PRACE ORYGINALNE/ORIGINAL PAPERS

Hyperandrogenism in adolescent girls with type 1 diabetes mellitus treated with intensive and continuous subcutaneous insulin therapy

Hiperandrogenizm u dojrzewających dziewcząt z cukrzycą typu 1 leczonych metodą wielokrotnych wstrzyknięć i ciągłym podskórnym wlewem insuliny

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Abstract

Introduction: Women with type 1 diabetes mellitus (T1DM) experience high prevalence of hyperandrogenic disorders. The aim of this study was to evaluate hormonal profile with respect to hyperandrogenic disorders in adolescents with T1DM.

Material and methods: Forty seven adolescent girls with T1DM were evaluated and compared to 19 healthy and 21 non-diabetic girls with polycystic ovary syndrome (PCOS). In all subjects, basal and GnRH analogue stimulated androgens, gonadotropins and SHBG were measured and ultrasonography of ovaries was performed.

Results: Girls with T1DM experienced first menses significantly later than healthy controls [13.1 (12.0–14.0) $v.$ 12.0 (11.0–12.0) years, p = 0.02]. Nine (19.2%) of them fulfilled PCOS criteria (T1DM+PCOS). They had significantly mean HbA_{1c} from the diagnosis of T1DM than T1DM girls with no PCOS [6.7 (6.6–7.2) $v.$ 7.3(6.4–7.8)%, p = 0.049]. Hormonal profile, hirsutism score and ovarian volume did not differ significantly between the two groups. HbA_{1c} at the study point and mean HbA_{1c} for the last 12 months correlated negatively with SHBG level (r = –0.5, p = 0.006; r = –0.04, p = 0.02). T1DM+PCOS girls had significantly lower FAI [3.0 (2.6–4.3) $v.$ 8.6 (6.5–10.8), p = 0.04] and ovarian volume than non-diabetic PCOS girls [4.6 (2.7–5.2) $v.$ 7.4 (4.3–10.0) mL, p = 0.007].

Conclusions: Clinical symptoms of PCOS in adolescent girls with T1DM are milder than in non-diabetic peers, probably due to the protective role of higher SHBG resulting in lower free androgen level. **(Endokrynol Pol 2013; 64 (2): 121–128)**

Key words: type 1 diabetes mellitus, polycystic ovary syndrome, hyperandrogenism, adolescent girls

Streszczenie

Wstęp: U kobiet z cukrzycą typu 1 (T1DM) stwierdza się zwiększoną częstość występowania klinicznych i biochemicznych objawów hiperandrogenizmu. Celem pracy była ocena profilu hormonalnego oraz częstości występowania hiperandrogenizmu u dojrzewających dziewcząt z T1DM.

Materiał i metody: Do badania włączono 47 dziewcząt z T1DM oraz 19 zdrowych i 21 dziewcząt z zespołem policystycznych jajników (PCOS). U wszystkich dziewcząt dokonano oceny klinicznej i ultrasonograficznej oraz przeprowadzono badania hormonalne przed i po stymulacji analogiem GnRH.

Wyniki: U dziewcząt z T1DM pierwsza miesiączka występowała istotnie później aniżeli u zdrowych dziewcząt [13,1 (12,0–14,0) $v.$ 12,0 (11,0–12,0) lat, p = 0,02]. Dziewięć (19,2%) z nich spełniało kryteria rozpoznania PCOS (T1DM+PCOS). U dziewcząt z cukrzycą, u których zdiagnozowano PCOS, stwierdzono istotnie niższą średnią HbA_{1c} od początku zachorowania na T1DM aniżeli u dziewcząt z T1DM bez PCOS [6,7 (6,6–7,2) $v.$ 7,3 (6,4–7,8)%, p = 0,049]. Hirsutyzm, objętość jajników oraz profil hormonalny nie różniły się pomiędzy grupami. HbA_{1c} oznaczona w momencie badań oraz średnia HbA_{1c} z ostatniego roku korelowały negatywnie ze stężeniem SHBG (r = –0,5, p = 0,006; r = –0,04, p = 0,02). Dziewczęta T1DM+PCOS miały istotnie niższy indeks wolnych androgenów [3,0 (2,6–4,3) $v.$ 8,6 (6,5–10,8), p = 0,04] i objętość jajnika aniżeli dziewczęta z PCOS nie chorujące na cukrzycę [4,6 (2,7–5,2) $v.$ 7,4 (4,3–10,0) ml, p = 0,007].

Wnioski: Kliniczne objawy PCOS u dojrzewających dziewcząt z T1DM są łagodniejsze aniżeli u dojrzewających dziewcząt nie chorujących na cukrzycę. Może być to spowodowane protekcyjną rolą SHBG i mniejszą ilością wolnych androgenów. **(Endokrynol Pol 2013; 64 (2): 121–128)**

Słowa kluczowe: cukrzyca typu 1, zespół policystycznych jajników, hiperandrogenizm, dojrzewające dziewczęta

Introduction

Polycystic ovary syndrome (PCOS) is the commonest endocrine disorder in women of reproductive age. According to National Institute of Health (NIH) 1990 criteria, it affects 6–8% and according to Rotterdam consensus about 12% of adult women [1]. PCOS is a heterogeneous condition, characterised by oligoovula-

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tion and clinical and/or biochemical hyperandrogenism, and is frequently associated with hyperinsulinaemia and insulin resistance. It has been suggested that hyperinsulinaemia may contribute to PCOS development by enhancing androgen production [2, 3].

Insulin receptors are present in many compartments of the ovary including granulosa, thecal, and stromal cells [4, 5]. Moreover, insulin can also bind to the IGF-1 receptors in the female gonad which could mediate the effects of hyperinsulinaemia in the ovary [6]. *In vitro* and *in vivo* studies have shown that insulin can act as co-gonadotropin and together with LH may stimulate androgens' production in theca cells [7, 8]. Insulin affects also other steroidogenic enzymes, stimulates synthesis of oestrogen and progesterone, activates IGF-1 system, enhances ovarian growth and cyst formation, stimulates theca cell proliferation, and influences ovulation [3].

Increased incidence of menstrual disturbances, hirsutism and PCOS have been reported in women with type 1 diabetes mellitus (T1DM) [9–11]. Some authors have suggested that this could be due to non-physiologic insulin replacement therapy, leading to increased insulin level in systemic circulation. Under the physiological condition pancreas secretes insulin into the portal circulation. The main target organ of insulin action is the liver, where 50-70% of insulin is eliminated [12]. In diabetic patients, to deliver an appropriate amount of insulin to the liver and achieve good metabolic control, supraphysiological doses of insulin are injected subcutaneously. Due mainly to co-gonadotropin action, hyperinsulinaemia can therefore stimulate androgens synthesis in the ovaries, leading to PCOS development [13, 14].

Girls with T1DM have been found to have different ovarian steroidogenic response to GnRH-analogue stimulation compared to healthy peers [15]. Some of them may develop hyperandrogenism, which may evolve towards PCOS in adulthood. However there is little data concerning the incidence of hyperandrogenism in this group of patients and it is not clear which factors related to T1DM can influence the ovarian steroidogenesis.

The aim of our study was to evaluate the clinical characteristics and ultrasonography of the ovary as well as the hormonal profile of adolescent girls with T1DM with respect to their metabolic control and the type of insulin therapy and compare these results with nondiabetic PCOS girls and healthy controls matched for age.

Material and methods

All the girls attending the diabetes clinic of the Paediatric University Hospital of Silesia, Katowice, who had

menstruated for at least one year, were consecutively invited to participate in the study. Forty seven adolescent girls were finally recruited. They had been diagnosed with T1DM at least one year earlier. In all patients, T1DM diagnosis was confirmed (C-peptide level < 0.05 nmol/L, presence of specific antibodies), and intensive insulin therapy was applied. Twenty seven (57%) girls were treated with intermediate (NPH) or long-acting insulin (glargine) and multiple daily injections (MDI) with at least three per day injections of rapid acting insulin analogues, and 20 (43%) girls — with continuous subcutaneous insulin infusion (CSII) by means of an insulin pump. Twenty one adolescents with PCOS and 19 healthy, regularly menstruating adolescent girls with no clinical signs of hyperandrogenism, matched for chronological and gynaecological age, served as the control groups. The exclusion criteria were: honeymoon period, specific types of diabetes, type 2 diabetes mellitus, abnormal thyroid function, hyperprolactinaemia, congenital adrenal hyperplasia (based on basal 17-0H progesterone level > 3.0 ng/mL), use of medications known to influence sex steroids or sex hormone binding globuline (SHBG) in last three months, chronic systemic disease and malnutrition. All the girls participating in the study were Caucasians. The study was conducted according to the Helsinki declaration and approved by the Ethics Committee of the Medical University of Silesia. Informed consent was obtained from each subject and parent/guardian.

In all participants, menstrual cycle pattern was evaluated and oligomenorrhoea was defined as menstrual cycles longer than 45 days in the last six months. Weight was measured with Seca scale with a precision of 100g and height with Harpenden stadiometer to 0.1 cm. BMI and BMI z-score were calculated. Hirsutism was evaluated by one of the two authors (A.Z. and A.G.) and diagnosed if the modified Ferriman-Gallwey score was ≥ 8. In each girl, a transabdominal pelvic ultrasound examination was performed by the same observer (A. D-C.) with 5 MHz convex transducer (Siemens Acuson Antares 5.0), and volume and structure of the ovaries were evaluated. Ovaries were considered polycystic (polycystic ovary morphology, PCOM) if 12 or more follicles 2–9 mm in diameter were present at least in one ovary and if increased ovarian volume (> 10 mL) occurred. In each subject with T1DM, detailed medical history was obtained, including age at the onset of T1DM, HbA$_{1c}$ records from the beginning of the disease, and daily insulin requirement (DIR) for the last three days, expressed as units per kilograms of body weight per day.

Basal plasma concentration of gonadotropins (LH, FSH), androstenedione (A), testosterone (T), 17-hydroxyprogesterone (17OHP), dehydroepiandrosterone sulfate (DHEAS), oestradiol (E$_2$), SHBG, IGF-1 and HbA$_{1c}$

were measured. Free andorgen index (FAI = Tx100/ /SHBG) and LH/FSH ratio were calculated. All subjects underwent GnRH-analogue test with triptorelin (0.1 mg) administered subcutaneously as previously described [16]. Blood samples were collected four hours post stimulation for measurement of LH and FSH concentrations, and 24 hours post stimulation for 17OHP, A, DHEAS, T and E_2 plasma level. All the tests were performed during the follicular phase of menstrual cycle (3–7 day of cycle). Biochemical hyperandrogenaemia was defined if T level exceeded 58 ng/dL, A — 2.44 ng/mL and DHEAS — 248 mg/dL [17]. PCOS was diagnosed according to the Androgen Excess and PCOS Society criteria [18].

Serum levels of LH, FSH, T, DHEAS, E_2, and IGF-1 were measured using chemiluminescent immunoassay by Immulite 2000 analyser (DPC, USA), HbA_{1c} was measured by high-performance liquid chromatography method, and SHBG was determined by immunoradiometric assay (Radim, Roma, Italy). 17OHP and A were measured by enzyme-linked immunosorbent assay (DRG Diagnostics GmbH, Germany).

Anthropometric data and hormonal results were compared using Statistica 8.0 PL. All values were expressed as mean ± standard deviation for normal or median (interquartile range) for skewed distribution. Correlation analysis was performed using Pearson's correlation coefficient for normally distributed samples, and Spearman correlation coefficient for non-normally distributed data. Gamma correlation was used for non-normal distributions with many tied ranks. Comparison between groups was performed using t-Student test for normally distributed data and Mann-Whitney U test for skewed distributions. Differences between the four groups (T1DM no PCOS, T1DM with PCOS, PCOS, and control) were assessed by one-way ANOVA or Kruskal-Wallis test, followed by the least-significant difference (LSD) test for multiple comparisons when applicable. P value < 0.05 was considered statistically significant and $0.05 < p \leq 0.1$ was considered as a trend toward statistical significance.

Results

Clinical and hormonal characteristics of the girls with T1DM and healthy, regularly menstruating girls are shown in Table I. Girls with T1DM experienced first menses significantly later, therefore their gynaecological age was shorter. Forty three (87.2%) diabetic girls experienced menarche after T1DM diagnosis, in three girls (6.4%) menarche and T1DM occurred at the same age, and in the other three (6.4%) T1DM was diagnosed after menarche. There were no significant differences in examined hormonal profile between girls with T1DM

and healthy controls. The clinical manifestations of hyperandrogenism were found in 18 (36.7%) subjects — menstrual disturbances in 16 (32.6%) girls and/or hirsutism of 8 points in three (6.4%) girls. None of the girls presented with a hirsutism score above 8. PCOM on ultrasound was seen in 18 (38.3%) diabetic girls and hyperandrogenaemia was present in 21 (44.7%) girls. The hyperandrogenic disorders, defined as the presence of polycystic ovaries or hirsutism, were found in 19 (40.4%) girls.

The criteria of PCOS diagnosis were fulfilled by nine (19.2%) diabetic girls (T1DM+PCOS) according to the Androgen Excess and PCOS Society {by one (2.1%) girl according to the NIH, and by 12 (25.5%) subjects by ESHRE/ASRM criteria [19]}. Three of the T1DM+PCOS girls had clinical manifestations of the syndrome (oligomenorrhoea and/or hirsutism) together with PCOM and/or hyperandrogenaemia and the other six — PCOM with hyperandrogenaemia only.

There were no significant differences in the age of T1DM diagnosis, T1DM duration, HbA_{1c} at the study point, mean HbA_{1c} for the last 12 months and DIR per kg between diabetic girls with and without PCOS (Table I). Although the mean HbA_{1c} from the beginning of T1DM was higher in diabetic girls without PCOS than in T1DM + PCOS girls, there were no significant differences between the two groups with respect to hormones concentrations and FAI.

When compared to non-diabetic girls with PCOS, girls with T1DM experienced less pronounced clinical manifestations of the syndrome (Table II). Their menstrual cycle was significantly shorter. They also had significantly lower volume of left ovary and lower FAI. Basal and stimulated testosterone, DHEAS and androstenedione concentrations were similar in both groups. There were also no differences in basal and stimulated other hormones levels between T1DM+PCOS girls and PCOS girls.

Following the recommendations of ISPAD [20], we divided girls with T1DM into two groups — well controlled (mean HbA_{1c} level for the last 12 months < 7.5%, 36 girls) and poorly controlled (mean HbA_{1c} for the last 12 months ≥ 7.5%, 12 girls) (Table III). The occurrence of PCOS components (menstrual disturbances, hirsutism, PCOM and/or hyperandrogenaemia) was similar in both groups [24 (68.6%) well controlled girls *v.* ten (83%) poorly controlled girls, p > 0.05]. Girls with well controlled T1DM had significantly lower stimulated T level than girls with poorly controlled T1DM. IGF-1 as well as basal and stimulated 17OHP concentrations were significantly higher in well controlled girls. Girls with poor metabolic control had significantly higher BMI z-score. There was no significant difference between the two groups with respect to DIR per kg.

Table I. *Clinical and hormonal characteristics of adolescent girls with type 1 diabetes mellitus (T1DM), adolescent girls with T1DM and no polycystic ovary syndrome (PCOS), adolescent girls with T1DM and PCOS, and control group of healthy girls*

Tabela I. *Charakterystyka kliniczna i hormonalna dojrzewających dziewcząt z cukrzycą typu 1 (T1DM), dziewcząt z T1DM bez zespołu policystycznych jajników (PCOS), dziewcząt z T1DM i PCOS oraz zdrowych dziewcząt (grupa kontrolna)*

	Girls with T1DM (n = 47)	T1DM no PCOS (n = 38)	T1DM with PCOS (n = 9)	Control group (n = 19)
Chronological age (years)	15.9 (15.0–17.1)	15.8 (15.0–16.8)	16.9 (15.7–17.2)	16.5 (15.2–17.3)
Age at menarche (years)	13.1 (12.0–14.0)[1]	13.0 (12.0–14.0)[2]	13.0 (13.0–14.0)[3]	12.0 (11.0–12.0)
Gynaecological age (months)	32.0 (20.0–42.0)[4]	29.0 (20.0–42.0)	36.0 (24.0–45.0)[5]	51.0 (38.0–65.0)
Cycle duration (days)	29.0 (28.0–36.0)	29.0 (28.0–36.0)	29.0 (27.0–34.0)	
Age of T1DM diagnosis (years)	9.0 (6.5–11.7)	8.8 (6.3–11.8)	9.0 (7.0–10.0)	
T1DM duration (years)	7.1 (5.0–9.0)	7.3 (4.9–9.0)	7.0 (6.0–8.7)	
HbA1c at study point (%)	7.0 (6.2–7.6)	7.1 (6.2–7.8)	6.7 (6.6–7.2)	
Mean HbA1c for last 12 months (%)	6.9 (6.4–7.5)	7.0 (6.4–8.0)	6.9 (6.4–7.0)	
Mean HbA1c from the beginning of T1DM (%)	7.0 (6.3–7.5)	7.3 (6.4–7.8)[6]	6.7 (6.2–7.0)	
Daily insulin requirement [U/kg/day]	0.8 (0.7–0.9)	0.8 (0.7–1.0)	0.8 (0.7–0.8)	
BMI z-score	0.4 (0.0–0.9)	0.5 (0.1–1.0)	0.0 (–0.2–0.7)	0.5 (–0.1–2.0)
Ferriman-Gallwey score	1.0 (0.0–3.0)	0.5 (0.0–2.0)	2.0 (1.0–8.0)	2.0 (0.0–7.0)
Volume of the right ovary [mL]	5.0 (3.0–6.5)	4.9 (2.7–6.5)	5.6 (3.9–6.7)	5.0 (3.0–6.5)
Volume of the left ovary [mL]	4.1 (3.0–5.8)	4.1 (3.2–6.8)	4.6 (2.7–5.2)	3.8 (2.9–6.0)
Basal LH [IU/L]	3.1 (1.7–5.5)	2.8 (1.4–5.9)	3.8 (2.6–4.6)	3.5 (2.6–6.7)
Basal FSH [IU/L]	4.7 (3.6–5.8)	4.8 (3.6–5.8)	4.5 (4.1–4.9)	5.0 (3.8–6.5)
LH/FSH	0.6 (0.4–1.3)	0.5 (0.4–1.3)	0.9 (0.6–1.3)	0.9 (0.6–1.4)
Basal testosterone [ng/dL]	35.0 (21.7–52.8)	34.5 (21.7–49.8)	54.7 (27.0–57.3)	35.5 (21.7–52.9)
Stimulated testosterone [ng/dL]	49.3 (33.4–71.8)	45.2 (30.8–69.8)	60.9 (47.4–73.8)	49.8 (38.3–68.4)
Basal androstenedione [nmol/L]	8.1 (5.2–9.7)	7.7 (51–8.7)	9.6 (8.4–14.3)	6.7 (5.3–8.8)
Stimulated androstendione [nmol/L]	8.7 (7.3–10.4)	8.6 (6.5–9.9)	10.4 (9.8–15.4)	8.6 (6.9–11.5)
Basal DHEAS [μmol/L]	4.0 (2.9–6.0)	3.7 (2.5–5.4)	5.4 (3.3–7.1)	5.6 (4.8–6.3)
Stimulated DHEAS [μmol/L]	4.7 (3.5–6.5)	4.3 (3.0–6.2)	7.9 (5.0–8.3)	6.3 (4.7–8.0)
Basal 17OHP [nmol/L]	3.3 (2.4–4.2)	3.3 (2.5–4.0)	4.1 (2.8–4.3)	3.1 (2.1–4.2)
Stimulated 17OHP [nmol/L]	7.6 (4.8–9.7)	7.2 (4.5–9.7)	7.9 (6.4–11.4)	5.7 (4.9–8.2)
Basal oestradiol [pmol/L]	114.0 (85.9–155.0)	114.0 (80.0–155.0)	117.5 (88.5–149.0)	113.0 (82.2–175.0)
Stimulated oestradiol [pmol/L]	592.0 (396.0–885.0)	580.5 (437.0–804.0)	830.0 (364.0–896.0)	516.1 (328.0–742.0)
SHBG [nmol/L]	43.1 (33.1–64.4)	43.8 (37.6–65.6)	40.1 (30.1–59.8)	25.4 (18.8–45.2)
FAI	2.8 (1.8–3.4)	2.6 (1.7–3.4)	3.0 (2.6–4.3)	4.0 (2.8–7.0)
IGF-1 [μg/L]	302.0 (236.0–377.0)	301.0 (235.0–377.0)	304.5 (293.0–375.0)	292.0 (247.0–405.0)

Values are median (interquartile range). SHBG — sex hormone binding globulin, FAI — free androgen index; [1]Girls with T1DM v. control group p = 0.02; [2]Girls with T1DM no PCOS v. control group p = 0.008; [3]Girls with T1DM with PCOS v. control group p = 0.04; [4]Girls with T1DM v. control group p = 0.001; [5]Girls with T1DM with PCOS v. control group p = 0.005; [6]Girls with T1DM no PCOS v. T1DM with PCOS p = 0.05

HbA$_{1c}$ at the study point and mean HbA$_{1c}$ for the last 12 months correlated negatively with SHBG level (r = –0.5, p = 0.006; r = –0.04, p = 0.02). Mean HbA$_{1c}$ from the beginning of T1DM correlated negatively with basal and stimulated 17OHP level (r = –0.5, p = 0.002; r = –0.5, p = 0.002, respectively). There was a negative correlation of HbA$_{1c}$ at the study point with stimulated E$_2$ concentration (r = –0.4; p = 0.006). T1DM duration correlated negatively with stimulated LH level (r = –0.3, p = 0.03), and stimulated 17OHP concentration (r = –0.4; p = 0.02). Correlation between hyperandrogenic disturbances occurrence and DIR per kg, age at T1DM diagnosis, T1DM duration, mean HbA$_{1c}$ for the last 12 months and HbA$_{1c}$ at the study point were not statistically significant.

To evaluate the influence of the type of therapy on hyperandrogenic disorders, we compared girls treated

Table II. *Clinical and hormonal characteristics of adolescent girls with type 1 diabetes mellitus (T1DM) with polycystic ovary syndrome (PCOS) and non-diabetic girls with PCOS (control group)*

Tabela II. *Charakterystyka kliniczna i hormonalna dojrzewających dziewcząt z cukrzycą typu 1 (T1DM) i zespołem policystycznych jajników (PCOS) oraz dziewcząt bez T1DM z rozpoznanym PCOS (grupa kontrolna z PCOS)*

	T1DM with PCOS (n = 9)	Control group with PCOS (n = 21)
Chronological age (years)	16.9 (15.7–17.2)	16.3 (15.5–17.3)
Age at menarche (years)	13.0 (13.0–14.0)	12.0 (12.0–13.0)
Gynaecological age (months)	36.0 (24.0–45.0)	48.0 (33.5–57.0)[1]
Cycle duration (days)	29.0 (27.0–34.0)	57.5 (34.6–92.6)[2]
BMI z-score	0.0 (–0.2–0.7)	1.1 (0.1–1.7)
Ferriman-Gallwey score	2.0 (1.0–8.0)	11.0 (3.0–13.0)
Volume of the right ovary [mL]	5.6 (3.9–6.7)	7.7 (6.0–9.0)
Volume of the left ovary [mL]	4.6 (2.7–5.2)	7.4 (4.3–10.0)[3]
Basal LH [IU/L]	3.8 (2.6–4.6)	6.2 (3.5–10.4)
Basal FSH [IU/L]	4.5 (4.1–4.9)	4.9 (4.1–6.6)
LH/FSH	0.9 (0.6–1.3)	1.4 (0.8–1.8)
Basal testosterone [ng/dL]	54.7 (27.0–57.3)	62.5 (37.5–74.7)
Stimulated testosterone [ng/dL]	60.9 (47.4–73.8)	73.9 (47.6–103.0)
Basal androstenedione [nmol/L]	9.6 (8.4–14.3)	10.4 (6.1–12.4)
Stimulated androstenedione [nmol/L]	10.4 (9.8–15.4)	12.3 (9.1–15.7)
Basal DHEAS [μmol/L]	5.4 (3.3–7.1)	7.3 (5.8–8.1)
Stimulated DHEAS [μmol/L]	7.9 (5.0–8.3)	8.6 (6.0–10.3)
Basal 17OHP [nmol/L]	4.1 (2.8–4.2)	5.2 (4.1–6.6)
Stimulated 17OHP [nmol/L]	7.9 (6.4–11.4)	9.9 (6.95–12.0)
Basal oestradiol [pmol/L]	117.5 (88.5–149.0)	111.0 (84.4–160.0)
Stimulated oestradiol [pmol/L]	830.0 (364.0–896.0)	738.0 (492.0–958.0)
SHBG [nmol/L]	40.1 (30.1–59.8)	22.8 (15.1–28.4)
FAI	3.0 (2.6–4.3)	8.6 (6.5–10.8)[4]
IGF-1 [μg/L]	304.5 (293.0–375.0)	297.0 (243.0–481.0)

Values are median (interquartile range); SHBG — sex hormone binding globulin; FAI — free androgen index; [1]$p = 0.01$; [2]$p = 0.005$; [3]$p = 0.007$; [4]$p = 0.04$

with CSII to girls with MDI. PCOS occurrence as well as PCOS components incidence were similar in both groups [five (25%) girls *v.* four (15%) girls, $p > 0.05$; 15 (75%) girls *v.* 19 (70.4%) girls, $p > 0.05$, respectively]. There were no significant differences between the two subgroups with respect to ovarian volume and hormonal profile. Only SHBG level was significantly higher in the CSII group than in the MDI group (54.6 ± 17.8 nmol/L *v.* 40.1 ± 17.9 nmol/L, $p = 0.02$).

Discussion

Although in women with T1DM, increased prevalence of hyperandrogenic disorders such as PCOS and hirsutism has been described, the occurrence of hyperandrogenism in adolescents with T1DM has not been extensively evaluated. Our study results demonstrate that hormonal profile and clinical manifestations of hyperandrogenaemia in girls with T1DM are significantly different from adolescent girls with PCOS alone.

There is no published epidemiological data on the prevalence of PCOS in adolescent girls with T1DM to compare to the results of our study. The hyperandrogenic disorders, defined as the presence of polycystic ovaries or hirsutism, were found in 40.4% of girls with T1DM and the prevalence of PCOS in our group may seem high as 19.2% of them fulfilled the criteria of the syndrome. Nevertheless, this prevalence was much lower than in adult women, as Codner et al., using ESHRE/ASRM criteria, found PCOS in 40.5% of diabetic women [9]. In the study of Escobar-Morreale et al. [10], PCOS was present in 18.8% of diabetic subjects, and hyperandrogenic disorders were found in almost 39%. However, the data from the study of

Table III. *Clinical and hormonal characteristics of adolescent diabetic girls with well controlled (mean HbA_{1c} level from the last 12 months < 7.5 %) and poorly controlled type 1 diabetes mellitus (mean HbA1c level from the last 12 months ≥ 7.5%)*

Tabela III. *Charakterystyka kliniczna i hormonalna dojrzewających dziewcząt z dobrze wyrównaną (średnia HbA_{1c} z ostatnich 12 miesięcy < 7,5%) i źle wyrównaną cukrzycą typu 1 (średnia HbA_{1c} z ostatnich 12 miesięcy ≥ 7,5%)*

	Well controlled (n = 35)	Poorly controlled (n = 12)
Chronological age (years)	15.9 ± 1.4	15.8 ± 1.4
Age at first menses (years)	13.0 (12.0–14.0)	13.0 (12.0–14.0)
Age at T1DM onset (years)	9.0 ± 3.5	7.4 ± 3.8
T1DM duration (years)	6.9 ± 3.2	8.2 ± 4.2
HbA_{1c} at the study point (%)	6.8 ± 0.8	10.0 ± 2.8[1]
Mean HbA_{1c} for last 12 months (%)	6.7 ± 0.6	9.5 ± 1.3[1]
Mean HbA_{1c} for T1DM's span (%)	6.7 ± 0.6	8.6 ± 1.2[1]
Daily insulin requirement [U/kg/day]	0.8 (0.7–0.9)	0.8 (0.8–1.0)
BMI z-score	0.4 ± 0.5	0.8 ± 0.7[3]
Right ovary volume [mL]	5.3 ± 2.5	4.4 ± 2.2
Left ovary volume [mL]	4.5 ± 1.9	5.0 ± 3.2
Basal LH [IU/L]	3.4 (1.7–5.9)	2.1 (1.5–4.8)
Basal FSH [IU/L]	4.5 (3.6–5.5)	5.2 (3.7–5.9)
LH/FSH	1.0 (0.4–1.3)	0.5 (0.4–1.2)
Basal testosterone [ng/dL]	34.8 (20.0–52.8)	39.6 (22.6–60.2)
Stimulated testosterone [ng/dL]	48.8 ± 20.4	70.9 ± 44.4[4]
Basal androstenedione [nmol/L]	8.8 (5.9–9.7)	7.7 (4.9–9.1)
Stimulated androstenedione [nmol/L]	8.9 (7.3–10.6)	8.4 (5.9–9.8)
Basal 17OH-progesterone [nmol/L]	3.5 (2.6–4.6)	2.7 (1.8–3.3)[3]
Stimulated 17OH-progesterone [ng/mL]	7.9 (5.8–10.3)	5.2 (3.9–7.6)[5]
Basal DHEAS [mmol/L]	4.3 ± 1.8	4.0 ± 1.4
Stimulated DHEAS [mmol/L]	5.3 ± 2.0	4.3 ± 1.4
Basal oestradiol [pmol/L]	110.5 (85.9–158.0)	117.0 (84.5–139.5)
Stimulated oestradiol [pmol/L]	617.0 (477.0–991.0)	332.3 (207.0–558.5)
SHBG [nmol/L]	49.7 ± 19.2	37.6 ± 16.6
FAI	2.7 (1.8–3.1)	2.9 (2.1-3.8)
IGF-1 [mg/L]	329.7 ± 84.9	235.6 ± 74.8[2]

Values are mean ± standard deviation for normal or median (interquartile range) for skewed distribution. SHBG — sex hormone binding globulin, FAI — free androgen index. Well-controlled *v.* poorly-controlled: [1]$p \leq 0.001$; [2]$p = 0.003$; [3]$p = 0.02$; [4]$p = 0.03$; [5]$p = 0.04$

Escobar-Morreale et al. [10] are not comparable to our group because of the different age frames of the subjects and various criteria applied by the authors. We used the Androgen Excess and PCOS Society criteria that are more liberal in PCOS diagnosis than the NIH criteria used by Escobar-Morreale et al. [10]. If NIH criteria had been used by us, the frequency of PCOS in our group of adolescent girls would have been 2.1% only, so even lower than the prevalence observed by Codner et al. in adult women with T1DM, which was 11.9% [9] and similar to that reported in the general population [1]. The lower incidence of PCOS in our group of adolescent girls than in the Codner et al. [9]

and Escobar-Morreale el al. [10] studies could be explained by the shorter time of exposure to exogenous insulin due to shorter diabetes duration, as well as younger gynaecological age.

The prevalence of hirsutism was much lower in our patients than in other studies [9,10]. Escobar-Morreale et al. [10] found hirsutism ≥ 8 score in 20% of regularly menstruating diabetic women, while Codner et al. [9] described hirsutism of low score in 28% of patients. In our study, only three (6.4%) girls had hirsutism scoring 8 on the Ferriman-Gallway scale and one of them also experienced menstrual disturbances. Lower hirsutism occurrence in adolescent diabetic girls compared to

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adult women could be associated with younger gynaecological age of our patients and shorter time of excessive hair growth stimulated by hyperandrogenaemia. Other authors also suggest that milder signs of hyperandrogenism in adolescent girls with T1DM are due to the fact that the clinical and laboratory findings of hyperandrogenaemia evolve slowly during the second decade of life [9,13]. Some of them even applied lower scoring for hirsutism diagnosed in girls than in adult women [15].

It may also seem that diabetic women experience a milder form of PCOS with scarce clinical features, the main manifestation being biochemical hyperandrogenaemia and PCOM [14]. In our study, clinical manifestations of the syndrome in diabetic girls were much less pronounced than in non diabetic adolescents with PCOS. This could be explained by the fact that the nonphysiological, subcutaneous way of insulin administration results in decreased insulin amount acting in the liver, leading to higher SHBG production and lower FAI. Only three out of nine girls with T1DM and PCOS had clinical manifestations of hyperandogenism, while the other six had an increased level of androgens and PCOM. Although these six girls fulfilled the criteria of PCOS, both ESHRE/ASRM and the Androgen Excess and PCOS Society [18,19], they had no clinical presentation of the syndrome. A diagnosis of PCOS should therefore be made with caution, especially given that PCOM seems to be a physiologic condition during early adolescence [21].

It has been suggested that the pathogenesis of hyperandrogenism in women with T1DM and PCOS is different than in non diabetic women with PCOS and that insulin replacement therapy could be crucial [22]. Escobar-Morreale et al. [10] as well as Codner et al. [9] did not find any predisposing factors of hyperandrogenic disorders among clinical variables such as duration of T1DM, age of onset or the type of therapy. In our study we also did not find such correlations or significant differences. However, in girls with poor metabolic control we found significantly higher stimulated testosterone level. Some association between hormones concentrations and mean HbA_{1c} and T1DM duration were also present in our group of patients, not reported before by other authors. Negative correlation between T1DM duration and basal LH level and between HbA_{1c} and stimulated E_2 as well as SHBG concentrations can reflect the influence of metabolic control on ovarian and liver function. Surprisingly, in girls with lower mean HbA_{1c} for the last 12 months and from the beginning of T1DM, higher concentrations of 17OHP were found. We suggest that the lower HbA_{1c} could result from higher doses of insulin used for treatment that can be responsible

for higher 17OHP level. Another explanation of this state could be just the stronger biological activity of insulin, manifested as better hypoglycaemic effect, better metabolic control but also excessive reaction of ovaries. In addition, higher IGF-1 concentration in girls with mean HbA_{1c} for the last 12 months below 7.5% could be a co-factor increasing, together with insulin, production of 17OHP [3].

Therefore, both good and poor metabolic control could be possible reasons for hormonal disturbances associated with systemic hyperinsulinaemia.

Codner et al. [9] suggested that intensive insulin treatment may be a predisposing factor for PCOS and PCOM. However, their study was conducted on women over 20 years of age and subjects with intensive insulin treatment were compared to those on conventional therapy (< 3 insulin injections per day). In our study, in which we compared hyperandrogenism in girls with intensive therapy — MDI and CSII, hormonal profile was the same in two types of insulin therapy, although SHBG level was significantly higher in girls treated with CSII.

Our data did not show the influence of obesity on the development of PCOS, as apart from the group with poor metabolic control, in which BMI z-score was significantly higher, all the other comparative groups did not differ in BMI z-score value.

Finally, we found that girls with T1DM experienced menarche significantly later than healthy girls. It has been reported that delay of menarche in diabetic girls could be associated with poor metabolic control, lower BMI, prepubertal onset of diabetes and longer duration of the disease [14]. We did not find any relationship of late menarche with HbA_{1c}, T1DM duration, age of onset of diabetes, or BMI z-score. Rohrer et al. [23] suggested that the cause of the delay in menarcheal age in T1DM girls may lie in the hypothalamic–pituitary level, especially in decreased LH secretion. In our study, LH levels as well as LH/FSH ratio in diabetic girls were not significantly lower then in healthy controls, which does not support that hypothesis.

Our study limitation was a heterogeneous duration of diabetes in the study group; however, the difference between girls with and without PCOS was statistically not significant. The other weak point was the relatively short gynaecological age of our patients. On the other hand, the aim of the study was to evaluate girls as young as possible to find out at what age they may start to develop hyperandrogenaemia.

We conclude that the frequency of the clinical features of PCOS and hyperandrogenaemia in adolescent girls with T1DM seems to be lower than in adult diabetic women. The clinical manifestations of PCOS in these girls are mild. PCOS components occurrence

is independent of the type of insulin therapy, but poor metabolic control may decrease the protective role of SHBG.

The results of our study suggest a different patho-physiology of hyperandrogenism in adolescent girls with T1DM than in their peers with PCOS only.

References

1. March WA, Moore VM, Willson KJ et al. The prevalence of polycystic ovary syndrome in a community sample assessed under contrasting diagnostic criteria. Hum Reprod 2010; 25: 544–551.
2. Olszanecka-Glinianowicz M, Banas M, Zahorska-Markiewicz B et al. Insulinooporność a stężenie androgenów jajnikowych i nadnerczowych w surowicy otyłych kobiet bez chorób towarzyszących i z zespołem policystycznych jajników. Polish J Endocrin 2005; 56: 921-6.
3. Poretsky L, Cataldo NA, Rosenwaks Z et al. The insulin-related ovarian regulatory system in health and disease. Endocr Rev 1999; 20: 535–582.
4. Poretsky L, Smith D, Seibel M et al. Specific insulin binding sites in the human ovary. J Clin Endocrinol Metab 1984; 59: 809–811.
5. Poretsky L, Grigorescu F, Seibel M et al. Distribution and characterization of the insulin and IGF-I receptors in normal human ovary. J Clin Endocrinol Metab 1985; 61: 728–734.
6. Poretsky L. On the paradox of insulin-induced hyperandrogenism in insulin-resistance states. Endocr Rev 1991; 12: 3–13.
7. Nahum R, Thong KJ, Hillier SG. Metabolic regulation of androgen production by human thecal cells in vitro. Hum Reprod 1995; 10: 75–81.
8. Cara JF, Rosenfield RL. Insulin like growth factor I and insulin potentiate luteinizing hormone-induced androgen synthesis. Endocrinology 1985; 123: 733–739.
9. Codner E, Soto N, Lopez P et al. Diagnostic criteria for polycystic ovary syndrome and ovarian morphology in women with type 1 diabetes mellitus. J Clin Endocrinol Metab 2006; 91: 2250–2256.
10. Escobar-Morreale HF, Roldan B, Barrio R et al. High prevalence of the polycystic ovary syndrome and hirsutism in women with type 1 diabetes Mellitus. J Clin Endocrinol Metab 2000; 85: 4182–4187.
11. Kjaer K, Hagen C, Sando SHet al. Epidemiology of menarche and menstrual disturbances in an unselected group of women with insulin-dependent diabetes mellitus compared to controls. J Clin Endocrinol Metab 1992; 75: 524–529.
12. Polonsky KS, Given BD, Hirsch L et al. Quantitative study of insulin secretion and clearance in normal and obese subject. J Clin Invest 1988; 81: 435–441.
13. Codner E, Escobaar-Morreale HF. Hyperandrogenism and polycystic ovary syndrome in women with type 1 diabetes mellitus. J Clin Endocrinol Metab 2007; 92: 1209–1216.
14. Codner E, Cassorla F. Puberty and ovarian function in girls with type 1 diabetes mellitus. Horm Res 2009; 71: 12–21.
15. Codner E, Mook-Kanamori D, Bazaes RA et al. Ovarian function during puberty in girls with type 1 diabetes mellitus: response to leuprolide. J Clin Endocrinol Metab 2005; 90: 3939–3945.
16. Malecka-Tendera E, Zachurzok-Buczynska A, Januszek-Trzciakowska A. Hormonal abnormalities in adolescent girls with menstrual disorders-incidence of functional ovarian hyperandrogenism and polycystic structure of ovaries. Adv Clin Exp Med 2004; 13(Suppl 1): 11–18.
17. Carmina E; Oberfield S, Lobo R. The diagnosis of polycystic ovary syndrome in adolescents. Am J Obstet Gynecol 2010; 203: 201.e1–5.
18. Azziz R, Carmina E, Dewailly D et al. The Androgen Excess and PCOS Society criteria for the polycystic ovary syndrome: the complete task force report. Fertil Steril 2009; 91: 456–488.
19. The Rotterdam ESHRE/ASRM-sponsored PCOS Consensus Workshop Group Revised 2003 consensus on diagnostic criteria and long-term health risks related to polycystic ovary syndrome (PCOS). Hum. Reprod 2004; 19: 41–47.
20. Rewers M, Pihoker C, Donaghue K et al. Assessment and monitoring of glycemic control in children and adolescents with diabetes. Pediatric Diabetes 2009; 10 (Suppl. 12): 71–81.
21. Codner E, Villarroel C, Eyzaguirre FC et al. Polycystic ovarian morphology in postmenarchal adolescents. Fertil Steril 2011; 95: 702–706.
22. Codner E, Iniguez G, Villarroel C et al. Hormonal profile in women with polycystic ovarian syndrome with or without type 1 diabetes mellitus. J Clin Endorinol Metab 2007; 92: 4742–4746.
23. Rohrer T, Stierkorb E, Heger S et al. Delayed pubertal onset and development in German children and adolescents with type 1 diabetes: cross-sectional analysis of recent data from the DPV diabetes documentation and quality management system. Eur J Endocrinol 2007; 157: 647–653.