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Prevalence and clinical features of polycystic ovarian syndrome in adolescents with previous childhood growth hormone deficiency

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Abstract

Background: Growth hormone (GH) plays a role in the regulation of ovarian function but there are limited data in women with GH deficiency (GHD). Our aim was to evaluate the features of polycystic ovarian syndrome (PCOS) in women with previous GHD.
Methods: Data of 22 adolescents previously GH-treated (group A) were compared with those of 22 women with classical PCOS (group B) and 20 controls (group C).
Results: Group A showed higher testosterone (p=0.048) and prevalence of menstrual irregularities (p<0.001) than group C. Compared to the group B, group A showed lower diastolic blood pressure (p=0.004), degree of hirsutism (p=0.005), testosterone (p=0.003) and prevalence of polycsytic ovaries (POC) morphology (p=0.024), with higher HDL-cholesterol (p=0.035) and 17-β-estradiol (p=0.009).
Conclusions: Adolescents with previous GHD show a higher prevalence of PCOS than controls, but with milder metabolic and hormonal features than adolescents with classical PCOS. A careful long-term follow-up is advisable in these patients.

Keywords: GH deficiency; growth hormone (GH); polycystic ovarian syndrome.

Introduction

The polycystic ovarian syndrome (PCOS) is a heterogeneous clinical entity that affects approximately 6%–8% of reproductive-aged women [1]. A number of growth factors, such as insulin, growth hormone (GH) and insulin growth factor (IGF) 1 and 2, play a role in the regulation of ovarian function [2–4]. GH exerts its effects on the ovarian function directly, by both gonadotropin-dependent and gonadotropin-independent actions, or by local production of IGF-1 [5, 6]. PCOS is highly prevalent in acromegaly and acromegalic women with PCOS have increased ovarian volume and PCO morphology compared to those without PCOS with a positive correlation between serum IGF-1 levels and mean ovarian volume, suggesting that IGF-1 is one of the critical factors involved in the development of PCOS [7]. Consequently, the treatment of GH hypersecretion in acromegaly seems to improve the ovarian function and to restore the reproductive dysfunction related to PCOS [8, 9]. Conversely, there are limited data on ovarian function in women affected by GH deficiency (GHD) and whether the GHD status is accompanied by ovarian disturbance or the GH treatment may affect ovarian function and morphology is still unclear.

The aims of this study were to evaluate, in a cohort of young women previously treated with GH for GHD during childhood and not confirmed in the transition age, the prevalence, metabolic status and phenotype of PCOS and to compare their clinical and biochemical features with a group of women with classical PCOS without previous GHD.

Materials and methods

This is a prospective case-control study. For the purpose of the study we enrolled 54 consecutive patients (mean age 17.1±2.1 years) admitted to the Section of Endocrinology of the University of Palermo.

The diagnosis of GHD was established by the clinical, auxological and biochemical criteria of the GH Research Society (20). At the time of GHD diagnosis the patients had a mean age of 8.8 years (range 7.4–10.1) and mean height of –2.28±1.0 SD. They showed a mean growth velocity of 3.4±1.4 cm/years (–2.4 SD), mean bone/chronological age ratio of 0.81 (indicating a delay of bone maturation of at least 1 year) and low serum IGF-1 levels (–1.6±0.8 SD).

The GH secretion was assessed by arginine and glucagon stimulation test. During the arginine test, blood samples were

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obtained at 0, 30, 60, 90, 120 min after the administration of the stimulus (arginine monohydrochloride: 0.5 g/kg up to 30 g given intravenously over 30 min) for GH measurements. During the glucagon test, blood samples were collected at 0, 30, 60, 90, 120, 150, 180 and 240 min after the injection of 30 μg/kg (up to 1.000 μg) intramuscularly of glucagon (GlucaGen, NovoNordisk, Bagsvaerd, Denmark). GHD was demonstrated by failure of GH to respond to two stimuli with GH peaks below 10 μg/L. All patients were treated with GH for at least 36 months (mean duration of treatment 54 months; range 42–66) and received GH once daily at bedtime with a pen injection system. In line with our internal fixed protocol, IGF-1 levels and the growth velocity have allowed us to determine the GH dose. The initial daily dose of GH was 0.025 mg/kg and it has been on average gradually increased by 0.002–0.003 mg/kg/day every 6 months (mean daily dose of 0.028 mg/kg from month 6 to 12; 0.031 mg/kg from month 12 to 18; 0.033 mg/kg from month 18 to 24; 0.035 mg/kg from month 24 to 36) to maintain IGF-1 levels within the normal range for age during the entire follow-up. We excluded from this analysis patients affected by multiple pituitary hormone deficiency or receiving other hormonal replacement treatment.

GH treatment was stopped at the end of the linear growth when the height outcome in line with the genetic target was achieved, after approximately 12 months of the menarche, and the follow-up has been continued annually after the GH treatment discontinuation. During this follow-up all patients have been evaluated for regularity of menses, degree of clinical or biochemical hyperandrogenism (HA) and ovarian morphology. Unlike adult PCOS, there are no agreed upon diagnostic criteria for adolescent PCOS, although hyperandrogenaemia remains the sine qua non for its diagnosis [10]. For these reasons, in this study we used the well standardized adult criteria [11]. Following the Rotterdam criteria, PCOS can be diagnosed when two of the following symptoms or signs are present: menstrual irregularity (MI) with <9 menstrual periods per year, HA either clinical or biochemical, and PCO morphology on pelvic ultrasonography, in the absence of other disorders known to cause the same symptoms or signs. According to this classification, 22/54 patients resulted affected by PCOS (group GHD-PCOS=group A). The clinical and biochemical findings of this subgroup of patients were compared with those of a group of 22 women with classical PCOS, matched for age and BMI, without previous GHD diagnosis, recruited from a consecutive series of women of reproductive age followed up in our Outpatients Clinic for PCOS (group PCOS=group B). The following subjects were excluded from the study: women treated with clomiphene citrate, oral contraceptives, antiandrogens and drugs to control their appetite or insulin-sensitizing drugs (metformin, pioglitazone and rosiglitazone) during the 6 months prior to the first examination; women with hyperprolactinemia; patients with basal 17OH-progesterone (17OH-Pg) levels >6.05 nmol/L or >30.26 nmol/L at 60 min after 250 mg Synacthen (synthetic analog of adrenocorticotrophic hormone); 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.

At the time of hospitalization, all patients signed a consent form for the scientific use of their data after a full explanation of the purpose of the study. This study was approved by the Institutional Review Board of the Faculty of Medicine, University of Palermo and the identity of the participants remained anonymous during database analysis. The study complies with the World Medical Association Declaration of Helsinki regarding ethical conduct of research.

Study protocol

The following data were obtained from our database: age of menarche, body mass index (BMI), waist circumference (WC) and hip circumference (HC), systolic blood pressure (SBP) and diastolic blood pressure (DBP).

In all enrolled patients, in line with our internal protocol, we evaluated the metabolic profile with lipid profile [total cholesterol (TC), HDL-cholesterol (HDL), LDL-cholesterol (LDL), triglycerides (TG)], hemoglobin A_{1c} (HbA_{1c}), fasting glucose and insulin levels, IGF-1. These samples also served as the baseline for an oral glucose tolerance test (OGTT). Blood samples were collected every 30 min for 2 h for glucose and insulin measurements. The area under the curve (AUC) of glucose (AUC_{GLU}) and insulin (AUC_{INS}) during OGTT was calculated using the trapezoidal rule. As surrogate estimates of insulin sensitivity we considered the homeostasis model assessment estimate of insulin resistance (Homa-IR): [glycemia (mmol/L)×insulinemia (μU/mL)/22.5] [12] and the insulin sensitivity index (ISI), a composite index derived from the OGTT and validated by Matsuda and DeFronzo [10,000/glucose (mg/dL)×insulin (μU/mL)×glucose mean×insulin mean] [13]. The stimulated total insulin secretion was evaluated by AUC_{INS}, while the oral disposition index (DIo) was used as index of the ability of the β-cell to regulate its insulin response to stimuli based on differences in insulin sensitivity. DIo was calculated at the time 0′ and 30′ during OGTT as described (18), using the following formula, where insulin levels are expressed in IU/mL and glucose levels in mmol/L: DIo=[Δ insulin 0′–30′/Δ glucose 0′–30′]×1/fasting insulin [14]. All subjects were also analyzed according to each criterion of the metabolic syndrome (MS) [15]. Visceral adiposity index (VAI) was calculated as described by Amato et al. [16]. Hormonal status was assessed by the evaluation of follicle (FSH) and luteinizing (LH) stimulating hormones, 17-β-estradiol (E2), 17OH-Pg, total testosterone (TT), DHEA-S, Δ4androstenedione (Δ4), and prolactin (PRL) during the follicular phase (day-7 from the beginning of the last period). Serum progesterone (PG) level was determined in the luteal phase (between days 20 and 24 from the beginning of the last period). In all subjects we also evaluated the presence of clinical (hirsutism, acne or seborrhea) and biochemical HA. Hirsutism was defined as Ferriman-Gallwey (FG) score >8 [17].

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Due to the limitations of defining androgen excess and the lack of normative data on the normal developmental fluctuations in testosterone levels during puberty, androgen concentrations should be considered elevated when they are persistently greater than the adult female normative values [18]. In this study, biochemical HA was arbitrarily established with an in-house range, as follows: TT >2.08 nmol/L, DHEA-S >11.69 mmol/L, Δ4 >10.72 nmol/L, calculated on the basis of the 95th percentile upper limits of basal serum androgen normality in a group of 20 healthy eumenorrhoic Sicilian women without clinical HA and family history of PCOS, matched for age (mean age 17.1±1.5 years) and BMI (22.3±2.5 kg/m^2), recruited consecutively among the medical and paramedical personnel of our Department and their relatives, and/or patients' relatives. This group of girls was used as a control group (group C) for the hormonal parameters and PCOS-features after informed consent for the scientific use of their data was obtained.

All subjects underwent transvaginal pelvic ultrasound scanning on a single occasion in the follicular phase, between days 5 and 10 from the beginning of the last period using a 2.5–8 MHz vaginal probe transducer (General Electric LOGIQ 400MD, Milwaukee, WI, USA). Both ovaries were measured in the sagittal, transverse and coronal planes. Ovaries were classified as polycystic using the threshold of ≥25 follicles measuring 2–9 mm in diameter [19].

Hormone and biochemical assays

All hormones were measured in our laboratory using commercial kits. These included enzyme-linked immunosorbent assay (DRG Diagnostics, DRG Instruments GmbH, Germany) for FSH (mUI/mL), LH (mUI/mL), E2 (pg/mL), 17OH-PG (ng/mL), PG(ng/mL), prolactin (ng/mL), TT (ng/mL), Δ4 (ng/mL; Arnika, Milan, Italy), insulin (mUI/L). Chemiluminescence assays were used for DHEA-S (μg/dL; Immulite, Diagnostic Products, Genoa, Italy) and serum SHBG (nmol/L; Immulite, Diagnostic Products). Serum IGF-1 was assayed in the same laboratory with the ELISA method (OCTEIA IGF-I kit, IDS Inc., Fountain Hills, AZ, USA). The sensitivity of the method was 1.9 μg/L. The normal range of IGF-1 levels (μg/L) was 146–415 (15–20 years). Total cholesterol, HDL and TG were measured in our laboratory using standard assays. LDL 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 (μg/dL vs. μmol/L: 0.0272), Δ4 (ng/mL vs. nmol/L: 3.492), 17OH-Pg (ng/mL vs. nmol/L: 3.026), FSH (mUI/mL vs. IU/L: 1), and LH (mUI/mL vs. IU/L: 1).

Statistical analysis

The Statistical Packages for Social Sciences SPSS version 17 (SPSS Inc., Chicago, IL, USA) was used for data analysis. Baseline characteristics were presented as mean±standard deviation (SD) for continuous variables; rates and proportions were calculated for categorical data. The normality of distribution of the quantitative variables was assessed by the Kolmogorov-Smirnov test. The differences between the two groups of subjects (group GHD-PCOS and group PCOS) were evaluated by the Student's t test for continuous variables with a normal distribution, by the Mann-Whitney U-test (non-parametric test) for continuous variables without normal distribution and by the χ^2-test and Fisher's exact test (when appropriate) for categorical variables. Simple univariate correlations among continuous variables with normal distribution were determined by Pearson's test. A p-value of 0.05 was considered statistically significant.

Results

Hormonal parameters and PCOS features

In Table 1 we showed the prevalence of each PCOS-feature in the two groups of patients (group A and B) and controls (group C). Group A showed a lower prevalence of PCO morphology on pelvic ultrasonography than group B (36.4% vs. 90.9%; p=0.024), while no difference was found in the prevalence of MI (p=0.635), clinical (p=0.445) or biochemical HA (p=0.388), as well as of complete PCOS-phenotype (p=0.183).

Both group A and B showed a higher prevalence of clinical (p<0.001) and biochemical (p<0.001) HA and of MI (p<0.001) than control subjects, while no difference was found in PCO morphology between group A and C (p=0.738).

The hormonal parameters of patients (group A and B) and controls (group C) are shown in Table 2. Group B

Table 1: Prevalence of PCOS-features in patients grouped in group GHD-PCOS (group A), group PCOS (group B) and control group (group C).

	GHD-PCOS (group A) n=22	PCOS (group B) n=22	Control group (group C) n=20	p-Value	p-Value[a]	p-Value[b]
	Subjects (%)	Subjects (%)	Subjects (%)			
Hyperandrogenism						
Clinical	16 (72.7)	20 (90.9)	0	0.445	<0.001	<0.001
Hirsutism	10 (45.4)	18 (81.8)		0.183	–	–
Acne/seborrhea	12 (54.5)	14 (63.6)	–	1	–	–
Biochemical	6 (27.2)	14 (63.6)	0	0.388	<0.001	<0.001
High TT levels	0	2 (9.1)	–	1	–	–
High DHEA-S levels	2 (9.1)	2 (9.1)	–	1	–	–
High Δ4 levels	4 (18.2)	10 (45.5)	–	0.361	–	–
MI	18 (81.8)	12 (63.6)	0	0.635	<0.001	<0.001
PCO	8 (36.4)	20 (90.9)	7 (35)	0.024	0.738	12
Complete phenotype (HA+MI+PCO)	7 (31.8)	12 (54.5)	0	0.183	<0.001	<0.001

HA, Hyperandrogenism; TT, total testosterone; DHEA-S, dehydroepiandrosterone sulfate; Δ4, Δ4androstenedione; MI, menstrual irregularity; PCO, polycystic ovaries on pelvic ultrasonography. p=Difference between group A and group B. [a]p=Difference between group A and group C. [b]p=Difference between group B and group C.

Q5: "Mean±SD" has been added in Table 2, column 4 under Control group (group C). Please check and confirm

Table 2: Hormonal parameters of patients grouped in group GHD-PCOS (group A), group PCOS (group B) and control group (group C).

	GHD-PCOS (group A) n=22	PCOS (group B) n=22	Control group (group C) n=20	p-Value	p-Value[a]	p-Value[b]
	Mean±SD	Mean±SD	Mean±SD			
FSH, IU/L	4.5±1.7	6±2.6	4.8±1.4	0.178	1	0.224
LH, IU/L	8.6±5.7	8±4.5	8.4±4.4	0.870	0.980	0.650
LH/FSH ratio	1.9±1	1.4±0.8	1.7±0.9	0.158	0.445	0.238
17-β-E2, pg/mL	58.5±24.8	39.5±16.6	55.7±28.2	0.009	0.766	0.007
17OHPg, nmol/L	1.3±0.6	1.4±0.9	1.3±0.5	1	1	0.980
Pg, ng/mL	5±6.7	5.1±5.2	5±6.5	0.888	0.890	0.870
PRL, ng/mL	15.9±9.8	11.3±6.6	10.7±8.1	0.139	0.122	0.545
Total testosterone, nmol/L	1.4±0.4	2±0.5	0.8±0.3	0.003	0.048	0.002
DHEA-S, µmol/L	6.8±3.1	7.8±3.3	6.9±4.2	0.450	0.755	0.520
Δ4androstenedione, nmol/L	9.6±7.1	10.2±5.4	9.5±7.4	0.669	0.868	0.702
IGF-1, µg/L	245±38.2	290±28	287.1±33.6	0.445	0.580	0.860

p=Difference between group A and group B. [a]p=Difference between group A and group C. [b]p=Difference between group B and group C.

showed lower E2 (39.5±16.6 vs. 58.5±24.8 and 55.7±28.2 pg/mL; p=0.009 and p=0.007, respectively) and higher TT (2±0.5 vs. 1.2±0.4 and 1.1±0.8 nmol/L; p=0.003 and p=0.002, respectively) than group A and group C, while no difference was found in others hormonal parameters. No difference was found in all hormonal parameters between group A and C, with the exception of higher TT levels in group A than C (1.4±0.4 vs. 0.8±0.3 nmol/L; p=0.048).

Clinical and metabolic parameters

The clinical and metabolic features of patients are shown in Table 3.

The mean age (17.2±2.7 vs. 17.6±1.2 years; p=0.615) and BMI (22.2±3.7 vs. 22.6±2.7 kg/m^2; p=0.818) were comparable in both groups of patients. Similarly, no difference was found in WC and HC. Group A showed lower DBP (56.3±6.7 vs. 67.2±7.8 mmHg; p=0.004) and FG score (8±3.1 vs. 14.2±5.4; p=0.005) than group B, while no difference was shown in SBP (100.9±10.2 vs. 110±10.9 mmHg; p=0.051). As regards the metabolic parameters, group A showed higher HDL-cholesterol than group B (1.7±0.3 vs. 1.4±0.3 mmol/L; p=0.035), while no significant difference was found in the others parameters evaluated. Using the above mentioned criteria of MS [15], no patients showed the presence of MS as a whole and we have not found a statistical difference in each of the components of MS between the two groups of patients. Only two patients (9.1%) of group A showed hypertriglyceridemia, two patients (9.1%) of group B showed impaired fasting glucose, while two patients (9.1%) of group A and six patients (27.3%) of group B showed low HDL-cholesterol. No patient showed systolic or diastolic hypertension or increased WC.

Discussion

To our knowledge, this is the first study that investigates the PCOS phenotype in patients with previous GHD. The results of this study reveal a high prevalence of PCOS in this group of women previously treated with GH, but with milder metabolic and hormonal features than the classical PCOS.

During the transition from adolescence to adulthood it is well known that several PCOS-features may be in evolution or transitory. The classic signs and symptoms of MI and HA seen in adults are not as clear in adolescents as the physiology of normal puberty may mimic PCOS and most authors agree that applying criteria for adults to adolescents can lead to over-diagnosis because of similarity in physiological changes during puberty and common PCOS symptoms [20, 21]. Indeed, approximately 40%–50% of adolescent girls have anovulatory cycles. In addition, some girls could show multifollicular ovaries as a stage of development and it may be misinterpreted. Further, if the ovary has features of polycystic morphology but does not meet the recommended volumetric criteria, the diagnosis of PCOS should not be ascribed on this basis. In this study we found in GHD-PCOS group a similar prevalence of HA and MI than PCOS-group, but higher than control subjects. Conversely, in both GHD-PCOS and controls we found a lower prevalence of PCO morphology. Actually, there are limited data that suggest the relationship between GHD and ovarian disturbance. It has been already suggested that some disturbance in reproductive function can be expected in women treated for GHD during childhood [22], while GHD as a cause for delayed puberty or primary amenorrhea has also been hypothesized [6, 23]. In line

Table 3: Clinical and metabolic features of patients grouped in group GHD-PCOS (group A) and group PCOS (group B).

	GHD-PCOS (group A) n=22	PCOS (group B) n=22	p-Value
	Mean±SD	Mean±SD	
Age, years	17.2±2.7	17.6±1.2	0.615
Age of menarche, years	12.7±1.3	12±1.1	0.200
BMI, kg/m^2	22.2±3.7	22.6±2.7	0.818
Waist circumference, cm	76.3±6.7	75.9±11.1	0.669
Hip circumference, cm	89.6±6.5	95.9±11.8	0.188
Waist/hip circumference ratio	0.85±0.08	0.79±0.07	0.053
Systolic blood pressure, mmHg	100.9±10.2	110±10.9	0.051
Diastolic blood pressure, mmHg	56.3±6.7	67.2±7.8	0.004
FG score	8±3.1	14.2±5.4	0.005
Fasting glucose, mmol/L	4.4±0.2	4.5±0.4	0.621
AUC_{GLU} (OGTT), mmol/L	651±116	701±154	0.577
Fasting insulin, pmol/L	12.3±5.3	13.3±6.4	0.793
AUC_{INS} (OGTT), pmol/L	7929±5658	9945±5687	0.061
HbA_{1c}, %	4.8±0.4	5±0.3	0.661
Homa IR	2.4±1.1	2.6±1.2	0.768
ISI Matsuda	5.2±2.2	4.2±1.7	0.200
Dio	6.4±7	5.2±6.3	0.870
Total cholesterol, mmol/L	4.3±0.6	4.7±1.1	0.459
HDL cholesterol, mmol/L	1.7±0.3	1.4±0.3	0.035
LDL cholesterol, mmol/L	84.8±16	110.9±45.7	0.453
Triglycerides, mmol/L	2.1±0.8	2.2±0.8	0.511
Visceral adiposity index	1±0.4	1.3±0.6	0.412
	Subjects (%)	Subjects (%)	
Metabolic syndrome	0	0	–
Increased waist circumference	0	2 (9.1)	1
Hypertriglyceridemia	2 (9.1)	0	1
Low HDL cholesterol	2 (9.1)	6 (27.3)	0.586
Increased SBP or DBP	0	0	–
Hyperglycemia	0	2 (9.1)	1

OGTT, Oral glucose tolerance test; FG, Ferriman-Gallwey; Dio, oral disposition index; SBP, systolic blood pressure; DBP, diastolic blood pressure.

with these evidences, in our study the previous GHD condition may have been the cause of the differences between the two groups of patients and these data are partially in line with previous studies. Indeed, De Boer et al. showed in 60 GHD women who had been treated for GHD during childhood that about 56% showed no spontaneous pubertal development and among the women who did, only half showed regular menstrual cycles, while the remaining showed secondary amenorrhea or oligomenorrhea after discontinuation of GH [22].

In the current study, although the age of menarche was not significantly different between the two groups of women, we found that about 81% of women of the GHD-PCOS group had MI and this finding is not different from that observed in PCOS-group, but it is higher than both our healthy controls and the general population age-matched, where chronic anovulation and MI are evident in approximately 40%–50% [24].

The Endocrine Society Clinical Practice Guidelines suggested that the diagnosis of PCOS in adolescents is based on the presence of clinical and/or biochemical HA in the presence of persistent oligomenorrhoea, while anovulatory symptoms and PCO morphology were deemed insufficient to make a diagnosis in adolescents [25]. Despite these findings, there is no overall consensus regarding the diagnosis of PCOS in adolescents. Currently it seems justifiable to diagnose PCOS in adolescents using the Rotterdam criteria, on condition that all three symptoms are present, HA is established in laboratory tests and pelvic ultrasound meets clear morphological criteria [19, 26, 27]. In line with these criteria, when the diagnosis was made by the presence of the complete phenotype, we found a higher prevalence of PCOS in girls with previous GHD than control subjects. These data are confirmed if we consider a recent epidemiological survey on the prevalence of PCOS-features in late adolescent and young women performed

by Gambineri et al. that showed a prevalence of MI of 10%–13%, clinical HA of about 16% and PCOS of <15% [28]. To explain our data of prevalence the relationship between GH-IGF-1 axis and ovarian morphology and function must be taken into account. It is well established that GH promotes sexual maturation and reproductive function [29]. On the other hand, ovarian dysfunction is often associated with altered GH secretion and anovulatory women often show lower GH pulses [30, 31], as well as women with PCOS can manifest a blunted GH secretion after GHRH stimulation [32, 33]. To evaluate the impact of the previous GHD condition or GH treatment on the metabolic and hormonal features of PCOS in these patients we compared them to a group of age-matched women with classical PCOS without previous GHD. These latter showed worse metabolic and hormonal parameters than the GHD-PCOS group. Higher blood pressure values and a worse lipid profile were found in classical PCOS than in GHD-PCOS women. These data are not surprising because the metabolic impairment in women with PCOS is well known [34–36], although a limitation of this study can be represented by the "non-obese" population in both groups of patients.

Similarly, the hormonal assessment and ovarian morphology were found worse in classical PCOS than in women with previous GHD, as showed by higher TT and lower E2 levels in the first group and by the concomitant higher prevalence of PCO morphology. Conversely, GHD-PCOS women showed a higher prevalence of MI and higher TT levels, with a consequent higher prevalence of clinical and biochemical HA, than healthy controls and these data lead to the higher prevalence of PCOS-phenotype observed in the patients of group A. These findings can lead to hypothesize the effect of GH therapy on the ovarian morphology and function. Indeed, the involvement of GH in the control of sexual maturation and gonadal growth and function, through both autocrine and paracrine effects, is well known [37–39] and a role of GH and IGF-1 in activating the ovarian androgen production has been shown [40, 41]. In addition, exogenous GH has been demonstrated to increase the concentration of testosterone [42] and these data are in line with our findings. Conversely, we can speculate that the presence of softer metabolic and hormonal features in the GHD-PCOS group than classical PCOS may be dependent on the previous condition of GHD, probably not treated from the real onset of the hormonal deficit but only from the time of the diagnosis. These data, with the concomitant higher evidence of MI in GHD-PCOS than healthy subjects, can be considered as a result of the delayed or reduced effect of GH on the ovarian maturation and function, probably destined to weaken over time.

In conclusion, the adolescents previously treated with GH for childhood GHD show a higher prevalence of PCOS than healthy subjects, but with milder features than adolescents with classical PCOS and it can be assumed that these findings may be due both to the previous lack of GH effects and to the subsequent effect of GH therapy. Given that it is known that during puberty the features of PCOS overlap normal pubertal development and caution should be taken before diagnosing PCOS without longitudinal evaluation, and given the well-known relationship between GH axis and ovarian morphology and function, in adolescents with a previous diagnosis of GHD who underwent GH treatment it is advisable to maintain a careful clinical follow-up after discontinuation of GH treatment to evaluate any hormonal or phenotypic change that can allow to review the eventual diagnosis of PCOS in these patients.

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