundamental importance, because the presence of PCOS, and therefore of a condition of insulin resistance, could be an additional cardiometabolic risk factor in women with DM1 (42, 43). Hyperandrogenemia is often the most reliable finding in these patients, and it may be prudent to define diabetic women with PCOS according to the AES criteria.

In addition, given the evidence for PCOS as a complex genetic disorder with familial clustering, it is important to ascertain the family history for both PCOS and metabolic disease. In diabetic women with poor glycemic compensation without pronounced hyperandrogenism, it is preferable to monitor the symptoms and repeat PCOS evaluation after improvement in glycemic profile.

Finally, in the future the scientific community should use unique diagnostic criteria to better understand the pathophysiological mechanisms underlying the syndrome and to define the best therapeutic approach.

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