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GAS CHROMATOGRAPHY-MASS SPECTROMETRY-BASED METABOLIC PROFILING OF ANDROGENS, PROGESTINS AND GLUCOCORTICOIDS IN WOMEN WITH POLYCYSTIC OVARY SYNDROME

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▪ ***Hypothesis/aims of study.*** Polycystic ovary syndrome (PCOS) is a common disease. Depending on the diagnostic criteria, the disease is seen in 10-20% of women of reproductive age and accounts for 70-80% of all forms of hyperandrogenic syndrome. PCOS is a heterogeneous condition of multifactorial etiology characterized by various clinical, endocrine and metabolic disorders. Therefore, it is important to clarify the specific features of steroid hormone biosynthesis and metabolism and steroidogenesis enzyme activity, as well as to search for new laboratory criteria for early diagnosis and prompt treatment. The aim of this study was to perform metabolic profiling of androgens, progestins and glucocorticoids using gas chromatography-mass spectrometry (GC-MS) in obese and non-obese women with PCOS.

Study design, materials and methods. We examined 53 women of reproductive age diagnosed with PCOS. The first group included 30 women aged 22 to 29 years with normal body weight. The second group comprised 23 obese patients aged 25 to 33 years with an average body mass index (BMI) of 35.3 ± 0.4 kg/m^2. The control group consisted of 25 healthy women aged 26 ± 0.6 years having a normal BMI without clinical and biochemical signs of hyperandrogenism. Immunoassay methods were used to determine the serum levels of luteinizing hormone, follicle-stimulating hormone, free testosterone, 17-hydroxyprogesterone, and sex hormone-binding globulin. A glucose tolerance test was performed to determine glucose and insulin levels before and after load. Urine steroid profiles were studied by GC-MS with the optimization of the sample preparation schedule. Statistical data processing was performed using the STATISTICA for WINDOWS software system (version 10). The main quantitative characteristics of the patients are presented as the median (Me), the 25th percentile and the 75th percentile (Q_{25}–Q_{75}). To compare the results obtained in the study groups, the nonparametric Mann-Whitney test was used. The 95% confidence interval was considered statistically significant.

Results. The article presents a metabolomics analysis of androgens, glucocorticoid hormones and progestins in women with PCOS compared to the control group. It was revealed that non-obese patients with PCOS had increased urinary excretion of androstenedione metabolites, dehydroepiandrosterone and its metabolites, 17-hydroxypregnanolone, pregnantriol, and 5-ene-pregnenes, while obese patients with PCOS had increased that of androsterone and dehydroepiandrosterone metabolites (16-oxo-androstenediol and androstenediol-17β) compared to the control group findings. Decreased ratios of cortisol and cortisone tetrahydro metabolite amount to the levels of 11-oxo-pregnanetriol, pregnanetriol and 17-hydroxypregnenolone, when compared to the control group, was obtained in non-obese patients with PCOS, which indicates 21-hydroxylase deficiency. In obese patients with PCOS, four signs of increased 5α-reductase activity were obtained, and in PCOS patients with a normal BMI, three signs were obtained, which indicates varying 5α-reductase activity in PCOS patients depending on the BMI.

Conclusion. Quantitative evaluation of androgen and progestin metabolites, as well as 5α- and 5β-metabolites of androstenedione and glucocorticoids in the study of urine steroid profiles by GC-MS method opens new opportunities for PCOS diagnostics.

▪ **Keywords: gas c**hromatography-mass spectrometry; metabolomics; steroid hormones; polycystic ovary syndrome; hyperandrogenism; obesity.

МЕТАБОЛОМИКА АНДРОГЕНОВ, ПРОГЕСТИНОВ И ГЛЮКОКОРТИКОИДОВ У ЖЕНЩИН С СИНДРОМОМ ПОЛИКИСТОЗНЫХ ЯИЧНИКОВ ПО ДАННЫМ ГАЗОВОЙ ХРОМАТО-МАСС-СПЕКТРОМЕТРИИ

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▪ ***Актуальность.*** Синдром поликистозных яичников является распространенным заболеванием. В зависимости от диагностических критериев заболевание наблюдается у 10–20 % женщин репродуктивного возраста и составляет 70–80 % всех форм синдрома гиперандрогении. Синдром поликистозных яичников — гетерогенное заболевание с многофакторной этиологией, характеризующееся различными клиническими, эндокринными и метаболическими нарушениями. В связи с этим актуальны уточнение особенностей биосинтеза и метаболизма стероидных гормонов, активности ферментов стероидогенеза, поиск новых лабораторных критериев для ранней диагностики и своевременного начала лечения.

Цель — изучить метаболомику андрогенов, прогестинов и глюкокортикоидов по данным газовой хромато-масс-спектрометрии у женщин с синдромом поликистозных яичников с нормальной массой тела и ожирением.

Материалы и методы исследования. Обследовано 53 женщины репродуктивного возраста с диагнозом «синдром поликистозных яичников». В первую группу включены 30 женщин в возрасте от 22 до 29 лет с нормальной массой тела. Вторую группу составили 23 пациентки с ожирением в возрасте от 25 до 33 лет со средним индексом массы тела 35,3 ± 0,4 кг/м^2. В группу контроля вошли 25 здоровых женщин в возрасте 26 ± 0,6 года с нормальным индексом массы тела без клинических и биохимических признаков гиперандрогении. Методами иммуноанализа в сыворотке крови определяли уровни лютеинизирующего гормона, фолликулостимулирующего гормона, свободного тестостерона, 17-гидроксипрогестерона и глобулина, связывающего половые гормоны. Проводили пробу на толерантность к глюкозе с определением уровней глюкозы и инсулина до и после нагрузки с 75 г глюкозы. Методом газовой хромато-масс-спектрометрии с оптимизацией регламента пробоподготовки исследовали стероидные профили мочи. Статистическую обработку данных осуществляли с использованием программной системы STATISTICA for WINDOWS (версия 10). Основные количественные характеристики больных представлены в виде медианы (Me), 25-го перцентиля и 75-го перцентиля (Q_{25}–Q_{75}). Для сравнения результатов, полученных в исследуемых группах, применяли непараметрический критерий Манна – Уитни. Статистически значимым считали критерий достоверности $p < 0,05$.

Результаты исследования. В статье приведен анализ метаболомики андрогенов, глюкокортикоидных гормонов и прогестинов у женщин с синдромом поликистозных яичников в сравнении с соответствующими показателями у женщин контрольной группы. У больных синдромом поликистозных яичников без ожирения выявлено увеличение экскреции с мочой метаболитов андростендиона, дегидроэпиандростерона и его метаболитов, 17-гидроксипрегненолона и прегнантриола, 5-ен-прегненов, а у женщин с синдромом поликистозных яичников и ожирением была повышена экскреция с мочой андростерона и метаболитов дегидроэпиандростерона (16-оксоандростендиола и андростендиола-17β) по сравнению с показателями в группе контроля. Снижение соотношений суммы тетрагидропроизводных кортизола и кортизона к 11-оксо-прегнантриолу, прегнантриолу и 17-гидроксипрегненолону в сравнении с группой контроля обнаружено у пациенток с синдромом поликистозных яичников без ожирения, что является признаками недостаточности фермента 21-гидроксилазы. У женщин с синдромом поликистозных яичников и ожирением отмечены четыре признака увеличения активности фермента 5α-редуктазы, а у больных синдромом поликистозных яичников с нормальным индексом массы тела — три признака, что указывает на различную степень активности 5α-редуктазы у больных синдромом поликистозных яичников в зависимости от индекса массы тела.

Заключение. Одновременное количественное определение метаболитов андрогенов, прогестинов, 5α- и 5β-метаболитов андростендиона и глюкокортикоидов при исследовании стероидных профилей мочи методом газовой хромато-масс-спектрометрии открывает новые возможности для диагностики синдрома поликистозных яичников.

▪ **Ключевые слова:** газовая хромато-масс-спектрометрия; метаболомика; стероидные гормоны; синдром поликистозных яичников; синдром гиперандрогении; ожирение.

Background

The diagnosis and treatment of polycystic ovary syndrome (PCOS) are relevant to gynecological endocrinology. PCOS is seen in 10%–20% of women of reproductive age and accounts for 70%–80% of all forms of hyperandrogenic syndrome depending on the diagnostic criteria [1, 2]. Women with PCOS account for approximately half of all patients with endocrine infertility [3]. The mean age of menarche in PCOS does not differ from that of the population (12–13 years). The disease predominantly begins at an early reproductive age. Menstrual disorders occur during menarche and are manifested by opsomenorrhea, primary (rare) or secondary amenorrhea, and dysfunctional uterine bleeding in 50% of women [4, 5]. Some patients with PCOS have an ovulatory menstrual cycle, and pregnancy is possible. Infertility is observed in 70%–75% of women. Studies by R. Hartet et al. showed that PCOS patients had a 10-fold increased risk of infertility in 2015 as compared with that of the general population [6]. Type 2 diabetes mellitus, cardiovascular disorders, and endometrial hyperplasia are commonly found in women with PCOS in the late reproductive period [7, 8]. The risk of endometrial cancer is found to be threefold higher in patients with chronic anovulation and PCOS, on average, as compared with healthy women [9]. PCOS is a multifactorial disease, which depends on various endogenous and exogenous factors [10]. The severity of hormonal and metabolic disorders and the clinical pattern of the disease depend on the PCOS phenotype.

The criteria used for diagnosing PCOS are the US National Institutes of Health criteria revised in 2012, the European Society for Human Reproduction and Embryology (ESHRE) and the American Society for Reproductive Medicine (ASRM) criteria adopted in Rotterdam in 2003, and the Androgen Excess and PCOS Society (AE-PCOS Society) criteria of 2006. PCOS is diagnosed when two of the following three signs are present: oligo-ovulation or anovulation, clinical or biochemical hyperandrogenism or a combination of both, and ultrasound signs of polycystic ovarian morphology. Four main phenotypes of PCOS have been identified as per the 2007 International Symposium of the ESHRE and ASRM because of the heterogeneity of the clinical pattern. The phenotypes are as follows: a classic (A) phenotype (hyperandrogenism, anovulation, and polycystic ovaries), an anovulatory (B) phenotype (hyperandrogenism and anovulation), an ovulatory (C) phenotype (hyperandrogenism and polycystic ovaries), and a nonandrogenic (D) phenotype (polycystic ovaries and anovulation) [11-13]. According to the studies on the prevalence of PCOS phenotypes in women of reproductive age, (A) phenotype occurs in 44%–65%, (B) phenotype in 8%–33%, (C) phenotype in 3%–29%, and (D) phenotype in 23% of women [14, 15]. (A) and (B) phenotypes are associated with menstrual disorders, insulin resistance, obesity, dyslipidemia, and an increased risk of metabolic syndrome. E.M. Neves reported in his study that metabolic syndrome was found in 71% of women with (A) phenotype and in 67.4% of women with (B) phenotype [16].

Obesity plays an important role in the PCOS pathogenesis. Overweight and obesity occur in 22%–65% of women and are major risk factors for metabolic disorders, impaired glucose tolerance (IGT), type 2 diabetes mellitus, and dyslipidemia [17, 18]. Some authors reported that IGT occurs in 30%–40%, and type 2 diabetes mellitus occurs in 10% of women with PCOS [19]. Adipose tissue is the most important endocrine organ, which produces steroid hormones and various biologically active substances. The following enzymes are found in adipose tissue: aromatase with its function of converting androgens to estrogens and 5alpha-reductase, which converts testosterone to the more active androgen, such

as dihydrotestosterone. Adipose tissue also produces several hydroxysteroid dehydrogenases, such as the 11beta-hydroxysteroid dehydrogenase (11beta-HSDH) type 1 enzyme, which catalyzes the conversion of the functionally inactive cortisone to cortisol, that is, the most active glucocorticoid hormone, and the enzyme 17beta-hydroxysteroid dehydrogenase, which converts androstenedione to testosterone and estrone to estradiol. The estrone/estradiol ratio changes in favor of estrone in obesity, resulting in positive feedback mechanism failure necessary for ovulation. The stimulating effect of luteinizing hormone (LH) on the ovaries leads to theca and stromal cell hyperplasia and increased androgen synthesis. Relatively low levels of follicle-stimulating hormone (FSH) contribute to a decrease in ovarian aromatase, while granulosa cells lose the ability to aromatize androgens to estrogens, primarily testosterone to estradiol. This results in an accumulation of testosterone and a deficiency of estradiol, with its peak secretion being necessary for the ovulatory LH release and normal ovulation [20].

The lack of peak cyclical estradiol secretion leads to anovulation. The association between obesity and hyperandrogenism is mediated by insulin resistance and hyperinsulinemia. C. Achard and J. Thiers first described the association between impaired carbohydrate metabolism and hyperandrogenism in 1921, and the term "diabetes of bearded women" appeared. G.A. Burgen et al. found that women with PCOS had both basal and glucose-stimulated hyperinsulinemia, which suggested insulin resistance [21, 22]. The effect of insulin on ovarian steroidogenesis is realized both through its own receptors and indirectly through insulin-like growth factor-1 receptors. Insulin can stimulate ovarian LH-dependent and adrenal adrenocorticotropic hormone (ACTH)-dependent cytochrome p450c17alpha activity [23]. Insulin can also inhibit the production of sex hormone–binding globulin (SHBG), which leads to higher blood levels of free androgens. Insulin resistance in PCOS occurs in 40%–70% of patients, as per several studies, in both obese and normal-weight women [24].

Traditional tests are not sufficient for the diagnosis of various forms of hyperandrogenic syndrome, and new highly specific and highly sensitive current diagnostic techniques are needed. This is particularly important for the differential diagnosis between PCOS and nonclassical forms of congenital adrenal cortical dysfunction. Chromatographic methods have been used to study the metabolic profiling of steroid hormones. Sporadic data on urinary steroid profiles (USP) using gas chromatography–mass spectrometry (GC-MS) in PCOS and obese patients were obtained. They included increased urinary excretion of pregnenes, dehydroepiandrosterone and androstenedione metabolites, 5alpha-metabolites and 5beta-metabolites of glucocorticoids, and decreased activity of type 1 11beta-hydroxysteroid dehydrogenase enzyme, which led to an accumulation of inactive glucocorticoids [25, 26]. Yuying Deng and Yifei Zhang examined 1,044 women with PCOS; of these, 350 women were with normal weight, 312 with excessive weight, and 382 with obesity [27]. A comparative analysis revealed that normal-weight women showed signs of 21-hydroxylase enzyme deficiency in contrast to overweight and obese women that confirmed steroidogenic disorders of both ovarian and adrenal geneses in patients in different groups. Recent studies have presented diagnostic signs of a 21-hydroxylase defect, such as increased urinary excretion of 17-OH-pregnenolone (17P) and its metabolite, pregnanetriol (P3), and increased metabolites of 21-deoxycortisol, that is, tetrahydro21-deoxycortisol (21-deoxy-THF) and 11-oxo-pregnanetriol (11-oxo-P3) in women with PCOS and obesity [28].

Study design, materials and methods

A total of 53 women of reproductive age diagnosed with PCOS were examined. The first group included 30 women aged 22 to 29 years (mean age, 25 ± 0.4 years) with normal body weight (NBW) and body mass index (BMI) of 22.5 ± 0.7 kg/m^2. The second group comprised 23 obese patients aged 25 to 33 years (mean age, 28 ± 0.9 years) with BMI of 32.7 kg/m^2 to 38.4 kg/m^2 (average BMI, 35.3 ± 0.4 kg/m^2). The control group (CG) consisted of 25 healthy women aged 23 to 30 years (mean age, 26 ± 0.6 kg/m^2) with BMI of 21 kg/m^2 to 24 kg/m^2 (average BMI, 22 ± 0.8 kg/m^2) without clinical and biochemical signs of hyperandrogenism. PCOS was diagnosed when two of the following

three signs were present: oligo-ovulation or anovulation, clinical or biochemical hyperandrogenism, and ultrasound signs of polycystic ovarian morphology. Immunoassay methods were used to determine the serum levels of LH, FSH, free testosterone, 17-hydroxyprogesterone (17-OHP), and SHBG. Fasting glucose and insulin (INS) levels in serum were determined by glucose tolerance test (GTT) that was performed 2 hours after 75 g glucose ingestion. USP were studied by GC-MS with the optimization of the sample preparation schedule. Liquid extraction was chosen; optimum amounts of derivatizing agents (methoxyamine and trimethylsilylimidazole) were determined; and chromatographic analysis conditions were selected [41]. A total of 69 steroids were identified. USP were obtained using SHIMADZU GCMS-QP2020 gas chromatography–mass spectrometer. Statistical data processing was performed using the STATISTICA for WINDOWS software system (version 10). The main quantitative characteristics of the patients are presented as the median (Me) and the 25th percentile and the 75th percentile (Q_{25}–Q_{75}). The nonparametric Mann–Whitney test was used to compare the results obtained in the study groups. The criterion (p) < 0.05 was considered statistically significant.

Results

The levels of LH, 17-OHP, and free testosterone (FT) were increased, the serum SHBG levels were decreased, and the LH/FSH ratio was increased in all the examined PCOS patients as compared with the CG. FT levels were found to be higher and 17-OHP and SHBG levels were lower in PCOS and obese patients as compared with patients with NBW. Serum LH and FSH levels and LH/FSH ratio did not differ between the NBW and obese groups (p > 0.05). Increased fasting and posttest glucose tolerance INS levels were found in PCOS and obese patients as compared with those in the CG and PCOS patients with NBW (Table 1). Negative correlations of SHBG with INS-1 and INS-2 levels were obtained.

GC-MS data from PCOS patients with NBW revealed increased urinary excretion of dehydroepiandrosterone (DHEA), DHEA-17beta-metabolites (dA2-17beta), 16beta-OH-DHEA,

Table 1 / Таблица 1

Serum hormone levels in normal-weight and obese patients with polycystic ovary syndrome (data obtained using immunoassay)
Содержание гормонов в сыворотке крови у больных синдромом поликистозных яичников с нормальным весом и ожирением по данным методов иммуноанализа

Indicators	Me (Q_{25}–Q_{75})		
	Control group, n = 25	PCOS patients with BMI <25 kg/m^2, n = 30	PCOS patients with BMI >30 kg/m^2, n = 23
Luteinizing hormone, IU/L	5.6 (4.8–7.3)	9.0 (5.2–12.7) p = 0.004	9.7 (6.1–17.5) p = 0.009
Follicle-stimulating hormone, IU/L	5.8 (3.6–6.4)	6.1 (4.6–7.1)	5.8 (5.4–7.0)
Luteinizing hormone/follicle-stimulating hormone ratio	1.1 (0.9–1.3)	1.4 (1.1–2.3) p = 0.02	1.7 (1.0–2.9) p = 0.04
17-Hydroxyprogesterone, ng/mL	0.7 (0.4–0.8)	2.2 (1.9–3.3) p < 0.0001	1.2 (0.8–1.4) p = 0.003
Free testosterone, pg/mL	1.0 (0.7–2.0)	4.3 (2.8–8.0) p < 0.0001	7.2 (2.6–14.8) p < 0.0001
Fasting insulin, μU/mL	5.5 (4.0–7.5)	5.1 (4.8–7.1)	15.5 (14.9–25) p = 0.0005
Insulin after GTT, μU/mL	13.5 (9.9–15.0)	22.6 (17.6–32.5)	75.2 (72.3–146) p < 0.0001
Sex hormone–binding globulin, nmol/L	66 (50–86)	40 (31–64) p = 0.003	19 (14–23) p < 0.0001

N o t e: p, significance of differences in PCOS patients compared with the CG; BMI, body mass index; GTT, glucose tolerance test.

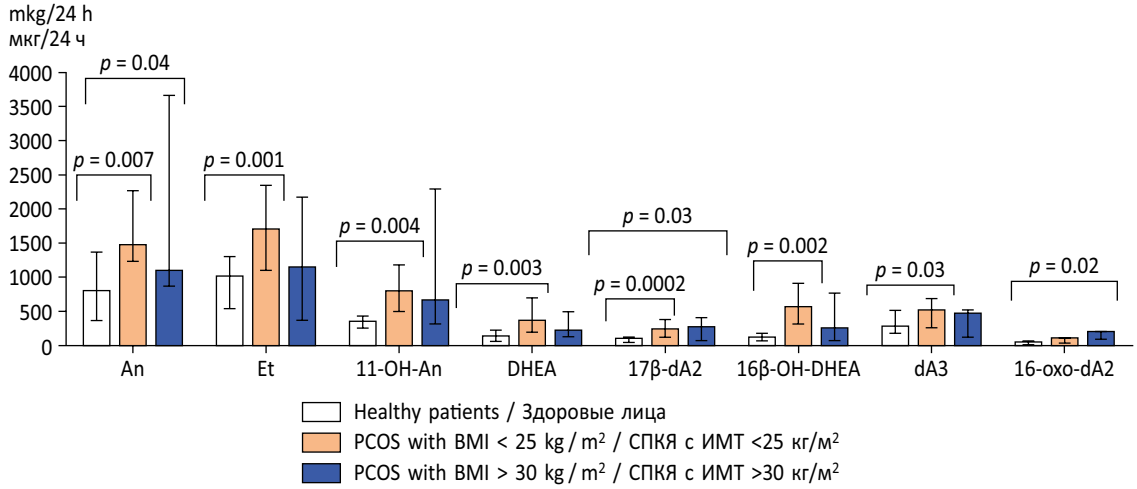

Fig. 1. Urinary excretion of androgens in normal weight and obese patients with polycystic ovary syndrome (data obtained using gas chromatography-mass spectrometry). PCOS, polycystic ovary syndrome; BMI, body mass index; An, androsterone; Et, etiocholanolone; DHEA, dehydroepiandrosterone; dA2, androstenediol; dA3, androstenetriol

Рис. 1. Экскреция с мочой андрогенов у пациентов с синдромом поликистозных яичников с нормальным весом и ожирением по данным газовой хромато-масс-спектрометрии: СПКЯ — синдром поликистозных яичников; ИМТ — индекс массы тела; An — андростерон; Et — этиохоланолон; DHEA — дегидроэпиандростерон; dA2 — андростендиол; dA3 — андростентриол

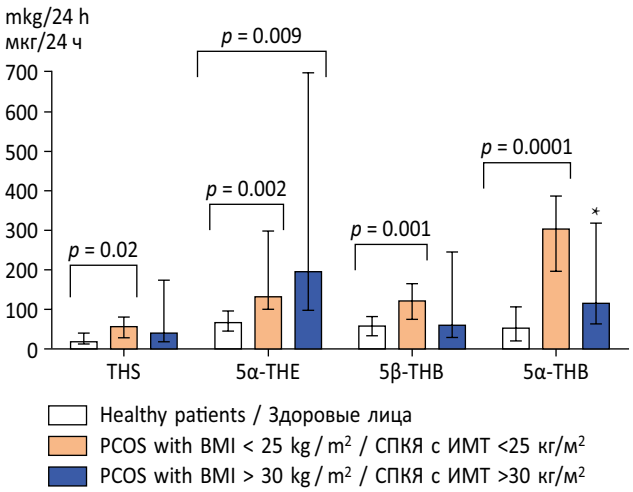

Fig. 2. Urinary excretion of glucocorticoid tetrahydro metabolites in normal weight and obese patients with polycystic ovary syndrome (data obtained using gas chromatography-mass spectrometry). PCOS, polycystic ovary syndrome; BMI, body mass index; THS, tetrahydro-11-deoxycortisol; THE, tetrahydrocortisone; THB, tetrahydrocorticosterone

Рис. 2. Экскреция с мочой тетрагидрометаболитов глюкокортикоидов у пациентов с синдромом поликистозных яичников с нормальным весом и ожирением по данным газовой хромато-масс-спектрометрии: СПКЯ — синдром поликистозных яичников; ИМТ — индекс массы тела; THS — тетрагидро-11-дезоксикортизол; THE — тетрагидрокортизон; THB — тетрагидрокортикостерон

androstentriol (dA3), and androstenedione metabolites, such as androsterone (An), ethiocholanolone (Et), and 11-OH-An as compared with those in the CG (Fig. 1). Urinary excretion of An, dA2-17beta, and 16-oxo-dA2 was increased in PCOS and obese patients as compared with the CG.

Urinary excretion of tetrahydrocortisone (THE) was increased in all PCOS patients (Fig. 2) while the tetrahydrocortisol (THF)/THE ratio was reduced (Table 2). The ratio (THF+5alpha-THF+alpha-cortol+beta-cortol)/(THE+5alpha-THE+alpha-cortolon+beta-cortolon) was reduced only in PCOS and obese patients (see Table 2). These findings indicate a decrease in 11beta-hydroxysteroid dehydrogenase type 1 activity, which leads to increased urinary excretion of inactive glucocorticoids.

Urinary excretion of 17-OH progesterone metabolites, such as 17P, P3, 11-oxo-P3, 5-ene-pregnenes (dP2, 16-OH-dP2, and dP3) was increased in PCOS and NBW patients compared to those in the CG (Fig. 3). The signs of decreased 21-hydroxylase activity were found, that is, decreased ratios of (THF+5alpha-THF+THE)/P3, (THF+5alpha-THF+THE)/11-oxo-P3, and (THF+5alpha-THF+THE)/17P compared with the GC. Urinary excretion of 11-oxo-P3 was found to be higher and ratios of (THF+allo-

Table 2 / Таблица 2

Impaired steroid metabolism in normal-weight and obese patients with polycystic ovary syndrome (data obtained using gas chromatography-mass spectrometry)

Нарушения метаболизма стероидов у пациентов с синдромом поликистозных яичников с нормальным весом и ожирением по данным газовой хромато-масс-спектрометрии

Product/substrate ratios	Me (Q_{25}–Q_{75})		
	Control group, *n* = 25	PCOS patients with BMI <25 kg/m², *n* = 30	PCOS patients with BMI >30 kg/m², *n* = 23
Signs of 21-hydroxylase activity			
(THE+THF+allo-THF)/P3	5.3 (3.6–7.4)	2.3 (1.3–2.9)***	4.7 (3.5–7.4)
(THE+THF+allo-THF)/11-oxo-P3	162 (129–203)	79 (39–204)*	306 (159–1190)
(THE+THF+allo-THF)/17P	29.6 (12.4–59.1)	10.9 (6.4–14.9)*	18.5 (14.7–37.5)
Signs of 3beta-hydroxysteroid dehydrogenase activity			
(THE+THF+allo-THF)/DHEA	17.7 (14.5–34.8)	4.9 (2.9–17.0)**	12.4 (5.6–27.9)
(THE+THF+5alpha-THF)/dP3	10.9 (8.5–13.1)	6.7 (5.1–8.8)*	11.8 (7.7–16.6)
Signs of 11beta-hydroxysteroid dehydrogenase activity			
(5beta-THF+5alpha-THF+cortols)/ (5beta-THE+5alpha-THE+cortolones)	0.51 (0.47–0.60)	0.47 (0.32–0.56)	0.44 (0.30–0.56)*
5beta-THF/5beta-THE	0.36 (0.34–0.45)	0.28 (0.23–0.35)**	0.29 (0.22–0.35)*
Signs of 5alpha-reductase activity			
An/Et	1.1 (0.7–1.3)	1.0 (0.7–1.3)	1.5 (1.4–2.4)**
11-OH-An/11-OH-Et	1.4 (1.2–1.5)	2.1 (1.3–3.3)*	4.5 (1.6–6.7)**
5alpha-THF/5beta-THF	0.7 (0.5–1.0)	1.1 (0.9–1.6)**	1.3 (1.1–1.6)**
5alpha-THB/5beta-THB	1.0 (0.7–1.5)	2.2 (1.6–3.3)**	1.8 (1.5–2.7)*

N o t e: * $p < 0.05$, ** $p < 0.01$, *** $p < 0.0001$, significance of differences compared with the CG; THE, tetrahydrocortisone; THF, tetrahydrocortisol; THB, tetrahydrocorticosterone; P3, pregnanetriol; 17P, 17-hydroxypregnanolone; DHEA, dehydroepiandrosterone; dP3, pregnentriol; dP2, pregnendiol; An, androsterone; Et, ethiocholanolone; PCOS, polycystic ovary syndrome; BMI, body mass index.

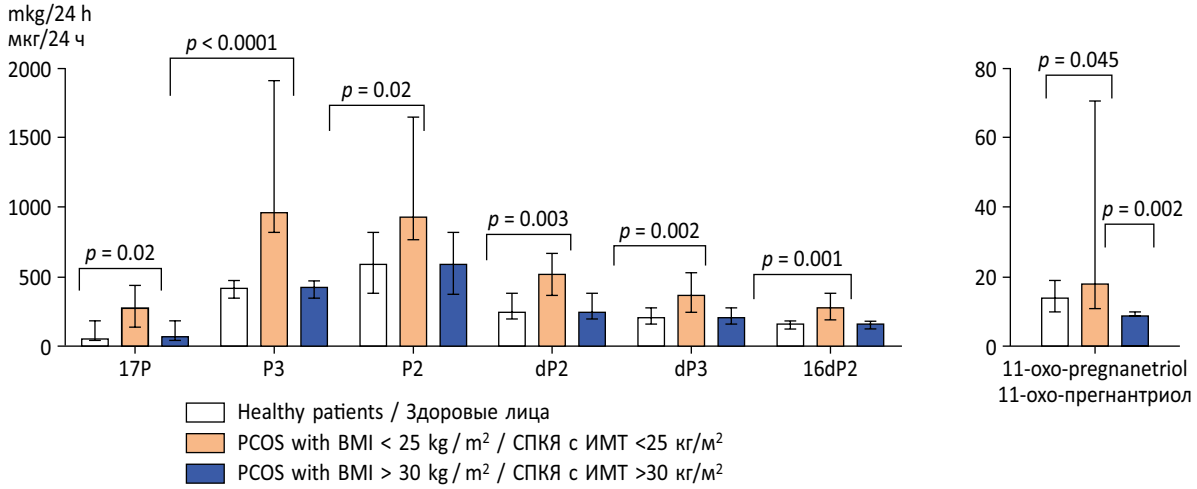

Fig. 3. Urinary excretion of 17-hydroxyprogesterone metabolites and 5-ene-pregnenes in normal weight and obese patients with polycystic ovary syndrome (data obtained using gas chromatography-mass spectrometry). PCOS, polycystic ovary syndrome; BMI, body mass index; 17P, 17-hydroxypregnenolone; P3, pregnanetriol; P2, pregnanediol; dP2, pregnenediol; dP3, pregnenetriol

Рис. 3. Экскреция с мочой метаболитов 17-гидроксипрогестерона и 5-ene-прегненов у пациентов с синдромом поликистозных яичников с нормальным весом и ожирением по данным газовой хромато-масс-спектрометрии: СПКЯ — синдром поликистозных яичников; ИМТ — индекс массы тела; 17P — 17-гидроксипрегнанолон; P3 — прегнантриол; P2 — прегнандиол; dP2 — прегнендиол; dP3 — прегнентриол

THF+THE)/11-oxo-P3 and (THF+allo-THF+THE)/P3 were lower in PCOS and NBW patients compared to those in PCOS and obese patients (see Table 2). Reduced ratios of (THF+5alpha-THF+THE)/DHEA and (THF+5alpha-THF+THE)/dP3 may indicate a decrease in 3beta-hydroxysteroid dehydrogenase activity. In PCOS and obese patients, these values did not differ from those in the CG (see Table 2).

All PCOS patients showed increased ratios of 11-OH-An/11-OH-Et, 5alpha-THF/5beta-THF, and 5alpha-THF/5beta-THF compared to those in CG, which indicates increased 5alpha-reductase activity. The An/Et ratio was increased only in PCOS and obese patients as compared with CG patients and PCOS and normal weight patients, which indicates greater 5alpha-reductase activity in PCOS and obese patients (see Table 2). In PCOS and obese patients, mild-to-moderate hirsutism was observed in 12 women (52.2%), and mild-to-moderate acne was observed in 5 women (21.7%). In PCOS and normal weight patients, mild hirsutism was found in 9 women (30%), and mild acne was reported in 8 women (26.7%). Patients with PCOS and obesity showed more significant signs of androgenic dermopathy, which confirms the higher 5alpha-reductase enzyme activity in this group of patients.

Discussion

PCOS is a heterogeneous disease with a multifactorial etiology characterized by various clinical, endocrine, and metabolic disorders. It is a leading cause of hyperandrogenism in women and a frequent cause of menstrual disorders, chronic anovulation, and infertility [29]. Although the ovaries are the main source of androgens in PCOS, the levels of adrenal androgens, such as dehydroepiandrosterone and dehydroepiandrosterone sulphate, are increased in 20%–30% of patients [30]. More than half of PCOS patients have increased 17-OHP levels, which are a marker of impaired adrenal steroidogenesis in congenital adrenal cortical dysfunction. A large number of women with PCOS have been found to have a mixed pattern of androgen overproduction. The GC-MS results allow the metabolic profiling of steroid hormones to be studied and differences in their metabolism in different PCOS forms to be identified.

When the results were analyzed, it was found that urinary excretion of androstenedione metabolites, DHEA, 17-OHP, and 5-ene pregnenes were increased, and signs of 21-hydroxylase deficiency were revealed in PCOS and NBW patients. Urinary excretion of DHEA metabolites and one An metabolite was increased in PCOS and obese patients.

One of the most important steroidogenic enzymes is 3beta-hydroxysteroid dehydrogenase, which is essential for the conversion of Δ5-steroids (pregnenolone, 17-hydroxypregnenolone, and dehydroepiandrosterone) to their corresponding Δ4-steroids (progesterone, 17-hydroxyprogesterone, and androstenedione). GC-MS data in PCOS and NBW patients in this study showed increased urinary excretion of dehydroepiandrosterone and pregnentriol and decreased ratios of tetrahydroderivatives of cortisol and cortisone to these steroids compared with those in the CG, which indicates 3beta-hydroxysteroid dehydrogenase deficiency. The study findings were consistent with those of several authors [31].

Androgen excess may result in varying degrees of clinical manifestations in women, such as acne, hirsutism, and alopecia. The 5alpha-reductase enzyme, which converts testosterone in the dermis and other androgen-dependent tissues into dihydrotestosterone, that is, the most active androgen, is responsible for the manifestations of androgenic dermopathy. There are two isoforms of 5alpha-reductase: type 1 and type 2 5alpha-reductase (SRD5A1, SRD5A2). Type 1 5alpha-reductase is expressed in the scalp, liver, ovaries, uterus, kidney, and brain, whereas type 2 5alpha-reductase is expressed in the liver and to a lesser extent in the scalp and skin [32]. Severe signs of hyperandrogenic dermopathy are observed in women with high 5alpha-reductase type 1 activity with no other manifestations of hyperandrogenism. The study results of an examination of girls from 1 to 3 years of age in the CG compared with those born to PCOS mothers showed that the latter had an increased 5alpha-reductase activity. These findings confirm a genetic predisposition to hyperandrogenism, which may further lead to the development of PCOS [33].

Increased 5alpha-reductase activity was found from the ratio of urinary excretion of 5alpha-metabolites and 5beta-metabolites of glucocorticoids and androgens in all PCOS patients, but

the degree of this enzyme activity differed between women in different groups. Four signs of increased 5alpha-reductase activity were observed in PCOS and obese patients, while three signs were observed in PCOS patients with a normal BMI, which indicates a different degree of 5alpha-reductase activity in PCOS patients depending on the BMI. Clinical signs of androgenic dermopathy were more significant in PCOS and obese women with more severe hirsutism and acne on the face, back, and chest.

Obesity is regarded as one of the clinical PCOS forms. A large number of steroidogenic enzymes have been identified in visceral and subcutaneous adipose tissue. Visceral adipose tissue contains the type 1 11beta-hydroxysteroid dehydrogenase enzyme (11beta-HSD1), which catalyzes the conversion of biologically inactive cortisone into the most active glucocorticoid hormone, cortisol. Androgen-induced adipose tissue dysfunction is an important PCOS feature. It has previously been found that urinary excretion of 5alpha-tetrahydrocortisone and cortolones was increased, and there were signs of decreased 11beta-HSD1 activity in PCOS and obese women [34, 35]. Similar findings were observed in normal weight patients in this study, but more evidence of decreased 11beta-HSD1 activity in PCOS and obese women was found.

Increased 5alpha-reductase activity or decreased 11beta-HSD1 activity increases cortisol metabolism, which results in a compensatory increase in ACTH secretion and stimulation of adrenal steroidogenesis, which confirms the mixed nature of hyperandrogenism in PCOS women. A direct correlation was found between the degree of abdominal and visceral adipose tissue development and the severity of insulin resistance. Our study showed the highest informative value for diagnosing PCOS in obese patients associated with increased fasting serum INS levels, IGT, and decreased serum SHBG levels.

The use of GC-MS to assess urine steroid profiles provides new opportunities for diagnosing various PCOS manifestations, including its differential diagnosis.

Conclusion

1. GC-MS data in PCOS and nonobese patients showed increased urinary excretion of androstenedione metabolites, dehydroepiandrosterone and its metabolites, 17-hydroxypregnenolone, pregnanetriol and 11-oxo-pregnanetriol, 5-ene-pregnenes, and 21-hydroxylase and 3beta-hydroxysteroid dehydrogenase deficiency. This finding suggests a mixed genesis of hyperandrogenism, while urinary excretion of dehydroepiandrosterone metabolites and one androstenedione metabolite was increased in PCOS and obese women.
2. Four signs of increased 5alpha-reductase activity were observed in PCOS and obese patients, while three signs were observed in PCOS patients with a normal BMI. This finding indicates a different degree of 5alpha-reductase activity in PCOS patients depending on the BMI.
3. Increased urinary excretion of 5alpha-tetrahydrocortisone and cortolones and decreased 11beta-hydroxysteroid dehydrogenase type 1 activity were observed in PCOS patients regardless of the BMI, which indicates functional hypercortisolism.

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