Polycystic ovary syndrome in adolescence

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LIST OF ABBREVIATIONS

PCOS- Polycystic Ovary Syndrome

FOH- Functional Ovarian Hyperandrogenism

LH- Luteinizing Hormone

FSH- Follicle Stimulating Hormone

GnRH- Gonadotropin Releasing Hormone

ACTH- Adrenocorticotropic Hormone

hCG- human Chorionic Gonadotropin

HDL- High Density Cholesterol

BMI- Body Mass Index

SDB- Sleep Disordered Breathing

CAH- Congenital Adrenal Hyperplasia

17-OHP- 17- Hydroxyprogesterone

DHEA- Dihydroepiandrosterone

SHBG- Sex Hormone Binding Globulin

DAST- Dexamethasone Suppression Testing

FAH- Fetal Adrenal Hyperplasia

OCP- Oral Contraceptive Pill

DUB- Dysfunctional Uterine Bleeding

CONTENTS

SUMMARY

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Polycystic ovary syndrome (PCOS) is the most common endocrine disorder in women of reproductive age, a demographic which includes adolescents as well. Accurate data on the prevalence and incidence in the adolescent population is unavailable. This is largely due to the variable nature of this disorder, uncertain etiology and diagnostic challenges that often lead to the late diagnosis of PCOS, typically during adulthood.

The diagnosis of PCOS should be considered in any adolescent female with a main complaint of hirsutism, menstrual irregularity and often followed by obesity. In general these are not rare complaints for adolescent females. There are also long term consequences associated with PCOS, which, in addition to infertility include type 2 diabetes mellitus, metabolic syndrome and possibly cardiovascular disease and endometrial carcinoma.Acanthosis nigricans, treatment-resistant acne, scalp hair loss or hyperhidrosis may alternatively present as the chief complaint, although these features are not always present.

Treatment for an adolescent with PCOS is primarily directed at the major clinical manifestations and complaints. Several treatment options have been developed for each of these and some options address more than one symptom. However, the initial measure is to adopt a healthy lifestyle, with a strong focus on reducing the body weight in obese patients, which, in combination with some other therapeutic procedures, leads to an optimal effect of therapy. It also helps reduce the risk of certain cancers and improve the quality of life.Targeting of the etiologic factor is not yet possible, although pathophysiologic explanation for it remains an area of intense study.

This review focuses on the pathophysiology, presentation and diagnosis of PCOS in adolescence together with its clinical management.

Keywords: Polycystic ovarian syndrome, adolescent, menstrual irregularity, hyperandrogenism, obesity

INTRODUCTION

In 1935, Stein and Leventhal published a case series of seven women with amenorrhea, hirsutism, and bilateral polycystic ovaries, a condition that later came to be known as polycystic ovary syndrome (PCOS). Since its original description, the definition has undergone several revisions [1].

PCOS is now recognized as the most common endocrine disorder in females affecting 5-10% of those of reproductive age [2]. The concept of polycystic ovary syndrome has progressed from primarily a reproductive disorder to largely a metabolic disorder. It has also been expanded from a disorder that presents at menarche at ends at menopause to a disorder that may be present form birth to old age. Characteristics of disorder are high levels of androgens from the ovary, anovulatory cycles and polycystic ovaries on ultrasound. Insulin resistance and obesity are oftenly associated with the syndrome.

Symptoms of hyperandrogenism which include excessive hair growth on the face and body (hirsutism), acne, alopecia as well as irregular or absent periods appear around the onset of puberty. Most women with PCOS have some level of insulin resistance and experience weight gain together with difficulties losing weight that additionally worsens clinical picture. The problem is in recognizing these symptoms on time because they are frequent in adolescent girls without PCOS and can be easily overlooked.It is estimated that PCOS is present in around 25% of adolescent population [3].

Metabolic disturbances which follow PCOS lead tohigher risk for type 2 diabetes mellitus, metabolic syndrome, cardiovascular diseases and endometrial cancer.This is the reason that PCOS has been recognized as a leading health risk among the woman of reproductive age.

Early detection in adolescence could prevent both emotional and financial suffering, as well as prevention of metabolic consequences in the future life of individual.

PATHOPHYSIOLOGY OF PCOS

PCOS is characterized by intraovarian androgen excess, which seems to be responsible for not only the cutaneous manifestations of the syndrome, but also both anovulation and the polycystic appearance of the ovaries. Intraovarian androgen excess stimulates the growth of small follicles but interferes with selection of a dominant follicle [4].The resultant excess of small follicles arrested in development yields the “polycystic” appearance of the ovaries. Historically, PCOS was considered to result from a disorder of hypothalamic-pituitary gonadotropin secretion. More recently, considerable evidence suggests that PCOS is fundamentally a disorder of ovarian steroidogenesis. In addition, there is evidence that the steroidogenic dysfunction is closely associated with a metabolic disorder, a key component of which is insulin resistance.

Abnormal intraovariansteroidogenesis

Recent studies have preferred the concept where the anovulation of PCOS is attributable to intraovarian androgen excess, which arises from functional ovarian hyperandrogenism (FOH) [4,5].The intraovarian level of androgens in FOH is higher than in most adrenal causes of androgen excess.Significantintraovarian androgen excess, stimulate excessive growth of small ovarian follicles while inhibiting follicular maturation necessary for the development of dominant follicle [10]. It also causes thecal and stromal hyperplasia. These androgen effects cause the anovulatory symptoms and the polycystic appearance of the ovaries [11]. Similar ovarian changes are found in poorly controlled classic congenital adrenal hyperplasia, which, thus, cause secondary PCOS.

Eighty percent or more cases of PCOS appear to arise from dysregulation of ovarian androgen secretion. The dysregulation of steroidogenesis is postulated to result from imbalance among the various intrinsic and extrinsic factors involved in the modulation of LH action in the ovary or ACTH action in the adrenal cortex. In the ovary, the major site of dysregulation occurs in theca cell cytochrome P450c17, which has 17-hydroxylase and 17,20lyase activity. The latter enzymatic activity is the rate-limiting step in androgen formation both in the ovaries and adrenal glands [5].

This combination of deficits seems to account for the characteristic hyper-responsiveness of excessive 17-hydroxyprogesterone to GnRH agonist.

Granulosa cells also exhibit steroidogenic dysregulation [5].They are excessively responsive to FSH, mostly at high dosage. The increased responsiveness of the granulosa cells to gonadotropins may be, in part, due to an increase in the percentage of follicles that are recruited to enter the growth cycle and in part because of intrinsic granulosa cell dysfunction [12]. This seems to contribute for the tendency of women with PCOS to develop ovarian hyperstimulation syndrome during fertility treatment. This concept is supported by a proof of persistently abnormal granulosa cell function after long-term suppression of ovarian androgen excess [13].

Abnormal gonadotropin secretion

Excessive luteinizing hormone (LH) relative to follicle-stimulating hormone (FSH) was the first laboratory abnormality identified in classic PCOS. Despite being a common characteristic of PCOS it is not included into current diagnostic criteria. Elevated LH is thought to play a role in the pathogenesis of PCOS by increasing androgen production and secretion by ovarian theca cells [5].

The latest research suggest that excess in LH may result from abnormal sex steroid feedback at the hypothalamic level rather than the cause of androgen excess [6]. This evidence indicates that the modest rise in androgen levels in patients with PCOS paradoxically stimulates LH pulsatility. This is because women with PCOS were less responsive to luteal phase levels of estradiol and progesterone than normal [7]. Further examination showed that antiandrogen treatment normalized the inhibitory effect of progesterone on LH pulse frequency [8].These findings suggest that androgen excess interferes with the hypothalamic inhibitory feedback of female hormones, principally progesterone.

Other lines of evidence also argue against the hypothesis that PCOS is primarily caused by abnormal pituitary function. About half of patients with PCOS, principally obese patients, do not have elevated LH levels or abnormal gonadotropin responses to gonadotropin-releasing hormone (GnRH) agonist testing [6, 9]. Furthermore, about half of PCOS subjects with a documented ovarian source of hyperandrogenism were demonstrated to have normal LH levels and LH responses to a GnRH agonist test, also suggesting that their ovarian dysfunction is independent of LH excess.

Disturbances in insulin secretion

The compensatory, insulin-resistant hyperinsulinism, is an important extrinsic factor in the steroidogenic dysregulation of PCOS [14, 15].There are many evidence to the metabolic effects of insulin excess. Insulin resistance is common in PCOS and is excessive for the degree of adiposity [14-16].Hyperinsulinism tends to account for for the epidermal hyperplasia that causes acanthosis nigricans. Beside contributing to pituitary LH excess, it potentially also affects hypothalamic GnRH release [17]. Clinically all forms of insulin resistance, from rare lipodystrophies to common type 2 diabetes mellitus, are related with PCOS. The evident increase in prevalence in type 1 diabetes mellitus among PCOS women may be explained by relative peripheral hyperinsulinemia [18]. Insulin- lowering treatments particularly improve ovarian function.

A summarymodel of PCOS pathogenesis

The core of PCOS is functional ovarian hyperandrogenism. This can be a reason for all the clinical features that characterize PCOS: hirsutism, anovulation, and polycystic ovaries. The hyperinsulinemia found in about half of cases,seems to act selectively on the ovary, regardless of resistance to the metabolic effects of insulin, so as to both upregulate androgen production in response to LH and stimulate adipogenesis [5].

Secondarily, two vicious cycles of feed-forward effects occur. The modest hyperandrogenemia causes secondary LH elevation by interfering with female hormone negative feedback. In the presence of insulin excess, this LH excess exacerbates the ovarian dysfunction. Insulin-resistant hyperinsulinemia also stimulates adiposity, which in turn aggravates the insulin-resistant state [14].

In most PCOS, the cause of ovarian hyperandrogenism seems to be intrinsic, and this appears to be the case for significant insulin resistance. In the absence of intrinsic ovarian dysfunction,hyperandrogenemia of extra-ovarian origin (adrenal or peripheral sources) is an unusual cause of anovulation and polycystic ovaries.

Several inborn and environmental factors are known that can contribute to ovarian hyperandrogenism and/or insulin resistance. Polycystic ovaries, androgen levels, andinsulin resistance are hereditary traits while environmental factors include obesity and intrauterine androgen exposure[5].

ETIOLOGY

PCOS is a complex genetic disorder which develops in carriers of susceptible genetic variations and as a response to complex environmental influences [20, 21].It seems to be a congenital disorder that is first diagnosable at the onset of puberty.A genetic basis for the disorder has been suggested by familial clustering cases [22].

Two studies propose that polycystic ovaries appear to occur as an autosomal dominant trait [23, 24]. In almost half of sisters of women with PCOS an elevated plasma testosterone level was found. However, only half of these sisters had symptoms of menstrual irregularity. Most adolescent with a polycystic ovary have either a mother with a polycystic ovary, or a father with metabolic syndrome [25].

The widespread concept is that PCOS is the result of one of a number of intrinsic ovarian genetic traits that interact with one or more other congenital or environmental factors to cause dysregulation of steroidogenesis. This is essentially a "two-hit model," that is, a minimum of two interactive factors is required for the disorder to become manifest.

The underlying cause for androgen excess is still unclear. Multiple subclinical steroidogenic defects have been reported to be risk factors for the syndrome[26]. Lately, theca cells were found to retain their capacity for excessive steroidogenesis through many doublings in culture, supporting the intrinsic mechanism [27].

Recently, evidence for a developmental basis for the syndrome has become visible too. Prenatal androgen excess or perinatal insults that cause intrauterine growth retardation may predispose to obesity, insulin resistance, and androgen excess later in life [28, 29].

An increase in insulin resistance sometimes follows the subtle increase in androgen levels that occur in ovulatory women with polycystic ovaries [30]. Accordingly, insulin resistance may develop in parallel with androgen excess. The association of PCOS with insulin resistance [31] and obesity accounts for the predisposition of these women to metabolic syndrome[32] and type 2 diabetes mellitus [33].

CLINICAL FEATURES

The most common complaints in adolescent girls with PCOS are hirsutism and menstrual irregularities, followed by obesity and/or acanthosis nigricans. An abnormal menstrual pattern may be of little concern to the patient, but emphasizing its presence is critical to the diagnosis in adolescents. Some patients have an abnormal degree of inflammatory acne instead of hirsutism, but cutaneous symptoms of hyperandrogenism are not necessarily present in every patient. Although obesity is commonly associated with PCOS, about half of patients are not obese.

Cutaneous manifestations

Hirsutism — Hirsutism is defined clinically as an excessive sexual hair that appears in a male pattern [37]. It is a classical feature of the high androgen levels and is found in about two thirds of cases.Ferriman-Gallwey system, which quantitates the extent of hair growth in the most androgen-sensitive areas, is commonly used for grading of hirsutism as shown in figure 1.The score above 8 is diagnostic of hyperandrogenism[34]. The normal score varies with ethnicity: hirsutism is defined in Asian populations as a score ≥2 to 3, and in Mediterranean populations as a score ≥9 to 10 [35].Sexual hair growth develops throughout puberty to achieve maturity by two years after menarche, at about 15 years of age, based on sparse normative data [36].“Focal hirsutism” (localized areas of hirsutism with a normal score) is a common cosmetic complaint. However, not all hyperandrogenic women are hirsute because their pilosebaceous unit is relatively insensitive to androgens. Some of the young adolescents whose hyperandrogenism has not fully evolved may not be hirsute. On the other side, hirsutism may occur without elevated circulating levels of androgen. That condition is termed “idiopathic hirsutism”. [37].

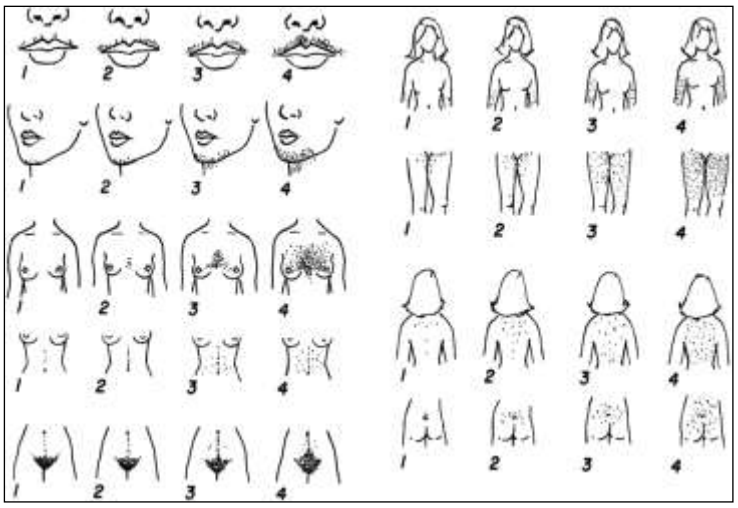

Figure 1. ModifiedFerriman-Gallwey scoring system [34]

Acne — Acne vulgaris is an important cutaneous manifestation of hyperandrogenemia in adolescents [37]. While comedonal acne is common in adolescent girls, less than 5 percent of girls have comedonal acne that is moderate or more (>10 facial lesions) in early puberty, or have moderate or more inflammatory acne through the perimenarcheal years [38]. In such adolescentsthe possibility of hyperandrogenism should be considered,as well as for those with acne that are persistent and poorly responsive to topical dermatologic therapy. These patients are often treated with hormonal therapy for their acne which masks hyperandrogenemia [39].

Other cutaneous manifestations

Balding is an unusual manifestation of hyperandrogenemia in adolescents.It may be either male-pattern (affecting the fronto-temporo-occipital scalp) or female-pattern (affecting the crown, typically manifesting early as a midline part widened in a "Christmas tree" pattern) [37]. Other cutaneous signs of androgen excess are seborrhea, hyperhidrosis, and hidradenitis suppurativa.

Menstrual irregularities

Anovulation- The distinction between abnormal and physiologic anovulation is frequently delayed because patients, families, and clinicians are often unsure about

the normal range of menstrual cycle variation in adolescents. We will mention here some menstrual dysfunctions that suggest abnormal degrees of anovulation in adolescence [40].

Primary amenorrhea is defined as lack of menarche by 15 years of age, or more than three years after the onset of breast development [40].

Secondary amenorrhea is defined as absence of menses for at least 90days (3 months), after previously menstruating [40].

Oligomenorrhea is defined as an infrequent or very light menstruation that occurs at intervals over 40 days (fewer than 9 periods yearly) as an adult criterion. The criteria changes based on the post menarcheal years [40].

Dysfunctional uterine bleeding (DUB) or anovulatory abnormal uterine bleeding is defined as irregular (more frequently than every 21 days) or excessive (bleeding lasting more than seven days) vaginal bleeding [40].

In one study based among school population, 51 percent of girls who became oligomenorrheic at 15 years of age after initially menstruating regularly remained so at 18 years; no data exist for intervening intervals [41]. Studies of healthy schoolgirls have shown that menstrual irregularity [41,42] and above-average androgen levels are significant risk factors for PCOS or infertility in adulthood.

Polycystic ovaries- Polycystic ovaries themselves have little clinical manifestation in adolescence other than their relationship to anovulation. Among the reasons for that is the use of abdominal route to image ovaries by ultrasound rather than the vaginal one. The absence of polycystic ovaries in adolescence may also relate to the fact that ovarian dysfunction may not be demonstrable until 3 years after menarche [43].

Additional features - Obesity and clinical manifestations of insulin resistance are strongly associated with PCOS but are not included into diagnostic criteria. The clinical manifestations of insulin resistance include acanthosis nigricans, metabolic syndrome, sleep-disordered breathing, and nonalcoholic fatty liver disease. Insulin resistance is an important factor in the pathogenesis of PCOS because it has a role in dysregulated steroidogenesis and excessive androgen production.

Obesity -Obesity is present in approximately half of patients with PCOS (prevalence varies from 30 to 75 percent) [33]. It is often the initial complaint beginning usually in

midchildhoodand is emphasizedduring puberty. PCOS is the most common obesity-related endocrine syndrome in females, though the possibility exists that the relationship of PCOS to obesity is due to referral bias [44]. It is typically android in type (central adiposity defined by a waist circumference ≥88 cm) after sexual maturity is reached [25]. Whether the insulin resistance of PCOS is more fundamentally related to central obesity than global adiposity is controversial [46].

Clinical manifestations of insulin resistance

The metabolic disturbances typical of adult women with PCOS are also found adolescents with PCOS.

Adolescents with PCOS are at increased risk for glucose intolerance, suggesting that the insulin resistance is related to the pancreatic beta-cell dysfunction that is seen in type 2 diabetes mellitus [47]. Glucose tolerance progressively deteriorates over time: approximately 10 percent of women with PCOS will have type 2 diabetes mellitus by 40 years of age [33]. American Diabetic Association (ADA) and International Diabetic Federation recognized PCOS as nonmodifible risk factor for type II diabetes in 2004.[19].

Metabolic syndrome – The metabolic syndrome results from the interaction of insulin resistance with obesity and age. It refers to the co-occurrence of metabolic risk factors for type 2 diabetes mellitus and cardiovascular disease, including abdominal obesity, hyperglycemia, elevated triglycerides, low HDL cholesterol, and hypertension. Three or more of these findings confer a high risk of cardiovascular disease in adults [48]. There is not yet consensus about the critical levels necessary for the diagnosis in adolescents.

Several interesting studies have been conveyed about connection of PCOS and metabolic syndrome.In the National Health and Nutrition Examination Survey (NHANES) report (1999 to 2000), the prevalence of the metabolic syndrome (as defined by the National Cholesterol Education Program/Adult Treatment Panel [ATP] III criteria) in normal women ages 20 to 39 years was approximately 18 to 19 percent [25].Using the same diagnostic criteria, Rossi et al. observed that, the prevalence appears to be much higher in women with PCOS: 43 percent of PCOS patients had

the metabolic syndrome, almost twofold higher than that of age-matched women in the general population, as reported by NHANES III [50].

A few studies among adolescents with PCOS have shown that 25 percent of them have metabolic syndrome which is approximately threefold higher than expected for age, ethnicity or BMI in the general population[25, 45].On contrary to those studies, one research found that 43 adolescents with PCOS(half of them obese) had similar rates of metabolic syndrome as compared with 31 control girls who were matched for weight and ethnicity but where somewhat younger than the group with PCOS [50]. Nevertheless, the adolescents with PCOS had evidence of significantly more insulin resistance.

Sleep-disordered breathing – Sleep disordered breathing (SDB) is another feature found in adolescents with PCOS and it is usually connected with metabolic syndrome. In a series of mildly obese adolescents, patients with PCOS had significantly longer sleep latency, poorer sleep efficiency, and lower percentage of REM sleep as compared with BMI-matched controls, although the prevalence of polysomnographically-determined SDB was similar [51]. A separate series of 103 somewhat older predominantly Hispanic-African American adolescents with PCOS had approximately twice the prevalence of questionnaire-determined SDB as compared with 90 BMI-matched controls (45 versus 27 percent) [52]. Among the group with PCOS, BMI was greater in those with SDB, and metabolic syndrome was a significant independent predictor of SDB [52]. Among a subset of these obese PCOS patients who underwent a sleep study, the prevalence of polysomnographically-determined SDB was significantly greater than in controls matched for age and BMI (71 versus 41 percent) and the prevalence of metabolic syndromewas greater. (56 versus 8 %) [52].

DIFFERENTIAL DIAGNOSIS

Making a correct diagnosis of PCOS in adolescence may be a difficult task. Symptoms such as hirsutism, acne, menstrual irregularities and obesity are common during normal puberty. Three fourths of adolescent girls develop acne, and one quarter have inflammatory acne [53]. Similar scenario applies for irregular menstrual cycles which are anovulatory in half of cases during the first two postmenarchal years [75]. Although PCOS accounts for around 85 percent of androgen excess in

adolescent girls, many conditions other than PCOS present with hyperandrogenism. Pelvic ultrasonography and specific endocrine tests are used to distinguish among the many causes of hyperandrogenism that will be reviewed below.

Virilizing congenital adrenal hyperplasia- Congenital adrenal hyperplasia (CAH) is an autosomal recessive disease with deficiency in the activity of an adrenocortical enzyme step necessary for corticosteroid biosynthesis.

Nonclassic ("late-onset") CAH caused by mild deficiency of 21- hydroxylase is the second most common cause of androgen excess in adolescence. It accounts for 2.5 to 5 percent of cases of hyperandrogenism in most populations [54]. Compared to classic CAH, this one is only mildly hyperandrogenic and lacks the genital ambiguity. Usually it presents with premature pubarche (appearance of sexual hair), adolescent-or- adult onset hirsutism, and/or symptoms of anovulation. Affected females may have polycystic ovaries and elevated serum luteinizing hormone (LH) levels. Diagnosis is suggested by elevated levels of serum 17-hydroxyprogesterone (17-OHP). CAH responds to therapy with glucocorticoid replacement [28].

Classic CAHdue to 21-hydroxylase deficiency is the most well-known form of CAH. It is almost always diagnosed during infancy, presenting with genital ambiguity due to congenital virilization of affected females, and may be associated with a salt-losing crisis. Individuals may develop signs and symptoms resembling PCOS during adolescence, especially if their disorder is poorly controlled by glucocorticoid therapy. Symptoms may include menstrual irregularity, hirsutism, and clitoromegaly. Polycystic ovaries can result from the direct effects of virilizingextraovarian androgen excess and from adrenal rests of the ovaries (ectopic adrenal tissue in the ovaries)[28]. Adrenal progesterone excess may be sufficient in the absence of androgen excess to cause ovarian dysfunction by inhibiting LH pulsatility [55].

Even in patients who are well-controlled on glucocorticoid therapy, classic CAH and, to a small extent, nonclassic CAH, are associated with a PCOS-type of functional ovarian hyperandrogenism that is responsible for persistent menstrual irregularity[28].

Virilizing tumors – Virilizing tumors of the adrenals or ovaries are a rare cause of hyperandrogenismand occur in only 0,2% of cases [56]. However, it should be

emphasized thatmore than half are malignant. These tumors cause rapid onset of virilizing symptoms, including hirsutism, temporal hair recession, increased muscle bulk, voice deepening, and onset of clitoromegaly without genital ambiguity. Other tumor manifestations may include Cushingoid changes and abdominal or pelvic masses. Acanthosis nigricans may occur with virilizing tumors, though it is an uncommon feature and more suggestive of PCOS [57].However, rapid onset of virilizing symptoms or high serum levels of androgens (total testosterone > 5 nmol/L) require thorough examination to exclude virilizing tumor.

Insulin-resistance disorders – Extreme states of insulin-resistant hyperinsulinemia, such as congenital diabetes mellitus caused by insulin-receptor mutations (eg, Donohue‘s syndrome or leprechaunism) or lipodystrophy, are accompanied by PCOS[58].Less extreme, but still severe, insulin resistance is also linked with PCOS in the setting of pseudo-Cushing’s syndrome and pseudo-acromegalic gigantism, disorders which mimic glucocorticoid excess and childhood growth hormone excess clinically, without excess production of these hormones. In these disorders, the symptoms of insulin resistance often precede the PCOS [59].

In addition, mild forms of insulin resistance also are associated with PCOS, including type 1[60] and type 2 diabetes mellitus [61]. Elevated insulin levels seem to promotePCOS by increasing the activity of steroidogenic enzymes in the ovaries and adrenal glands, similarly to insulin-like growth factor-I (IGF-I).

Drugs – Anabolic steroids cause virilization in women and may havecharacteristics similar to those of virilizing tumors. Virilizing amounts of endogenous androgens [28] or exogenous androgens [62] can also cause polycystic ovaries. Valproic acid directly enhances the transcription of the steroidogenic gene *CYP17* that encodes cytochrome P450c17 and can cause elevated serum testosterone [63].

In addition to aforementioned ones, diseases that also sometimes mimic PCOS are Hyperprolactinemia, Cushings syndrome [64] and acromegaly [65].

DIAGNOSTIC CRITERIA

In the absence of clear etiology and pathogenesis, the diagnosis of PCOS is based on consensus about diagnostic criteria.At the National institutes of Health(NIH) consensus conference held in 1990, diagnosing of PCOS was defined as chronic anovulation with clinical and/or biochemical hyperandrogenism, with exclusion of other mimicking etiologies, such as thyroid and adrenal dysfunction. [66] In 2003, the Rotterdam European Society for Human Reproduction (ESHRE) / American Society of Reproductive Medicine (ASRM) consensus workshop group proposed that diagnosis of PCOS include two of the following three criteria: oligo-and/or anovulation, clinical and /or biochemical hyperandrogenism, and polycystic ovaries on ultrasound; other etiologies must be excluded [67]. The Rotterdam definition extended the diagnosis of PCOS by adding the polycystic ovaries criterion. Women with oligo-ovulation and polycystic ovaries (nonhyperandrogenic) as well as women with hyperandrogenism and polycystic ovaries (ovulatory) could be diagnosed with this new criteria compared to narrower NIH criteria. In 2009, The Androgen Excess and PCOS (AE-PCOS) Society published report emphasizing that PCOS is primarily a hyperandrogenic disorder and suggest revising the definition to hyperandrogenism and ovarian dysfunction, thereby involving the Rotterdam ultrasound criteria but requiring hyperandrogenism for the diagnosis [68].In adolescent patients, the criteria mentioned above present a particular diagnostic problem because characteristics of normal puberty often overlap with signs and symptoms of PCOS.

Carmina and colleagues suggested that during adolescence, a definitive diagnosis of PCOS should require all elements of Rotterdam criteria andnot just 2 out of 3 [69]. The criteria proposed by these authors are stricter than their adult counterparts and may limit inappropriate early diagnosis but we suggest reevaluating the diagnosis every 6-12 months. Rotterdam diagnostic criteria is used in majority of the world countries as well as in Croatia and proposed strict criteria for diagnosing PCOS in adolescents is presented in Table 1.[3].

Table 1.Suggested criteria for diagnosis of PCOS in adolescents [69].

Criteria	Oligoanovulation	Hyperandrogenism	PCO-US
Dg PCOS	+	+	+
Dg PCOS possible, not confirmed	+	+	-
Dg PCOS not possible during adolescense	+	-	+
Dg PCOS not possible during adolescense	-	+	+
Not PCOS	-	+	-
Not PCOS	-	-	+

Several steps that should be done for the evaluation of PCOS together with importance ofdiagnosing PCOS in adolescence will be discussed next. Before starting our diagnostic approach we need to determine who should be screened, then define the presence of androgen excess, rule out disorders that mimic PCOS; and finally determine the source of androgen excess.

Screening for polycystic ovary syndrome

Screening for PCOS in adolescent girls is recommended if they fulfill one or more of the following characteristics: hirsutism (or hirsutism equivalent such as acne vulgaris, poorly responsive to topical therapies), menstrual irregularities(amenorrhea, oligomenorrhea or abnormal uterine bleeding) or obesity.The history and physical examination should start with assessment of the clinical symptoms and signs suggestive of polycystic ovary syndrome and for disorders that can mimic it[70]. The cutaneous manifestations that can be observed such as hirsutism, acne and other equivalents provide clinical evidence of hyperandrogenism.While mild hirsutism is often idiopathic, its connection with menstrual irregularities and obesity suggest androgen excess. The degree and distribution of sexual hair growth should be identified (eg, using the Ferriman-Gallwey score) and interpreted in the context of norms for the patient's age and ethnicity.The history should also explore whether the patient shaves excess hair or uses depilatory agents, which may dim the physical findings, and whether the patient is taking medications that cause hirsutism (eg, anabolic steroids). The degree of acne should be evaluated in the context of the patient gynecological age. Possibility of hyperandrogenism is suggested by moderate or more inflammatory (>10 facial lesions) acne through the perimenarcheal years [53]. Acne that is persistent and poorly responsive to topical dermatologic therapy is an alternative indication for evaluation of PCOS.

Furthermore, failure to establish a normal adult menstrual cycles by 2 years after menarchecarries a greater than two third risk of persistent oligo-ovulation [89].Recommendation is to start the work up at this time or sooner if the other signs of PCOS coexist. Although the problem of obesity is unfortunately becoming common among adolescents, the presence of acanthosis nigricans or a family history of metabolic syndrome or type 2 diabetes mellitus is also an indicator for work-up for PCOS [70].

Defining the presence of androgen excess

The next step in diagnosing PCOS is to show an excess of androgen. Although the diagnostic criteria for hyperandrogenism can be based on clinical findings, biochemical testing establishes diagnosis more securely. This is because only about half cases of mild hirsutism are associated with hyperandrogenemia [34]. Moreover, Rosenfield and group of authorsdescribed the best laboratory choices for the

evidence of hyperandrogenism. They recommend initiating workup with an androgen panel of plasma total testosterone, free testosterone and DHEA sulfate [70]. The interpretation of results is sometimes complicated because some laboratories provide broad normal ranges for total testosterone. Results by the best current assays vary by an average of approximately 6 to 26 percent [9]. Reason for that lies in inaccurate commercial direct assays and another reason is that the general population includes apparently normal women with unrecognized androgen excess [71].

An elevation of plasma free testosterone is the best single most sensitive indicator of androgen excess [72]. The combination of an upper- normal total testosterone and a lower- normal sex hormone-binding globulin(SHBG) gives a high free testosterone concentration. The serum free testosterone concentration is about 50 percent higher in hirsute women than the total testosterone concentration. This is because they have relatively low level of SHBG, which is the main determinant of the fraction of serum free testosterone. SHBG production by the liver is raised by estrogen and suppressed by hyperinsulinemia of insulin resistance and by androgen excess itself [73]. Disadvantage of assaying free testosterone is that there is no uniform laboratory standard, so test specific normal ranges differ. The most reliable methods report free testosterone that is calculated as the product of total testosterone and a function of sex hormone binding globulin [74].Direct tests of the free testosterone serum concentration are inaccurate and should be avoided.

The Androgen Excess-PCOS Society (AES) also recommends androgen measurement before beginning hormonal therapy for hirsutism [35].

Ultrasonography

Performing an ultrasound for diagnosing PCOS in adolescence presents special difficulties. First, ovarian appearance and volume may vary during adolescence; it has been reported that ovaries can develop a polycystic morphology over time, and enlarged ovaries with a polycystic appearance can subsequently become normal in size[76]. Second, transabdominal approach is standard and appropriate method in virginal adolescent girls. Although this approach helps in screening of adrenal mass, it tends to underestimate the prevalence of polycystic ovaries in comparison with transvaginal approach used in adult women. That is particularly limited in overweight and obese individuals.

In practice it differs whether ultrasonography should be performed for all girls with hyperandrogenemia. Some experts perform adrenal and ovarian ultrasonography for all patients with anovulatory symptoms and documented hyperandrogenemia, primarily to exclude rare but serious androgen-producing tumors.Some other experts perform it only for selected patients with features that are atypical for PCOS, such as very high testosterone levels (eg,>5nmol/L), clitoromegaly, or rapidly progressive hirsutism [77].

The primary purpose of ultrasonography is to exclude the rare but serious adrenal or ovarian tumor and ovarian pathology not related to PCOS. Other pelvic pathology such as ovotesticular disorder of sex development and the functional hyperandrogenism of pregnancy can also be detected by ultrasonography [78]. Girls who have an ultrasound that shows an ovarian tumor or other explanation for hyperandrogenism should be referred for further evaluation and treatment of the underlying disorder. Otherwise, hyperandrogenic girls need further endocrine studies irrespective of whether the ovaries are polycystic.

A secondary purpose of ultrasonography is to determine whether the ovaries are polycystic. In adolescents, the high frequency of polycystic-appearing ovaries makes this an unreliable criterion for the diagnosis of PCOS.

Exclusion of other disorders that mimic polycystic ovary syndrome

According to Rotterdam criteria, polycystic ovary syndrome is a diagnosed after exclusion of diseases that mimic the syndrome [67]. Therefore, the diagnosis of PCOS is made in hyperandrogenic adolescents who have anovulatory symptoms or polycystic ovaries but after we exclude other disorders with androgen excess.

For practical purposes the endocrine screening panel and an ultrasonography examination are often performed at the same time as the initial testing for hyperandrogenemia, especially in patients with high clinical suspicion for PCOS. (eg, oligomenorrheic girls with moderate or severe hirsutism).

This screening evaluation excludes most of the non-PCOS causes of hyperandrogenism and is consistent with the American College of Obstetricians and Gynecologists (ACOG) and Endocrine Society guidelines for the diagnosis of PCOS [75]. According to the guidelines for adults, the blood sample is used to for detection

of major mimicking disorders such as: congenital adrenal hyperplasia, Cushing syndrome, hyperprolactinemia, thyroid dysfunction and acromegaly [67-68, 75].In adolescents such guidelines do not exist because these disorders are less likely to occur than in adults.

Therefore, the actual range of practice varies considerably among expert clinicians in this field. Some of them perform full range of screening tests for all adolescent patients with suspected PCOS, while some other experts select among tests based on clinical symptoms and signs that raise concerns about for a particular disorder.In the absence of a cost-effectiveness analysis, the optimal approach to the workup seems to be an individualized one.

The most clinically relevant of these simple screening tests, along with the free testosterone at the initial evaluation for hyperandrogenemia includes a serum cortisol since so many patients have central obesity. Initial evaluation of patients with anovulatory symptoms ordinarily also includes serum luteinizing hormone (LH), follicle-stimulating hormone (FSH), and estradiol, as well as history about diet. Pregnancy should be excluded in amenorrheic patients.

Determining the source of androgen excess

American association of Clinical Endocrinologists guidelines (2001) recommended a complete evaluation of hyperandrogenism to determine the source of androgen in PCOS and rule out rare congenital adrenal disorders, because the treatment and prognosis differ [79]. The authors found that combination of dexamethasone suppression testing (DAST) and cosyntropintesting, will give a diagnosis of FOH or FAH in most patients.

Dexamethasone is given in dosage of 0.5mg four times daily for 4 days, and the following morning after a final dose, free testosterone (alternatively 17-hydroxyprogesterone), DHEA sulfate, and cortisol are measured. Dexamethasone suppression of ACTH- dependent adrenal function normally causes plasma cortisol to fall below 1.5mcg/dl, DHEA sulfate to fall by 75% to below 80mcg/dl, and total testosterone to fall below 35 ng/dl [80].

The DAST is interpreted on the following way: If testosterone excess is not suppressed by dexamethasone, but cortisol and DHEAS are suppressed normally, then this is typical diagnosis of PCOS. Virilizing tumors and adrenal rests must be considered in the presence of suggestive clinical factors.

If neither androgen (testosterone and DHEA) excess nor corticoids are suppressed normally by dexamethasone, then the androgen excess may be secondary to Cushing syndrome, defective cortisol metabolism or noncompliance with taking dexamethasone.

If both androgen and corticoids suppression are normal, an ovarian source for androgen is unlikely. In that case,cosyntropin stimulation testing is recommended to assess 17-hydroxyprogesterone and other steroids intermediates. A clear diagnosis of non-classic congenital adrenal hyperplasia requires a steroid peak that is more than five standard deviations above average [70]. Early morning sampling is critical to detect the 17-OHP elevation of non-classic CAH because it wanes rapidly thereafter due to the diurnal variation of adrenal steroid secretion. Mildly elevated or normal responses to cosyntropin are consistent with FAH or idiopathic hyperandrogenism.

Additional evaluation of PCOS patients

Once a diagnosis of polycystic ovary syndrome (PCOS) has been established, the possibility of insulin resistance manifestations and quality of life issues should be considered. As already mentioned in paragraph with clinical features, PCOS is a risk factor for the early development of type 2 diabetes mellitus, metabolic syndrome, and their associated risks for sleep-disordered breathing, and possibly cardiovascular disease [81]. The proposed screening of PCOS population is summarized in Table 2.

Table 2. Screening of PCOS population for metabolic abnormalities [3].

BMI (Body mass index) kg/m2	• **Every visit**
Waist to hip ratio (WHR)	• **Every visit**
RR	• **1x year in BMI <25 kg/m2** • **Every visit BMI >25 kg/m2**
Lipidogram	• **Every 2 years BMI< 25 kg/m2** • **Every year in BMI> 25 kg/m2**
OGTT	• **Every 2 years in BMI< 25 kg/m2** • **Every year in BMI> 25 kg/m2**
INZULIN 0 i 120 min	• **HOMA- IR > 2.5** • **HOMA- IR = insulin x GUK / 22.5**

Parents and siblings of PCOS patients are also at increased risk for metabolic syndrome and diabetes mellitus, particularly if they are obese. So screening is recommended for family members of both sexes. That can be realized by measurement of hemoglobin A1c or oral glucose tolerance testing [33].

THERAPY

The question on which majority of the patients are waiting the answer for, after being diagnosed with PCOS sounds: “How can we treat it?” To answer on that particular question we need to make the clear goal of our therapy. Is that the establishment of a regular menstrual period or reducing in formation of androgen with the improvement in cosmetic problems? Are we planning to induce an ovulation, prevent the action of unopposed estrogen on the endometrium or we want to decrease hyperinsulinemia with the aim in avoidance of long term cardiovascular and metabolic consequences.

The universal drug and scheme for management of all the symptoms of PCOS do not exist, so individualized approach is used. It depends on the patient age, symptoms, risks and wishes for the future [70].

What should be unique as a first line therapy in obese individuals is a lifestyle modification because weight itself contributes to the worsening of all the other PCOS symptoms.On the other side, loss of only 5% of body mass leads to better cycle regulation and spontaneous ovulation. Commonly, combination of oral contraceptive pills (OCP) is used for the treatment in adolescents when there are expressed menstrual cycle disturbances, acne and mild hirsutism. (becausethey correct both menstrual abnormalities and hyperandrogenemia). In cases of severe hirsutism antiandrogen therapy is added to combination OCPs. Metformin is used as the therapy of choice if the abnormal glucose tolerance or lipid abnormalities cannot be solved just by loosing of weight.

Treatment of hirsutism and acne

The cornerstone of treatment of hirsutism and removal of excess hair are numerous cosmetic measures. Some of the most commonly used are shaving, depilation, bleaching and waxing techniques. They are often efficacious, relatively inexpensive and in general safe. Additional more expensive measures are for example:eflornithine hydrochloride cream (topical agent that inhibits the local hair growth) and laser therapy or electrolysis (thermal destruction of dermal papilla). If these measures are not satisfactory enough for the patient, then options are physical therapy, hormonal therapy or combination of the two.The decision among these options involves patient preference including cost of the measure, tolerance of discomfort/pain, risk of complications, and outcome [70].

Combination oral contraceptive pills

The combination oral contraceptive pills (OCPs), which contain both estrogen and progestin, are the first line endocrine treatment for women with dermatologic and menstrual abnormalities of PCOS. This combination suppresses hypothalamic-pituitary-ovarian axis and reduces excess androgen production by the ovary [37]. Result of that is improvement in menstrual irregularity, hirsutism and acne. Progestin component also inhibits endometrial proliferation, preventing hyperplasia and the

associated risk of endometrial carcinoma [90]. Those with larger estrogen doses may be used in larger women to provide menstrual regularity. Endocrine Society guidelines suggest OCPs as first-line pharmacologic therapy of menstrual irregularity [82] and hirsutism [34].

In general, it is suggested to choose OCPs that contain at least 30 μg ethinyl estradiol. Those OCPs containing ≤20 μg ethinyl estradiol pose lesser cardiovasular risk, but may inadequately promote normal growth of bone mass and may be less effective in controlling irregular menstrual bleeding, especially in obese hyperandrogenic girls [83]. There has been concern that OCPs may cause harmful metabolic effect in women with PCOS compared to the one without it [84]. Although consistent clinically significant differences in metabolic effects have not been found among commonly used OCPs [85]. The improvement effects on hirsutism and acne is primarily due to estrogenic component, and any differences in the androgenicity of the progestin are minimal at these doses. The choice of the most adequate agent depends on the clinical picture of the individual and cost/insurance considerations.

OCPs therapy usually normalizes androgen levels within 18-21 days. It is recommended to recheck patient serum free testosterone three months after beginning of therapy to assess the efficacy of treatment and normalization of androgen level. Androgen suppression is supportive of diagnosis, but not diagnostic [68].

Antiandrogens

Antiandrogens are used as a therapy for significant improvement of hirsutism. They act as competitive antagonists of steroid binding to the androgen receptor and inhibit the androgen- induced transformation of vellus to terminal hairs [37]. Therefore, the effects of these agents are not valued for 9 to 12 months due to long growth cycles of sexual hair follicles. All antiandrogens should be administered with oral contraceptives. The reason for that is their potential teratogenic feminization of the male fetus and they may cause menstrual disturbance. Antiandrogens also have an uncertain effect on metabolic abnormalities associated with PCOS [89].

Spironolactone, mostly known as a weak diuretic, is the strongest antiandrogen available. Beside itsantiandrogenic and antimineralcorticoid effect, it is also a weak progestin and a weak glucocorticoid. The recommendation is to start by 100mg twice daily until achieving a maximal effect and then reducing the dose to 50 mg twice a day for maintenance therapy. Spironolactone administered in these doses is well tolerated, with exception of hyperkalemia that may limit its usefulness in certain patients, so electrolytes should be monitored. It is contraindicated in women with adrenal, hepatic and renal failure [69].

Treatment of menstrual irregularity

Menstrual irregularities should be controlled in patients with PCOS, because chronic anovulation is associated with increased risk of developing endometrial hyperplasia and carcinoma. Major treatment options for menstrual irregularity include progestin and OCPs.

Cyclic progestin can be used alone for treatment of menstrual irregularities in sexually mature adolescents. It relies on direct inhibitory effects on endometrial proliferation. Dose of micronized progesterone is 100-200 mg daily at bedtime for 7-10 days out of each month or cycle. In most patients it will induce withdrawal bleeding, but some will not respond, probably because of an antiestrogenic effect of androgen excess on the endometrium[90]. Unlike OCPs, progestin therapy causes transient reduction of androgen excess but does not normalize it, what is generally insufficient to expect improvement in hirsutism [91]. Side effects of progestin include mood symptoms (depression), bloating and breast soreness. Patients must be also informed that oral progestin dosed in this way is not a method of contraception.

Dysfunctional uterine bleeding (DUB) usually can be treated with cyclic progestin, but heavy DUB may require adding of estrogen. Treatment of heavy menstrual bleeding is similar in adolescents with or without PCOS. The estrogen doses required to treat heavy DUB may be three- to fourfold higher than the doses needed to treat irregular menses [92]. Estrogen can be given as OCPs, one tablet three to four times daily for 7 days. Treatment is then stopped for 5 days, and patient should be warned that heavy withdrawal bleeding may occur. Usage of OCP with relatively large estrogenic component is preferred. Once active bleeding is controlled, therapy with cyclic OCP or progestin should be started to prevent recurrence of DUB.

Several potential limitations exist in the management of PCOS in adolescents by OCPs. Combination OCPs have about fourfold risk of venous thromboembolism in first-time users [93].Risk is related to the dose and duration of estrogen use, and to use of recent- generation of progestins which carry about a twofold independent risk [94].In patients with migraine headaches, OCPs should be used for caution and in the lowest possible dose [93]. Another situation where OCPs are contraindicated is in perimenarcheal girls with short stature and whose epiphysis are still opened determined by radiographic imaging. OCPs contain growth- inhibitory amounts of estrogen so they will bring growth of those girls to an end.For most women with PCOS, the benefits of oral contraceptives outweigh the potential risks of venous thromboembolism [70].

Treatment of obesity and insulin resistance

It has been proven that low glycemic index diet leads to correction of hormonal and metabolic parameters resulting in more regular cycles and spontaneous ovulations. Considering that patients with PCOS are prone of gaining extra weight, it is of utmost importance to counsel them about healthy nutrition and low calorie intake [88].

The first line therapy for obese adolescents with or without diagnosis of PCOS is healthy diet and exercises [82]. Lifestyle modification improves menstrual frequency, ovulation and androgen excess. About half of obese patients who managed to lose weight had improvement of their symptoms [95].

Insulin lowering drugs, metformin and thiazolidinediones are used as adjuncts for treating of PCOS. Metformin acts to inhibit hepatic glucose productionand decrease peripheral insulin resistance. Thiazolidinediones increase insulin sensitivity by promoting fat mobilization from the bloodstream. Both of these drugs stimulate ovulation and lower androgen levels. However, these agents are not likely to improve hirsutism and normalize androgen levels that are greater than two times normal [87].

Metformin has the most usefulness in treating adolescents with advanced risk of developing PCOS (high risk ethnicity, obesity, strong family history, acanthosis nigricans), because it decreases appetite and improves weight loss although to moderate degree [96].Therapy should be started with 500 mg daily before the

evening meal, with an increase in the dose by 500mg per week to the maximal dose of 1500-2000mg daily as tolerated [97].Result of treatment cannot be expected before three months.Individual that is on metformin therapy is advised to obtain comprehensive metabolic panel as a baseline because of rare complications of lactic acidosis [98].Due to that complication it is contraindicated in patients with impaired hepatic or renal function, alcoholism as well as cardiopulmonary insufficiency.

Many questions remain unanswered about the use of metformin in adolescents. Duration of treatment and the primary indication(hyperinsulinemia vs. hyperandrogenemia vs. oligomenorrhea) blurs treatment guidelines.

Multidisciplinary approach to PCOS

There are many aspects to evaluating and treating the adolescents with PCOS, such as normalizing nutrition, regulating menses, addressing future concerns (e.g. infertility, endometrial cancer), screening for metabolic consequences, behavioral counseling and modification(low self- esteem, depression, anxiety). Because all of the mentioned requires a lot of knowledge, time and patience to deal with, a multidisciplinary adolescent PCOS clinic should be established.

CONCLUSION

PCOS is a serious disease with severe consequences. The formation of uniform guidelines is of a vital importance for the sake of establishing a clear diagnosis, treatment plan and follow up care. Irregular menses, anovulatory cycles, acne and obesity are fairly common during adolescence.Therefore, making a correct diagnosis in adolescent is more difficult than in adult women as the main symptoms of PCOS often overlap with the physiological changes that occur during puberty. With the rising prevalence of childhood obesity, the early identification and treatment of PCOS is of significant importance. Current treatment options target the specific symptoms of PCOS but should also include patient goals and preferences.Regular exercise, balanced diet and healthy way of living are of the main targets, especially in adolescents who have a better opportunity to establish and maintain a healthy lifestyle than their adult counterparts.The importance of even minor weight loss in obese individuals and the benefits that it has on cycle regulation and the return of spontaneous ovulation must have particular emphasis placed upon it. Cosmetic measures, hormonal contraceptives and antiandrogens are also commonly used.

Primary prevention of long term consequences connected with PCOS such as diabetes mellitus, metabolic syndrome and cardiovascular disease can also be achieved by lifestyle modifications. Besides the advantage of treating distressing symptoms, early intervention aims to improve self- esteem and quality of life as a whole. Educating adolescents about PCOS and its risks, as well as providing emotional support, allows the patient to make informed choices in favor of establishing and maintaining a healthy lifestyle.

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REFERENCES

1. Hsu Roe A, Dokras A. The diagnosis of Polycystic Ovary Syndrome in Adolescents.Rev Obstet Gynecol. 2011; 4:45-51
2. Azziz R, Woods KS, Reyna R, et al. The prevalence and features of the polycystic ovary syndrome in an unselected population. J Clin Endocrinol Metab 2004; 89:2745.
3. Šimunić V. Sindrom policističnih jajnika. Kliničke smjernice. Konsenzus s 2. Hrvatskog simpozija o policističnim jajnicima. Zagreb, Foto Soft, 2006. Str.34.
4. Jonard S, Dewailly D. The follicular excess in polycystic ovaries, due to intra-ovarian hyperandrogenism, may be the main culprit for the follicular arrest. Hum Reprod Update 2004; 10:107.
5. Rosenfield RL. Ovarian and adrenal function in polycystic ovary syndrome. Endocrinol Metab Clin North Am 1999; 28:265.
6. Rosenfield RL, Bordini B. Evidence that obesity and androgens have independent and opposing effects on gonadotropin production from puberty to maturity. Brain Res 2010; 1364:186-197.
7. Pastor CL, Griffin-Korf ML, Aloi JA,et al. Polycystic ovary syndrome: evidence for reduced sensitivity of the gonadotropin-releasing hormone pulse generator to inhibition by estradiol and progesterone. J Clin Endocrinol Metab. 1998; 83:582-90.
8. Eagleson CA, Gingrich MB, Pastor CL, et al. Polycystic ovarian syndrome: evidence that flutamide restores sensitivity of the gonadotropin-releasing hormone pulse generator to inhibition by estradiol and progesterone. J Clin Endocrinol Metab. 2000; 85:4047-52.
9. Rosenfield RL, Mortensen M, Wroblewski K, et al. Determination of the source of androgen excess in functionally atypical polycystic ovary syndrome by a short dexamethasone androgen-suppression test and a low-dose ACTH test. Hum Reprod 2011; 26:3138.
10. Maciel GA, Baracet EC, Benda JA, et al. Stockpiling of transitional and classy primary follicles in ovaries of women with polycystic ovary syndrome. J Clin Endocrinol Metab. 2004; 89:5321
11. Pache TD, Chadha S, Gooren LJ, et al. Ovarian morphology in long-term androgen-treated female to male transsexuals. A human model for the study of polycystic ovarian syndrome? Histopathology 1991; 19:445.
12. Coffler MS, Patel K, Dahan MH, et al. Evidence for abnormal granulosa cell responsiveness to follicle- stimuating hormone in women with polycystic ovary syndrome. J Clin Endocrinol Metab. 2003; 88:1742.
13. Hirshfeld-Cytron J, Barnes RB, Ehrmann DA, et al. Characterization of functionally typical and atypical types of polycystic ovary syndrome. J Clin Endocrinol Metab 2009; 94:1587.
14. Rosenfield RL. Polycystic ovary syndrome and insulin-resistant hyperinsulinemia. J Am Acad Dermatol 2001; 45:S95.
15. Diamanti-Kandarakis E, Dunaif A. Insulin resistance and the polycystic ovary syndrome revisited: an update on mechanisms and implications. Endocr Rev 2012; 33:981.
16. Lewy VD, Danadian K, Witchel SF, Arslanian S. Early metabolic abnormalities in adolescent girls with polycystic ovarian syndrome. J Pediatr 2001; 138:38-44.
17. Bruning JC, Gautam D, Burks DJ, et al. Role of brain insulin receptor in control of body weight and reproduction. Science. 2000; 289:2122
18. Roldan B, Escobar- Morreale HF, Barrio R, et al. Identification of the source of androgen excess in hyperandrogenic type 1 diabetic patients. Diabetes Care. 2001; 24:1297.
19. American Diabetes Association Screening for type 2 diabetes. Diabetes Care 2004;27:11–14

20. Escobar-Morreale HF, Luque-Ramirez M, San Millan JL. Molecular-genetic basis of functional hyperandrogenism and the polycystic ovary syndrome. Endocr Rev 2005;26:251-82.
21. Rosenfield RL. Clinical review: Identifying children at risk for polycystic ovary syndrome. J Clin Endocrinol Metab 2007; 92:787-96.
22. Kahsar-Miller MD, Nixon C, Boots LR, et al. Prevalence of polycystic ovary syndrome (PCOS) in first degree relatives of patients with PCOS. Fertil Steril. 2001; 75:53.
23. Carey AH, Chan KL, Short F, et al. Evidence for a single gene effect causing polycystic ovaries and male pattern baldness. Clin Endocrinol (Oxf) 1993; 38:653.
24. Govind A, Obhrai MS, Clayton RN. Polycystic ovaries are inherited as an autosomal dominant trait: analysis of 29 polycystic ovary syndrome and 10 control families. J Clin Endocrinol Metab 1999; 84:38.
25. Leibel NI, Baumann EE, Kocherginsky M, Rosenfield RL. Relationship of adolescent polycystic ovary syndrome to parental metabolic syndrome. J Clin Endocrinol Metab 2006; 91:1275-83.
26. Witchel SF, Lee PA, Suda-Hartman M, Hoffman EP. Hyperandrogenism and manifesting heterozygotes for 21-hydroxylase deficiency. Biochem Mol Med 1997; 62:151.
27. Nelson VL, Legro RS, Strauss JF 3rd, McAllister JM. Augmented androgen production is a stable steroidogenic phenotype of propagated theca cells from polycystic ovaries. Mol Endocrinol 1999; 13:946.
28. Barnes RB, Rosenfield RL, Ehrmann DA, et al. Ovarian hyperandrogynism as a result of congenital adrenal virilizing disorders: evidence for perinatal masculinization of neuroendocrine function in women. J Clin Endocrinol Metab 1994; 79:1328-33.
29. Abbott DH, Dumesic DA, Franks S, et al. Developmental origin of polycystic ovary syndrome- a hypothesis. J Endocrinol. 2002; 174:1-5.
30. Adams JM, Taylor AE, Crowley WF Jr, et al. Polycystic ovarian morphology with regular ovulatory cycles: insights into the pathophysiology of polycystic ovarian syndrome. J Clin Endocrinol Metab. 2004.; 89:4343-50.
31. Dunaif A. Insulin resistance and the polycystic ovary syndrome: mechanism and implications for pathogenesis. Endocr Rev. 1997; 18:774-800
32. Glueck CJ, Papanna R, Wang P,et al. Incidence and treatment of metabolic syndrome in newly reffered women with confirmed polycystic ovarian syndrome. Metabolism. 2003; 52:908
33. Ehrmann DA, Barnes RB, Rosenfield RL, et al. Prevalence of impaired glucose tolerance and diabetes in women with polycystic ovary syndrome. Diabetes Care. 1999; 22:141-6.
34. Hatch R, Rosenfiled RL, Kim MH, Tredway D. Hirsutism: implications,etiology and management. Am J Obstet Gynecol 1981; 140:815-30.
35. Escobar-Morreale HF, Carmina E, Dewailly D, et al. Epidemiology, diagnosis and management of hirsutism: a consensus statement by the Androgen Excess and Polycystic Ovary Syndrome Society. Hum Reprod Update 2012; 18:146-170.
36. Lucky AW, Biro FM, Daniels SR, et al. The prevalence of upper lip hair in black and white girls during puberty: a new standard. J Pediatr 2001; 138:134-6.
37. Deplewski D, Rosenfield RL. Role of hormones in pilosebaceous unit development. Endocr Rev 2000; 21:363-92.
38. Lucky AW, Biro FM, Simbartl LA, et al. Predictors of severity of acne vulgaris in young adolescent girls: results of a five-year longitudinal study. J Pediatr 1997; 130:30-39.
39. Eichenfield LF, Krakowski AC, Piggott C, et al. Evidence-based recommendations for the diagnosis and treatment of pediatric acne. Pediatrics 2013; 131 Suppl 3:S163.
40. Rosenfield RL. Clinical review: Adolescent anovulation: maturational mechanisms and implications. J Clin Endocrinol Metab 2013; 98:3572-3583.
41. van Hooff MH, Voorhorst FJ, Kaptein MB, et al. Predictive value of menstrual cycle pattern, body mass index, hormone levels and polycystic ovaries at age 15 years for oligo-amenorrhoea at age 18 years. Hum Reprod 2004; 19:383-92.

42. West S, Lashen H, Bloigu A, et al. Irregular menstruation and hyperandrogenaemia in adolescence are associated with polycystic ovary syndrome and infertility in later life: Northern Finland Birth Cohort 1986 study. Hum Reprod 2014; 29:2339.
43. Ibáñez L, de Zegher F, Potau N. Anovulation after precocious pubarche: early markers and time course in adolescence. J Clin Endocrinol Metab 1999; 84:2691-95.
44. Ezeh U, Yildiz BO, Azziz R. Referral bias in defining the phenotype and prevalence of obesity in polycystic ovary syndrome. J Clin Endocrinol Metab 2013; 98:E1088.
45. Coviello AD, Legro RS, Dunaif A. Adolescent girls with polycystic ovary syndrome have an increased risk of the metabolic syndrome associated with increasing androgen levels independent of obesity and insulin resistance. J Clin Endocrinol Metab 2006; 91:492-497.
46. Barber TM, Golding SJ, Alvey C, et al. Global adiposity rather than abnormal regional fat distribution characterizes women with polycystic ovary syndrome. J Clin Endocrinol Metab 2008; 93:999-1004.
47. Palmert MR, Gordon CM, Kartashov AI, et al. Screening for abnormal glucose tolerance in adolescents with polycystic ovary syndrome. J Clin Endocrinol Metab 2002; 87:1017-23.
48. Grundy SM, Brewer HB Jr, Cleeman JI, et al. Definition of metabolic syndrome: Report of the National Heart, Lung, and Blood Institute/American Heart Association conference on scientific issues related to definition. Circulation 2004; 109:433-438.
49. Apter D, Bützow T, Laughlin GA, Yen SS. Metabolic features of polycystic ovary syndrome are found in adolescent girls with hyperandrogenism. J Clin Endocrinol Metab 1995; 80:2966-73.
50. Rossi B, Sukalich S, Droz J, et al. Prevalence of metabolic syndrome and related characteristics in obese adolescents with and without polycystic ovary syndrome. J Clin Endocrinol Metab 2008; 93:4780-4786.
51. de Sousa G, Schlüter B, Buschatz D, et al. A comparison of polysomnographic variables between obese adolescents with polycystic ovarian syndrome and healthy, normal-weight and obese adolescents. Sleep Breath 2010; 14:33-38.
52. Nandalike K, Agarwal C, Strauss T, et al. Sleep and cardiometabolic function in obese adolescent girls with polycystic ovary syndrome. Sleep Med 2012; 13:1307-1312.
53. Lucky AW, Biro FM, Simbartl LA, et al. Predictors of severity of acne vulgaris in young adolescent girls: results of a five-year longitudinal study. J Pediatr 1997; 130:30-39.
54. Escobar-Morreale HF, Sanchón R, San Millán JL. A prospective study of the prevalence of nonclassical congenital adrenal hyperplasia among women presenting with hyperandrogenic symptoms and signs. J Clin Endocrinol Metab 2008; 93:527-533.
55. Bachelot A, Chakhtoura Z, Plu-Bureau G, et al. Influence of hormonal control on LH pulsatility and secretion in women with classical congenital adrenal hyperplasia. Eur J Endocrinol 2012; 167:499-505.
56. Azziz R, Sanchez LA, Knochenhauer ES, et al. Androgen excess in women: experience with over 1000 consecutive patients. J Clin Endocrinol Metab 2004; 89:453-462.
57. Givens JR, Kerber IJ, Wiser WL, et al. Remission of acanthosis nigricans associated with polycystic ovarian disease and a stromal luteoma. J Clin Endocrinol Metab 1974; 38:347-55.
58. Lungu AO, Zadeh ES, Goodling A, et al. Insulin resistance is a sufficient basis for hyperandrogenism in lipodystrophic women with polycystic ovarian syndrome. J Clin Endocrinol Metab 2012; 97:563-67.
59. Littlejohn EE, Weiss RE, Deplewski D, et al. Intractable early childhood obesity as the initial sign of insulin resistant hyperinsulinism and precursor of polycystic ovary syndrome. J Pediatr Endocrinol Metab 2007; 20:41-51.

60. Codner E, Escobar-Morreale HF. Clinical review: Hyperandrogenism and polycystic ovary syndrome in women with type 1 diabetes mellitus. J Clin Endocrinol Metab 2007; 92:1209-16.
61. Peppard HR, Marfori J, Iuorno MJ, Nestler JE. Prevalence of polycystic ovary syndrome among premenopausal women with type 2 diabetes. Diabetes Care 2001; 24:1050-52.
62. Pache TD, Chadha S, Gooren LJ, et al. Ovarian morphology in long-term androgen-treated female to male transsexuals. A human model for the study of polycystic ovarian syndrome? Histopathology 1991; 19:445-452.
63. Nelson-DeGrave VL, Wickenheisser JK, Cockrell JE, et al. Valproate potentiates androgen biosynthesis in human ovarian theca cells. Endocrinology 2004; 145:799-808.
64. Glickman SP, Rosenfield RL, Bergenstal RM, et al. Multiple androgenic abnormalities, including elevated free testosterone, in hyperprolactinemic women. J Clin Endocrinol Metab. 1982; 55:251-257.
65. Rosenfield RL. Current concepts of polycystic ovary syndrome. Baillieres Clin Obstet Gynaecol. 1997;11:307
66. Zawadski JK, Dunaif A. Diagnostic criteria for polycystic ovary syndrome: towards a rational approach. In: Dunaif A, Givens JR, Haseltine FP, Merriam GR, eds. Polycystic Ovary Syndrome. Boston: Blackwell Scientific Publications; 1992:377-384
67. Rotterdam ESHRE/ASRM-Sponsored PCOS Consensus Workshop Group. Revised 2003 consensus on diagnostic criteria and long-term health risks related to polycystic ovary syndrome. Fertil Steril 2004; 81:19-25
68. 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.
69. Carmina E, Oberfield SE, Lobo RA. The diagnosis of polycystic ovary syndrome in adolescents. Am J Obstet Gynecol 2010; 203:201.e1-e5.
70. Buggs C, Rosenfield RL. Polycystic ovary syndrome in adolescence. Endocrinol Metab Clin North Am. 2005; 34: 677.
71. Legro RS, Driscoll D, Strauss JF 3rd, et al. Evidence for a genetic basis for hyperandrogenemia in polycystic ovary syndrome. Proc Natl Acad Sci U S A 1998; 95:14956.
72. Moll GW Jr, Rosenfield RL. Testosterone binding and free plasma androgen concentrations under physiological conditons: chararacterization by flow dialysis technique. J Clin Endocrinol Metab 1979; 49:730.
73. Pugeat M, Nader N, Hogeveen K, et al. Sex hormone-binding globulin gene expression in the liver: drugs and the metabolic syndrome. Mol Cell Endocrinol 2010; 316:53-59.
74. Miller KK, Rosner W, Lee H, et al. Measurment of free testosterone in normal women and women with androgen deficiency: comparison of methods. J Clin Endocrinol Metab. 2004; 89:525-533.
75. ACOG Committee on Practice Bulletins--Gynecology. ACOG Practice Bulletin No. 108: Polycystic ovary syndrome. Obstet Gynecol 2009; 114:936.
76. Venturoli S, Porcu E, Fabbri R, et al. Longitudinal change of sonographic ovarian aspects and endocrine parameters in irregular cycles of adolescence. Pediatr Res 1995; 38:974-80.
77. Bremer AA. Polycystic ovary syndrome in the pediatric population. Metab Syndr Relat Disord 2010; 8:375
78. Prassopoulos V, Laspas F, Vlachou F, et al. Leydig cell tumour of the ovary localised with positron emission tomography/computed tomography. Gynecol Endocrinol 2011; 27:837-39.
79. Goodman NF, Bledsoe MB, Futterweit W, et al. American Association of Clinical Endocrinologists medical guidelines for clinical practice for the diagnosis and treatment of hyperandrogenic disorders. Endocr Pract. 2001; 7:120-34.

80. Rosenfield RL, Barnes RB, Ehrmann DA, et al. The value of the low-dose dexamethasone suppresion test in the differential diagnosis of hyperandrogenism in women. J Clin Endocrinol Metab. 2003; 88:6115
81. Fauser BC, Bouchard P. Uncertainty remains in women with PCOS regarding the increased incidence of cardiovascular disease later in life, despite the indisputable presence of multiple cardiovascular risk factors at a young age. J Clin Endocrinol Metab 2011; 96:3675-77.
82. Legro RS, Arslanian SA, Ehrmann DA, et al. Diagnosis and treatment of polycystic ovary syndrome: an Endocrine Society clinical practice guideline. J Clin Endocrinol Metab 2013; 98:4565.
83. Gordon CM, Pitts SA. Approach to the adolescent requesting contraception. J Clin Endocrinol Metab 2012; 97:9-15.
84. Diamanti-Kandarakis E, Baillargeon JP, Iuorno MJ, et al. A modern medical quandary: polycystic ovary syndrome, insulin resistance, and oral contraceptive pills. J Clin Endocrinol Metab 2003; 88:1927-32.
85. Visser J, Snel M, Van Vliet HA. Hormonal versus non-hormonal contraceptives in women with diabetes mellitus type 1 and 2. Cochrane Database Syst Rev 2013; 3:CD003990.
86. Martin KA, Chang RJ, Ehrmann DA, et al. Evaluation and treatment of hirsutism in premenopausal women: an endocrine society clinical practice guideline. J Clin Endocrinol Metab 2008; 93:1105.
87. Baillargeon JP, Jakubowicz DJ, Iuorno MJ, et al. Effects of metformin and rosiglitazone, alone and in combination, in nonobese women with polycystic ovary syndrome and normal indices of insulin sensitivity. Fertil Steril 2004; 82:893.
88. Cummings DM, Henes S, Kolasa KM, et al. Insulin resistance status: predicting weight response in overweight children. Arch Pediatr Adolesc Med 2008; 162:764.
89. Ibáñez L, Potau N, Marcos MV, de Zegher F. Treatment of hirsutism, hyperandrogenism, oligomenorrhea, dyslipidemia, and hyperinsulinism in nonobese, adolescent girls: effect of flutamide. J Clin Endocrinol Metab 2000; 85:3251-3255.
90. Futterweit W, Deligdisch L. Histopathological effects of exogenously administered testosterone in 19 female to male transsexuals. J Clin Endocrinol Metab 1986; 62:16-21.
91. Woods KS, Reyna R, Azziz R. Effect of oral micronized progesterone on androgen levels in women with polycystic ovary syndrome. Fertil Steril 2002; 77:1125-1127.
92. Benjamins LJ. Practice guideline: evaluation and management of abnormal vaginal bleeding in adolescents. J Pediatr Health Care 2009; 23:189-93.
93. Lidegaard Ø, Løkkegaard E, Svendsen AL, Agger C. Hormonal contraception and risk of venous thromboembolism: national follow-up study. BMJ 2009; 339:b2890.
94. Jick SS, Hernandez RK. Risk of non-fatal venous thromboembolism in women using oral contraceptives containing drospirenone compared with women using oral contraceptives containing levonorgestrel: case-control study using United States claims data. BMJ 2011; 342:d2151.
95. Moran LJ, Noakes M, Clifton PM, Norman RJ. The use of anti-mullerian hormone in predicting menstrual response after weight loss in overweight women with polycystic ovary syndrome. J Clin Endocrinol Metab 2007; 92:3796-3802.
96. Ibáñez L, Valls C, Marcos MV, et al. Insulin sensitization for girls with precocious pubarche and with risk for polycystic ovary syndrome: effects of prepubertal initiation and postpubertal discontinuation of metformin treatment. J Clin Endocrinol Metab 2004; 89:4331-37.
97. Schwartz SL, Wu JF, Berner B. Metformin extended release for the treatment of type 2 diabetes mellitus. Expert Opin Pharmacother 2006; 7:803-809.
98. Domecq JP, Prutsky G, Mullan RJ, et al. Adverse effects of the common treatments for polycystic ovary syndrome: a systematic review and meta-analysis. J Clin Endocrinol Metab 2013; 98:4646-4654.

BIOGRAPHY

TomislavPuževski was born on September 27,1990 in Zagreb, Croatia. After finishing primary and high school, he passed the entrance exam and enrolled into University of Zagreb, School of Medicine in September 2009. During study,he worked as a student demonstrator in the course propaedeutics on the internal intensive care unit at KBC Zagreb under the mentorship of prof.dr.sc.R. Radonić. He also attended summer school of emergency medicine and gained Immediate Life Support (ILS) certificate. Once graduating in 2015, he is looking forward to gain the experience abroad and to start working.