Polycystic ovary syndrome and the differential diagnosis of hyperandrogenism

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Key content
• The presence of clinical or biochemical evidence of hyperandrogenism is a defining feature of polycystic ovary syndrome (PCOS).
• As the criteria for diagnosing PCOS becomes more inclusive, there is an increased risk of misdiagnosing women with other causes of hyperandrogenism.
• Biochemical testing for serum testosterone concentrations in women has important limitations.
• Patients with concerning features should be investigated for other causes of hyperandrogenism.

Learning objectives
• To review the different criteria for diagnosing PCOS and the repercussions for disease prevalence.
• To learn about the differential diagnosis of hyperandrogenism.
• To learn about the limitations of biochemical testing for testosterone in women.

Ethical issues
• Hyperandrogenism causing hirsutism and virilisation can have significant effects on physical and psychological wellbeing.
• Misdiagnosis can delay appropriate treatment and may affect future fertility.

Keywords: hirsutism / hyperandrogenism / polycystic ovary syndrome

Introduction

Hyperandrogenism is one of the most common and distressing endocrine disorders in women of reproductive age.¹ Androgen excess results in the development of hirsutism, androgenic alopecia, acne, ovulatory dysfunction, and virilisation or masculinisation if prolonged or severe. Bullock and Sequeira² were the first to describe an association between masculinisation and endocrine pathology in 1905, linking the adrenals and gonads. Many conditions can cause hyperandrogenism in women, but the most common cause worldwide is polycystic ovary syndrome (PCOS).

Some androgen production occurs in all healthy women and is required for the synthesis of estrogens. In hyperandrogenic states, there is either dysfunctional production of androgens or inadequate conversion to estrogens or both. This review focuses on the pathophysiology, biochemistry and differential diagnoses of hyperandrogenism in women.

Normal androgen production in females

Adrenal androgens

The adrenal gland is responsible for the production and secretion of aldosterone, cortisol, and androgens, as summarised in Figure 1. Dehydroepiandrosterone (DHEA) is the predominant androgen produced by and released from the adrenal gland. DHEA-sulfate (DHEA-S), the sulfuric acid ester of DHEA, is predominantly produced in the adrenal gland and is therefore a useful measure of adrenal androgen production.³
Ovarian androgens

The ovary also produces and releases androgens, including 20% of DHEA, 50% of androstenedione and 25% of circulating testosterone. The control of ovarian androgen production is summarised in Figure 1.

Androgen metabolism

In healthy women, 80% of circulating testosterone is bound to sex hormone binding globulin (SHBG), 19% is bound to albumin, and 1% circulates freely in the blood stream. Traditionally, only the unbound fraction is considered metabolically active although this is now disputed. Of the circulating androgens, only testosterone and its active metabolite dihydrotestosterone (DHT) are able to activate androgen receptors. The remaining androgens, DHEA-S, DHEA, and androstenedione are almost entirely bound to albumin and can be converted to testosterone in peripheral tissue. Most of the circulating testosterone is metabolised in the liver into androsterone and etiocholanolone, which are conjugated with glucuronic acid or sulfuric acid and excreted in the urine as 17-ketosteroids.

Androgen action

In the target tissues, androgens diffuse across cell membranes and bind to nuclear androgen receptors. The androgen-receptor complex attaches to a specific DNA site and stimulates the production of messenger RNA, which, in turn, stimulates the production of the enzymes and proteins necessary to affect androgen action.

In the brain, the highest concentrations of androgen receptors are present in the preoptic area of the hypothalamus – in close proximity to estrogen receptors – and are thought to be involved in behaviour and sexual function. In bone, androgens have an important role in bone mineralisation both directly and through the aromatisation to estrogen. Lower androgen concentrations have been associated with bone loss in various age groups. Androgen receptors are also present in mammary epithelial cells in addition to estrogen and progesterone receptors. The role of androgens in human breast tissue remains unclear, although no increase in breast cancer risk has been observed in hyperandrogenaemic women with PCOS.

Clinical assessment of hyperandrogenism

Symptomatic androgen excess in women of reproductive age is a common presentation to the general endocrinology clinic. The physical effects of hyperandrogenism are distressing for many patients but nonetheless a detailed history and thorough clinical examination are essential. Clinical assessment facilitates diagnosis of the underlying cause of androgen excess and appropriate investigation and management.

The history and examination should elucidate common symptoms and signs of hyperandrogenism including hirsutism, acne, menstrual cycle irregularities, weight gain, alopecia and virilisation (the development of classic male characteristics). The chronology of symptom commencement and progression is important and can be indicative of specific disease processes. For example, rapid excessive hair growth, deepened voice and breast atrophy would be more indicative of an adrenal tumour, compared with the slow development of hirsutism and menstrual irregularities occurring soon after puberty, classically due to PCOS. Disruption of the timing of puberty can be associated with congenital adrenal hyperplasia (CAH) and Cushing’s syndrome. A family history is also important as both PCOS and CAH can occur in other family members.

Clinical evaluation required in women with hyperandrogenism is outlined in Box 1. An objective grading of

![Figure 1. Androgen production in women. The secretion of corticotrophin releasing hormone (CRH) by the hypothalamus stimulates the pituitary to release adrenocorticotropic hormone (ACTH). ACTH acts on the adrenal cortex and thus controls synthesis rates of cortisol, aldosterone and adrenal androgens. Cortisol exerts a negative feedback on its own production. This is important in congenital adrenal hyperplasia (CAH). The release of gonadotrophin releasing hormone (GnRH) from the hypothalamus stimulates release of luteinising hormone (LH) and follicle stimulating hormone (FSH) from the pituitary. These act upon the ovary and ovarian androgens are synthesised. The main ovarian androgen is androstenedione.](image-url)
the severity of symptoms such as hirsutism is advisable due to significant cultural and social differences in perceptions of normal body appearance. The most common scoring system used by clinicians for hirsutism is the Ferriman-Gallwey score,8 which rates hair growth severity from 0–4 and scores 11 different body areas (upper lip, chest, chin, lower back, upper back, lower abdomen, upper abdomen, forearm, arm, thigh, and lower leg). A score of 8 or above defines hirsutism.

**Box 1. Clinical features associated with hyperandrogenism**

- Height, weight and body mass index.
- Distribution and extent of adiposity.
- Skin thinning or bruising (seen in Cushing’s syndrome).
- Acne, especially over the face, neck, back and chest.
- Degree, pattern and severity of hirsutism.
- Acanthosis nigricans (velvety skin hyperpigmentation), associated with insulin resistance.
- Deepened voice.
- Male pattern balding.
- Breast atrophy.
- Clitoromegaly.
- Loss of normal feminine body shape.

**Biochemical assessment of hyperandrogenism**

It is widely accepted that an elevated serum testosterone level provides biochemical evidence of hyperandrogenism. However, different analytical methods demonstrate significant differences in analytical specificity for testosterone – some methods have interference from non-testosterone androgens. Many practitioners believe that a serum total testosterone of <5.0 nmol/L, measured using an extraction immunoassay, makes serious pathology in a female unlikely.4 Mild hyperandrogenism with a serum total testosterone level of around 2–5 nmol/L is thought to be consistent with PCOS, while marked elevations (>5 nmol/L) should prompt investigation for other causes, such as an androgen-secreting tumour.

Most laboratories in secondary care institutions in the UK use an immunoassay to measure total testosterone. This method is fast, inexpensive and technically simple with the advantage of being suitable for automation.9 However, while this method reliably identifies testosterone concentrations in the adult male range, it often performs poorly in females, where even in PCOS, concentrations are much lower. Automatic immunoassays consistently overestimate the serum testosterone concentrations, some by as much as 100%.4 Suggested reasons for this include the presence of cross-reacting compounds, which may include immunoglobulins, drug metabolites and plasma proteins.4 Unfortunately, the presence and degree of interference from these cross-reactants is unpredictable.

The gold standard method for testosterone quantitation is considered to be based upon mass spectrometry (MS), which is only available in some tertiary centres. However, this technique requires expensive equipment and significant operator skill, and currently lacks widespread standardisation.

**Disorders associated with hyperandrogenism**

The differential diagnoses of hyperandrogenism are summarised in Box 2. Although PCOS is the most common cause in women of reproductive age, other important disorders should be considered and excluded.

**Box 2. Differential diagnoses of hyperandrogenism**

- PCOS
- Ovarian hyperthecosis
- Congenital adrenal hyperplasia
- Cushing’s syndrome
- Androgen-secreting tumour
  - Adrenal origin
  - Ovarian origin
- Exogenous androgen administration
- Gestational hyperandrogenism

**Polycystic ovary syndrome (PCOS)**

PCOS is a heterogeneous condition associated with multiple ovarian cysts, menstrual irregularity, subfertility, mood disturbance, hyperandrogenism, obesity, acne and hirsutism.10 It is the most common reproductive endocrine disorder of women and has significant short-term and long-term health sequelae. Although PCOS was first described in 1935 by Stein and Leventhal,11 its diagnosis and management still represent a significant challenge to the clinician. This is partially due to the heterogeneous nature of the condition and the conflicting guidance on diagnostic criteria.12

PCOS has also been associated with long-term metabolic effects, including the development of insulin resistance, diabetes mellitus, hyperlipidaemia, non-alcoholic steatohepatitis (NASH) and an adverse risk profile for cardiovascular disease.13 As PCOS is common, perhaps affecting up to 10–20% of women of reproductive age, this represents a significant population burden of disease for the future.
Pathophysiology of PCOS

There is controversy about the pathophysiology of PCOS. It is likely related to inherited and environmental susceptibilities, with possible excessive androgen stimulation in utero. Lifestyle factors such as weight gain can exacerbate PCOS. Biochemically, there is hyperinsulinism beyond that expected in an age- and BMI-matched group, which causes luteinsing hormone (LH) hypersecretion and potentiates the action of LH and the insulin-like growth factor-1 (IGF-1). Both LH and IGF-1 upregulate synthesis of ovarian and adrenal androgens causing production of testosterone and DHEA-S.10

In the ovaries, hyperinsulinism, hyperandrogenism and disordered paracrine signalling cause arrest of follicular development, typically when the follicle acquires aromatase activity and is around 7 mm in size. Excessive ovarian androgens impair aromatase function, which is required for further follicular growth. Overproduction of anti-mullerian hormone (AMH) antagonises follicle-stimulating hormone (FSH) action and reduces availability of estrogen, which is required for developing follicles.10 Arrest of follicular development leads to menstrual disturbance, anovulation and subfertility.

Diagnosing PCOS

Stein and Leventhal11 first described the association of polycystic ovaries and menstrual disturbance. However, it soon became apparent that some women with polycystic ovaries are otherwise healthy and do not have menstrual disturbance. Additionally, some patients with obesity, menstrual disturbance and hirsutism resemble the PCOS pattern but lack polycystic ovaries. This has made the diagnosis of PCOS very challenging and many authorities disagree on the value of different criteria in reaching a diagnosis.

The Rotterdam international consensus group14 described PCOS as a syndrome of ovarian dysfunction, characterised by hyperandrogenism and polycystic ovaries. They recommended that the diagnosis be made if two of the following three criteria were met:

- oligomenorrhoea or anovulation,
- clinical and/or biochemical evidence of hyperandrogenism,
- polycystic ovaries on ultrasonography: multiple peripheral follicles with ovarian volume >10 ml.

Other diseases that can cause similar symptoms, such as androgen secreting tumours, Cushing’s syndrome and congenital adrenal hyperplasia must also be excluded.

These criteria require a diagnosis to be reached on the basis of history-taking, clinical examination, blood testing for hyperandrogenism and ovarian imaging. The role of biochemistry in PCOS diagnosis is therefore crucial and may provide the only objective measurement contributing to a diagnosis. However, there is also controversy about what exactly constitutes biochemical evidence of hyperandrogenism. The typical biochemical profile in PCOS includes mild to moderate elevations in free and total testosterone, elevated DHEA-S and low SHBG concentrations. In some patients, the LH concentration is elevated while the FSH concentration remains normal, giving an elevated LH/FSH ratio.10

Ovarian hyperthecosis

Ovarian hyperthecosis accounts for most of the cases of hyperandrogenaemia in postmenopausal women,15 although its prevalence in younger women is much lower, affecting <1% of women with elevated androgens in their reproductive years. Ovarian hyperthecosis describes the presence of luteinised theca cell nests in the ovarian stroma. When compared with the closely related condition of PCOS, hyperthecosis is typically associated with more severe hyperandrogenism and virilisation. Testosterone concentrations are much higher than in PCOS and may exceed 7 nmol/L.15

Congenital adrenal hyperplasia

CAH is a group of autosomal recessive disorders, each of which involves a deficiency of one of five enzymes involved in the synthesis of cortisol in the adrenal cortex.16 In CAH, insufficient cortisol is produced, which stimulates hypothalamic CRH secretion, due to the absence of normal feedback inhibition (see Figure 1). This leads to chronically elevated adrenocorticotrophic hormone (ACTH), which in turn stimulates the adrenal gland to become hyperplastic with excess androgen hormones and steroid precursors being produced and secreted from the normally functioning metabolic pathways. The most common form of CAH is due to a deficiency of 21-hydroxylase activity, which accounts for 90–95% of cases of CAH. It is associated with a wide range of clinical effects and severity can vary depending on the mutation.16

Classic 21-hydroxylase deficiency commonly presents in infancy. Female infants can be severely virilised leading to ambiguous genitalia. Salt wasting and adrenal crises can occur in some patients and are important causes of neonatal death. Non-classic 21-hydroxylase deficiency tends to present in puberty, or later in adult life. In this condition, the mutated enzymes in the cortisol biosynthetic pathways maintain 20–60% of normal function.17 Typical patients have features of hyperandrogenism but have preserved cortisol and aldosterone production, so salt wasting and adrenal crises are not common features of this condition. Many female patients can present in early adulthood with menstrual disturbance or hirsutism.18 Affected males and females may exhibit precocious puberty with tall stature at pubarche, advanced bone age with early epiphyseal fusion, infertility and severe acne, which is refractory to treatment. It
Hyperandrogenism in women is a common and distressing condition which is due to disruption of normal ovarian or adrenal androgen production. The most common cause is PCOS, which is diagnosed using clinical, biochemical and ultrasonographic criteria. The biochemical quantitation of testosterone in females using the immunoassay method is significantly flawed and clinical judgement is crucial to prevent unnecessary investigation. Testosterone concentrations around 2–5 nmol/l are associated with PCOS while concentrations above 5 nmol/l should prompt investigation for another cause. MS-based methods will usually achieve lower results due to reduced interference from non-testosterone androgens but are not widely available. Prior to making a diagnosis of PCOS, other possible causes of hyperandrogenism should be excluded.

**Conclusions**

Hyperandrogenism in women is a common and distressing condition which is due to disruption of normal ovarian or adrenal androgen production. The most common cause is PCOS, which is diagnosed using clinical, biochemical and ultrasonographic criteria. The biochemical quantitation of testosterone in females using the immunoassay method is significantly flawed and clinical judgement is crucial to prevent unnecessary investigation. Testosterone concentrations around 2–5 nmol/l are associated with PCOS while concentrations above 5 nmol/l should prompt investigation for another cause. MS-based methods will usually achieve lower results due to reduced interference from non-testosterone androgens but are not widely available. Prior to making a diagnosis of PCOS, other possible causes of hyperandrogenism should be excluded.

**Disclosure of interests**

None to declare.

**References**

PCOS and hyperandrogenism