

RESEARCH

Predictive factors of polycystic ovary syndrome in girls with precocious pubarche

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Abstract

Objective: The aim of this study is to clarify, in girls with premature pubarche (PP), the influence of premature androgenization on the prevalence of polycystic ovary syndrome (PCOS).

Design and patients: Ninety-nine PP girls, 63 who developed PCOS and 36 who did not develop PCOS, were retrospectively included. Clinical, anthropometric, and metabolic parameters were evaluated at the time of diagnosis of PP and after 10 years from menarche to find predictive factors of PCOS.

Results: Young females with PP showed a PCOS prevalence of 64% and showed a higher prevalence of familial history of diabetes ($P = 0.004$) and a lower prevalence of underweight ($P = 0.025$) than PP-NO-PCOS. In addition, girls with PP-PCOS showed higher BMI ($P < 0.001$), waist circumference ($P < 0.001$), total testosterone ($P = 0.026$), visceral adiposity index (VAI) ($P = 0.013$), total cholesterol ($P < 0.001$), LDL-cholesterol ($P < 0.001$), non-HDL cholesterol ($P < 0.001$) and lower age of menarche ($P = 0.015$), ISI-Matsuda ($P < 0.001$), Dlo ($P = 0.002$), HDL cholesterol ($P = 0.026$) than PP-NO-PCOS. Multivariate analysis showed that WC ($P = 0.049$), ISI-Matsuda ($P < 0.001$), oral disposition index (Dlo) ($P < 0.001$), VAI ($P < 0.001$), total testosterone ($P < 0.001$) and LDL-cholesterol ($P < 0.001$) are independent predictive factors for PCOS in girls with PP.

Conclusions: Our study established a strong association between multiple risk factors and development of PCOS in PP girls. These risk factors are predominantly related to the regulation of glucose, lipid, and androgen metabolism. Among these factors, WC, ISI-Matsuda, Dlo, VAI, total testosterone, and LDL-cholesterol predict PCOS.

Key Words

- ▶ hyperandrogenism
- ▶ insulin resistance
- ▶ dyslipidemia
- ▶ visceral obesity
- ▶ precocious menarche

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Introduction

Girls with precocious pubarche (PP) (<8 years) tend to develop early and rapidly progressive puberty leading to early menarche (<8 years), to an adult height below the target level, and features of polycystic ovary syndrome (PCOS) (1, 2). Insulin resistance with total, visceral, and hepatic adiposity is thought to be a major driver of such early maturation (3, 4). Non-classical congenital adrenal

hyperplasia or heterozygous carriers of 21-hydroxylase deficiency can sometimes show PP (5).

PCOS is characterized by a combination of signs and symptoms of androgen excess (hirsutism and/or hyperandrogenaemia) and ovarian dysfunction (oligo-ovulation) and/or polycystic ovarian morphology (PCOM) (6). The most severe clinical manifestation is the classic PCOS

phenotype, which presents with both hyperandrogenism and oligo-ovulation, irrespectively of the presence of PCOM (6). A less severe phenotype is ovulatory PCOS (characterized by the co-presence of hyperandrogenism and PCOM), while the non-hyperandrogenic phenotype, consisting of oligo-ovulation and PCOM, is the least severe phenotype (6). In this light, PCOS is a heterogeneous disorder in terms of its link with insulin resistance and metabolic dysfunction. This association is much stronger in women with the classic PCOS phenotype than in those with ovulatory PCOS (7). Interestingly, women with the non-hyperandrogenic phenotype (8) rarely show insulin resistance (7) or metabolic dysfunction, although they might have mildly decreased levels of sex hormone-binding globulin (8). The reported prevalence of PCOS in women of reproductive age ranges from 6 to 20% depending on the different diagnostic criteria applied (9, 10, 11, 12, 13).

The age at menarche in women with PCOS may be earlier than in the general population on the basis of various conditions, such as genetic variants, androgen levels, and low birth weight (2, 14, 15). Both PP and PCOS are characterized by an excess of androgen levels showing an adverse metabolic phenotype, including hyperinsulinism, dyslipidaemia, and augmented risk for metabolic syndrome and diabetes mellitus in adulthood (16).

The aim of the current study was to identify metabolic factors in girls with PP that are associated with the development of PCOS and to establish predictive factors for PCOS in this population.

Materials and methods

This retrospective cohort study was carried out in patients with PP who developed or did not develop PCOS, attending the Unit of Endocrinology, University of Palermo (Italy), from January 2010 to December 2020. The study was approved by the Ethical Committee of Policlinico Paolo Giaccone, University of Palermo and carried out in accordance with the Declaration of Helsinki for experiments that involved humans. At the time of observation, the parents, regularly informed of the aim of the study, signed an informed consent for the scientific use of their data.

Population study

Ninety-nine Caucasian treatment-naive girls with PP (pubic hair < 8 years) and exclusion of congenital adrenal hyperplasia who developed or did not develop PCOS (PP-PCOS and PP-NO-PCOS) followed up from childhood

to adulthood in our dedicated Outpatients Clinic were retrospectively enrolled. Girls were evaluated at the time of diagnosis of PP and after 10 years from menarche to evaluate the presence of PCOS.

The diagnosis of PCOS was based on the diagnostic criteria of the European Society for Human Reproduction and Embryology/American Society for Reproductive Medicine (ESHRE/ASRM) Rotterdam Consensus (17) including the presence of at least two of the three following characteristics: clinical or biochemical hyperandrogenism, oligomenorrhea and/or PCO morphology in ultrasound. Applying the diagnostic criteria of the Rotterdam Consensus (17), 64/99 (64.6%) girls with a history of PP developed PCOS.

Exclusion criteria were the following: congenital adrenal hyperplasia or Cushing's syndrome.

The following relevant data were obtained from our databases: family history of diabetes, age of menarche, weight, BMI, waist circumference (WC), and Ferriman-Gallwey (FG) score.

At the time of diagnosis of PP, girls were tested for FSH, LH, 17 β -estradiol (E2), 17OH-Pg, basal prolactin, total and free testosterone, DHEA-S and Δ 4androstenedione, total cholesterol, high-density lipoprotein (HDL) cholesterol, low-density lipoprotein cholesterol, triglycerides, glutamic-pyruvic transaminase (GPT), glutamic-oxaloacetic transaminase (GOT). On the same day, an oral glucose tolerance test (OGTT) (75 g glucose) measuring glycaemia and insulinaemia was performed at 0, 30, 60, 90, and 120 min. The Matsuda index of insulin sensitivity (ISI-Matsuda) ($10000/\text{glucose (mg/dL)} \times \text{insulin (mU/mL)} \times \text{glucose mean} \times \text{insulin mean}$) (18), the oral disposition index (DIo) ($(\Delta\text{Insulin}_{0-30}/\Delta\text{Glucose}_{0-30}) \times (1/\text{fasting insulin})$) (19), and the area under the curve for insulin (AUC2h insulinaemia) and glucose (AUC2h glycaemia) were calculated.

The classes of obesity were defined according to BMI values. Girls were defined as underweight if having a BMI below the third percentile, overweight as having a BMI between 90th and 95th percentile, and obese for a BMI above the 95th percentile (20).

At the time of re-evaluation, after 10 years of menarche, women were trained to track a menstrual cycle calendar. 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 β -estradiol (E2), 17OH-Pg, basal prolactin, total and free testosterone, DHEA-S, Δ 4androstenedione, and SHBG, during the follicular phase (from the third to the fifth day of the spontaneous menstrual cycle or 7 days after withdrawal of bleeding, administering

a single 100-mg dose of medroxyprogesterone acetate in cases of secondary amenorrhoea). On the same day, we also tested for total cholesterol, high-density lipoprotein (HDL) cholesterol, low-density lipoprotein cholesterol, triglycerides, glutamic-pyruvic transaminase (GPT), glutamic-oxaloacetic transaminase (GOT) and performed an OGTT (75 g glucose) measuring glycaemia and insulinaemia at 0, 30, 60, 90, and 120 min. The 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)). Biochemical hyperandrogenism was diagnosed when androgen levels were as follows: total testosterone > 2.84 nmol/L, DHEA-S > 12.14 mmol/L, Δ 4androstenedione > 10.72 nmol/L (calculated on the basis of the 95th percentile upper limits of basal serum androgen concentrations in 144 healthy normal Sicilian eumenorrhoeal women without hirsutism and with no family history of PCOS (used as a control group in a previous study)) (21). 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). Ovaries were classified as polycystic if 12 or more follicles measuring 2–8 mm in diameter were present in each ovary, and/or there was an increase in ovarian volume (>10 mL) (11).

Metabolic syndrome was diagnosed according to the National Cholesterol Education Program Adult Treatment Panel (NCEP-ATP) III definition (22) and diagnosis of DM according to the American Diabetes Association criteria (23).

Assay methods

The samples were collected in the morning between 08:00 and 10:00 h after an overnight fasting of at least 8 h and stored at -80°C until processed. All hormones were measured in our centralized laboratory by chemiluminescence assays (Immulite, Diagnostic Products). These included FSH (mUI/mL), LH (mUI/mL), 17- β -E2 (pg/mL), PRL (ng/mL), total testosterone (ng/mL), DHEA-S ($\mu\text{g}/\text{dL}$), and insulin (mUI/L; the intra- and interassay coefficient of variance were $\leq 4\%$ and $\leq 3.6\%$, respectively). Mass spectrometry assays were used for Δ 4androstenedione (ng/mL) and 17OHPg (ng/mL).

Blood glucose levels, total cholesterol, HDL, and triglycerides were measured in our laboratory using standard assays. 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), DHEA-S ($\mu\text{g}/\text{dL}$ vs $\mu\text{mol}/\text{L}$: 0.0272), Δ 4androstenedione (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), PRL (ng/mL vs $\mu\text{g}/\text{L}$: 1), FSH (mUI/mL vs IU/L: 1), and LH (mUI/mL vs IU/L: 1).

Visceral adiposity index (VAI) was calculated as described (24, 25) 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:

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

Statistical analysis

SPSS version 17 and MedCalc version 11.3 were used for data analysis. Baseline characteristics were presented as mean \pm S.D. 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 PP-PCOS and PP-NO-PCOS were detected by Student's *t*-test for continuous variables and by the chi-square test for categorical variables. Crude odds ratios (ORs) and their 95% CI for the association of PCOS with potential risk factors in PP were calculated by univariate analysis at the time of diagnosis of PP. Adjusted ORs were calculated by stepwise logistic regression analysis to identify factors independently associated with the development of PCOS. Only factors significantly associated with PCOS by univariate analysis were included in the logistic regression analysis. The receiver operating characteristic (ROC) analysis was performed to investigate the diagnostic ability of significantly associated risk factors to predict PCOS development. The ROC curve is plotted as sensitivity vs 1-specificity. The area under the ROC curve (AUC) was estimated to measure the overall performance of the predictive factors for PCOS. A *P* value of <0.05 was considered statistically significant.

Results

Out of 99 girls with PP, 63 (64.6%) developed PCOS (PP-PCOS), while 36 did not develop PCOS (PP-NO-PCOS). Girls with PP-PCOS showed higher prevalence of familial history of diabetes (*P*=0.003) and lower prevalence

of underweight ($P=0.001$) than PP-NO-PCOS. In addition, PP-PCOS showed higher BMI ($P < 0.001$), WC ($P < 0.001$), total testosterone ($P=0.026$), VAI ($P=0.013$), total cholesterol ($P < 0.001$), LDL-cholesterol ($P < 0.001$), non-HDL cholesterol ($P < 0.001$) and lower age of menarche ($P=0.015$), ISI-Matsuda ($P < 0.001$), DIo ($P=0.002$), HDL cholesterol ($P=0.026$) compared with PP-NO-PCOS (Table 1). After 10 years from menarche, girls with a history of PP who developed PCOS had higher WC ($P < 0.001$), LH ($P=0.001$), LH/FSH ratio ($P=0.040$), VAI ($P=0.001$), DIo ($P=0.031$), total ($P=0.017$), LDL ($P=0.008$) and non-HDL cholesterol ($P=0.005$) and lower frequency of underweight ($P=0.001$), lower 17- β -E2 ($P=0.006$), progesterone

($P=0.018$) and ISI-Matsuda ($P=0.001$) values than those who did not develop PCOS (Table 2).

A multiple logistic regression model was fitted by using the above-mentioned risk factors as potential predictors for PCOS (Table 3). Our model demonstrates that ISI-Matsuda ($P < 0.001$), oral disposition index (DIo) ($P < 0.001$), VAI ($P < 0.001$), total testosterone ($P < 0.001$) and LDL-cholesterol ($P < 0.001$) at the time of diagnosis of PP were statistically significant factors for predicting the development for PCOS (Fig. 1). A ROC curve was constructed, and a prediction model was established with a moderately robust power (AUC=0.73) to predict PCOS in PP women. A P value <0.005 was statistically significant.

Table 1 Clinical, anthropometric, and biochemical characteristics of girls with premature pubarche developing or not PCOS at baseline.

	Premature pubarche who develop PCOS (n = 63)	Premature pubarche who do not develop PCOS (n = 36)	P
	Mean \pm s.d.	Mean \pm s.d.	
BMI (kg/m ²)	18.3 \pm 2.09	15.3 \pm 1.48	<0.001
Waist circumference (cm)	68.6 \pm 3.81	64.2 \pm 3.92	<0.001
FG score	14.5 \pm 5.38	15.8 \pm 5.99	0.574
Birth weight (g)	2930 \pm 642.1	3150 \pm 173.2	0.244
	Subjects (%)	Subjects (%)	
Familial history of diabetes mellitus	21 (33%)	6 (16.7%)	0.004
Classes of obesity			
Underweight	2 (3.2%)	6 (16.7%)	0.025
Normal weight	60 (96.8%)	30 (83.3%)	0.025
Overweight	1 (1.6%)	0	0.636
	Mean \pm s.d.	Mean \pm s.d.	
Hormonal profile			
FSH (IU/L)	0.66 \pm 0.28	0.77 \pm 0.23	0.901
LH (IU/L)	0.67 \pm 0.28	0.58 \pm 0.17	0.126
LH/FSH ratio	0.87 \pm 0.35	0.81 \pm 0.34	0.574
17- β -E2 (pmol/L)	20.1 \pm 12.8	20.1 \pm 8.86	0.996
17OHPg (nmol/L)	0.64 \pm 0.31	0.74 \pm 0.32	0.417
Prolactin (μ g/L)	11.1 \pm 3.84	11.2 \pm 3.19	0.989
Total testosterone (nmol/L)	0.28 \pm 0.31	0.11 \pm 0.04	0.026
DHEA-S (μ mol/L)	2.16 \pm 0.91	2.12 \pm 0.87	0.857
Δ 4androstenedione (nmol/L)	4.32 \pm 1.02	4.42 \pm 1.13	0.722
Metabolic profile			
ISI Matsuda	2.70 \pm 1.13	4.48 \pm 1.75	<0.001
AUC _{2h} insulin (pmol/L•120 min)	8639.9 \pm 604.8	7799.9 \pm 624.9	0.880
AUC _{2h} glucose (mmol/L•120 min)	747.7 \pm 190.2	735.5 \pm 120.1	0.959
Oral disposition index (DIo)	2.14 \pm 0.92	3.34 \pm 1.76	0.002
VAI	1.91 \pm 0.53	1.48 \pm 0.43	0.013
Total cholesterol (mmol/L)	3.31 \pm 0.44	2.68 \pm 0.67	<0.001
HDL cholesterol (mmol/L)	1.10 \pm 0.13	1.18 \pm 0.13	0.026
Calculated LDL cholesterol (mmol/L)	1.97 \pm 0.45	1.28 \pm 0.62	<0.001
Non-HDL cholesterol (mmol/L)	2.21 \pm 0.44	1.49 \pm 0.63	<0.001
Triglycerides (mmol/L)	1.14 \pm 0.29	1.07 \pm 0.23	0.256
GOT (U/L)	15.4 \pm 2.96	23.8 \pm 12.6	0.216
GPT (U/L)	20.4 \pm 12.4	28.8 \pm 16.6	0.392

17- β -E2, 17 β -estradiol; 17OHPg, 17OH progesterone; AUC_{2h} glucose, area under curve 2 h of glucose; AUC_{2h} insulin, area under curve 2 h of insulin; VAI, visceral adiposity index.

Table 2 Clinical, anthropometric, and biochemical characteristics of women with a history of premature pubarche at the time of diagnosis of PCOS.

	Premature pubarche with PCOS (<i>n</i> = 63)	Premature pubarche without PCOS (<i>n</i> = 36)	<i>P</i>
	Mean ± s.d.	Mean ± s.d.	
Age at evaluation (years)	21.2 ± 4.48	22.4 ± 3.42	0.509
BMI (kg/m ²)	32.6 ± 7.99	30.2 ± 7.36	0.127
Waist circumference (cm)	104.6 ± 16.1	98.1 ± 12.5	<0.001
FG score	16.6 ± 6.42	18.1 ± 6.45	0.574
	Subjects (%)	Subjects (%)	
Classes of obesity**			
Underweight	2 (3.1%)	8 (22.8%)	0.001
Normal weight	27 (42.1%)	14 (40%)	0.840
Overweight	15 (23.4%)	6 (17.1%)	0.465
Obese class I	7 (10.9%)	3 (8.5%)	0.705
Obese class II	5 (7.8%)	2 (5.8%)	0.712
Obese class III	8 (12.5%)	2 (5.8%)	0.293
Obesity	20 (31.7%)	7 (19.4%)	0.188
Metabolic syndrome***	10 (15.6%)	2 (5.8%)	0.154
Impaired fasting glucose (IFG)	1 (1.5%)	0	0.468
Impaired glucose tolerance (IGT)	0	0	/
IFG + IGT	0	0	/
Diabetes mellitus	0	0	/
	Mean ± s.d.	Mean ± s.d.	
Hormonal profile			
FSH (IU/L)	5.66 ± 1.74	5.13 ± 2.48	0.538
LH (IU/L)	7.77 ± 2.73	4.54 ± 2.02	0.001
LH/FSH ratio	1.47 ± 0.61	1.01 ± 0.56	0.040
17-β-E2 (pmol/L)	151.9 ± 97.3	325.9 ± 203.7	0.006
17OHPg (nmol/L)	5.71 ± 3.05	3.87 ± 1.91	0.066
Prolactin (μg/L)	18.1 ± 6.99	15.6 ± 6.51	0.279
Pg (nmol/L)	11.1 ± 10.4	23.8 ± 11.7	0.018
DHEA-S (μmol/L)	10.5 ± 4.92	9.35 ± 6.75	0.801
Δ4androstenedione (nmol/L)	13.4 ± 5.37	12.1 ± 6.56	0.707
Metabolic profile			
Homa2-IR	3.75 ± 2.41	3.33 ± 1.13	0.868
ISI Matsuda	2.78 ± 1.14	5.51 ± 1.64	0.001
AUC _{2 h} Insulin (pmol/L•120min)	8639.9 ± 604.8	7799.9 ± 624.9	0.729
AUC _{2 h} glucose (mmol/L•120min)	747.7 ± 190.2	735.5 ± 120.1	0.961
Oral disposition index (Dio)	2.08 ± 1.09	1.56 ± 1.18	0.031
VAI	2.65 ± 1.41	1.38 ± 0.42	0.001
Total cholesterol (mmol/L)	5.11 ± 0.71	3.27 ± 0.38	0.017
HDL cholesterol (mmol/L)	1.25 ± 0.31	1.29 ± 0.15	0.789
Calculated LDL cholesterol (mmol/L)	3.55 ± 0.82	1.78 ± 0.38	0.008
Non-HDL cholesterol (mmol/L)	3.84 ± 0.86	1.98 ± 0.39	0.005
Triglycerides (mmol/L)	1.27 ± 0.74	0.98 ± 0.19	0.104
GOT (U/L)	15.4 ± 2.96	23.8 ± 12.6	0.216
GPT (U/L)	20.4 ± 12.4	28.8 ± 16.6	0.392

WHO classification (2010); *According to Adult Treatment Panel (ATP) III criteria.

17-β-E2, 17β-estradiol; 17OHPg, 17OH progesterone; AUC_{2 h} glucose, area under curve 2 h of glucose; AUC_{2 h} insulin, area under curve 2 h of insulin; VAI, visceral adiposity index.

Discussion

This retrospective cohort study of PP girls confirms the interesting relationship between PCOS and PP and the higher prevalence of PCOS in girls with PP compared to that in the general population (26). We established

a significant association with a number of metabolic factors after diagnosis of PP, with the development of PCOS, including WC, age of menarche, ISI-Matsuda, Dio, VAI, total testosterone, total, LDL, non-HDL, and HDL cholesterol, and familial history of diabetes. In the multivariate analysis, WC, ISI-Matsuda, Dio, VAI,

Table 3 Risk factors associated with PCOS in girls with premature pubarche (PP) at diagnosis.

Variable	PP-PCOS (n = 63)	PP-NO-PCOS (n = 36)	Crude OR (95% CI)
Age of menarche			
11.1–13.11 years	8 (12.7%)	14 (38.9%)	1
8.6–11 years	55 (87.3%)	22 (61.1%)	4.37 (1.61–11.8)
Total testosterone			
≤0.13 nmol/L	23 (36.5%)	34 (94.4%)	1
>0.13 nmol/L	40 (63.5%)	2 (0.6%)	14.8 (3.08–70.9)
ISI-Matsuda			
>4.3	9 (14.3%)	23 (63.9%)	1
≤4.3	54 (85.7%)	13 (36.1%)	12.6 (4.21–38.1)
Dlo			
>4	6 (9.5%)	25 (69.4%)	1
≤4	57 (90.5%)	11 (30.6%)	29.8 (3.62–245.6)
VAI			
≤1.75	21 (33.3%)	25 (69.4%)	1
>1.75	42 (66.7%)	11 (30.6%)	4.74 (1.86–12.1)
Total cholesterol			
≤2.88 mmol/L	11 (17.5%)	27 (75%)	1
>2.88 mmol/L	52 (82.5%)	9 (25%)	8.18 (2.9–23.1)
LDL-cholesterol			
≤1.66 mmol/L	23 (36.5%)	31 (86.1%)	1
>1.66 mmol/L	40 (63.5%)	5 (13.9%)	12.3 (3.84–39.3)
Non-HDL cholesterol			
≤1.86 mmol/L	21 (33.3%)	25 (69.4%)	1
>1.86 mmol/L	42 (66.7%)	11 (30.6%)	5.72 (1.67–19.5)
Familial history of diabetes			
No	42 (66.7%)	30 (83.3%)	1
Yes	21 (33.3%)	6 (16.7%)	2.5 (0.9–6.94)
Underweight			
No	61 (96.8%)	30 (83.3%)	1
Yes	2 (3.2%)	6 (16.7%)	0.16 (0.03–0.86)

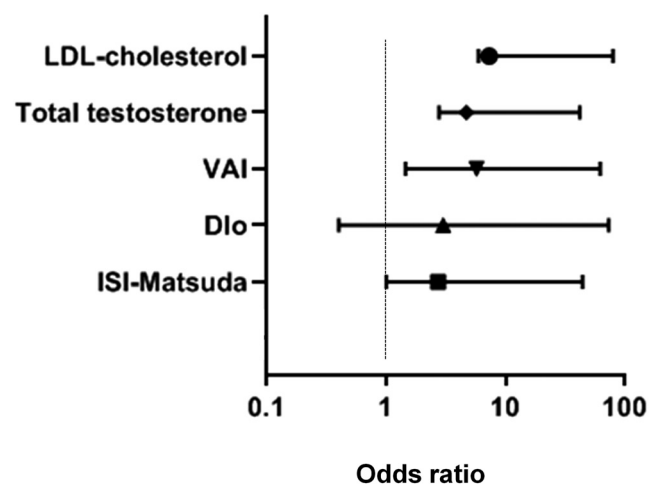


Figure 1 A forest plot showing odds ratio (OR) values and 95% CI for PP and PCOS, adjusted by age of menarche, total, non-HDL and LDL-cholesterol, familial history of diabetes, and underweight. The x-axis represents the OR (circles, square, and triangles) and 95% CI (whiskers). The dashed vertical line indicates an OR value of 1.

total and LDL-cholesterol were found to be significant predictive factors for PCOS. In addition, our model performs moderately well in predicting the incidence of PCOS.

The relationship between PP and PCOS has been widely investigated. The first hypothesis of a relationship between PP and PCOS was reported by Ibanez *et al.* who found that 15 out of 35 girls with PP developed PCOS (27). The coexistence of PCOS and PP is compatible with the concept that the ovarian and adrenal dysfunction of PCOS represents an inborn dysregulation of steroidogenesis (28). Besides the pubarchal androgen levels, other factors such as low birth weight and genotype have been suggested to be associated with the development of PCOS in girls with PP (2, 29). Although, some studies reported that low birth weight was associated with greater hyperinsulinism leading to PP and PCOS (2, 29, 30), other studies did not confirm these data (31).

Interestingly, some genetic variants and the presence of high BMI values were also reported to strongly influence menarche age in women with PCOS (29, 30). Girls with PCOS who were overweight in the pubertal window had an

earlier menarche age, whereas those who were thin tended to have a later menarche age (29, 30, 31).

In another set of studies, Ibañez *et al.* (32, 33) reported that, as in PCOS, PP girls were excessively insulin-resistant from mid-childhood through puberty. This was associated with central adiposity and dyslipidemia but not obesity (34). In agreement with these authors, other studies documented that PP patients were insulin-resistant and obese and reported a correlation between insulin resistance and androgen levels (35).

Early metformin treatment in low birth weight girls with PP was associated with a delay in the menarche age of about 1 year compared to girls who were not treated with metformin (36). In addition, early treatment was more effective in reducing hirsutism, androgen excess, oligomenorrhea, and PCOS than late treatment (37). Early adrenarche was, therefore, correlated with a high risk of adipose tissue dysfunction as a consequence of androgen excess (38).

A Parisian collaborative group identified Caucasian PP patients with no hormone abnormality who were postpubertal (31) and showed that these girls did not differ from controls in hirsutism score, acne, the prevalence of menstrual irregularity, BMI, or glucose and insulin during an OGTT. The investigators concluded that only 15–20% of their PP population developed hyperandrogenism after menarche. Their study did not clarify whether the subgroup of girls with exaggerated adrenarche was also at increased risk of developing PCOS.

In line with these studies, our study shows that girls with PP who develop PCOS are more insulin-resistant than PP who do not develop PCOS. In the current study, the insulin resistance degree was evaluated by ISI-Matsuda, and the insulin secretion by D_{IO}. ISI-Matsuda is a useful tool to evaluate the insulin resistance degree of the whole body. It derives from OGTT and more closely correlates with the M-value of the euglycaemic hyperinsulinaemic clamp, which represents the gold standard of insulin sensitivity measurement (18). Lower ISI-Matsuda values in patients with PP-PCOS clearly show an insulin resistance condition in these patients, compared to PP-NO-PCOS. Interestingly, girls with PP-NO-PCOS showed higher values than PCOS-NO-PP, suggesting that the coexistence of PP and PCOS may have an additive adverse effect on insulin sensitivity. D_{IO} is an interesting tool that expresses the ability of β -cells to adequately compensate insulin resistance through increased insulin secretion and is a predicting factor of diabetes development in adults (19). Girls with PP-PCOS showed lower D_{IO} values PP-NO-PCOS, further supporting the hypothesis of an additive effect of premature

androgenization, and the development of PCOS tends to worsen insulin sensitivity and secretion.

An interesting finding in this study is the evaluation of cardiometabolic risk by VAI. VAI is an index of altered visceral fat function and distribution. It has been shown that VAI is a marker of visceral adiposity, insulin resistance, impaired adipocytokine production, and inflammatory product secretion, which better correlates with the computed tomographic scan (38, 39, 40, 41) in women with PCOS. Although, VAI is not validated in paediatric population, many studies report its usefulness in predicting metabolic syndrome in children (42) and its association with insulin resistance, adipokines, and subclinical inflammation (43). Girls with PP-PCOS showed higher VAI compared to PP-NO-PCOS both at the time of diagnosis of PP and PCOS. These data suggest that the coexistence of PP and PCOS is responsible for an increased cardiometabolic risk. This hypothesis is confirmed by the knowledge that increasing androgen levels in females, as documented in PCOS women, result in abdominal adiposity, adipose tissue dysfunction, insulin resistance, and metabolic disturbances (44). On this concept, there has been created the theory of the 'metabolic valley of death', as a window of circulating androgen excess associated with adverse metabolic manifestations in both sexes.

There remains to be further explored the physiological role of androgens in regulating multiple aspects of insulin signalling in females which becomes evident early *in utero* and possibly determines PP. Indeed PP may represent the most pronounced example, where clinical signs of androgen action (pubic/axillary hair, acne, greasy hair, and accelerated stature growth) appear early in girls and are associated with an increase in adrenal androgen precursors, such as dehydroepiandrosterone and its dehydroepiandrosterone sulphate (44). These girls are often more overweight than control groups of the same age and show an adverse metabolic phenotype, including hyperinsulinism, dyslipidemia, and augmented risk of metabolic disturbances in adulthood.

Although the exact mechanisms leading to the development of PCOS in patients with PP are not clearly and definitely established, on the basis of our data, we hypothesize that both early endogenous hyperandrogenism together with a higher metabolic dysfunction may lead to enhanced hyperinsulinemia contributing to PCOS development.

Using multivariate analysis, we identified six risk factors – WC, ISI-Matsuda, D_{IO}, VAI, total testosterone, and LDL-cholesterol – for the development of PCOS. A risk prediction model was constructed using these six risk

factors. Our risk prediction model was moderately robust in predicting PCOS with an AUC of 0.73. Interestingly, our study is the first to identify the above-mentioned parameters as predictive factors for PCOS in girls with PP. However, these findings need to be further validated in larger studies.

The limitations of the study are the small sample of patients with PP and the retrospective design of the study. Our study is the first to report a high percentage of PCOS in girls with PP, compared to other studies conducted on smaller populations, which may be due to a longer period of observation.

In conclusion, in this study, we identified a number of metabolic factors in PP that are associated with the development of PCOS. These risk factors represent some of the key metabolic abnormalities of PCOS. Our prediction model proposes that in PP, WC, ISI-Matsuda, DIO, VAI, total testosterone, and LDL-cholesterol are significant predictive factors for PCOS. Taken together, these findings support the metabolic pathogenesis of PCOS and suggest easy tools to identify girls who may develop PCOS in order to establish an early therapeutic approach.

Declaration of interest

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research reported.

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