

Dynamic Testing for Evaluation of Adrenal and Gonadal Function in Pediatric and Adult Endocrinology: An Overview

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Abstract

Dynamic tests are often considered as the backbone of endocrinology. These tests involve the use of an exogenous agent to manipulate the body's hormonal milieu for the diagnosis and characterization of an endocrine disorder. They are especially helpful in the evaluation of certain endocrine conditions, such as disorders of growth and pubertal maturation and disorders of sex development. A great deal of heterogeneity exists across clinicians with regard to the usage, methodology, and interpretation of these tests. This review outlines various dynamic tests used to evaluate adrenal and gonadal function in pediatric and adult endocrinology, along with their clinical application and interpretation.

Keywords: Adrenals, CAH, DSD, Dynamic testing, GnRH stimulation, gonads, HCG stimulation

INTRODUCTION

Dynamic testing is integral to the diagnosis of endocrine dysfunction. Although useful for the diagnosis of some endocrine disorders (such as hypothyroidism and premature ovarian insufficiency), basal hormone measurement fails to characterize many other disorders. For instance, basal serum cortisol levels may be normal in patients with Cushing syndrome and adrenal insufficiency, and gonadotropin levels need not be elevated in patients with central precocious puberty (CPP). Dynamic tests are helpful to diagnose and better characterize such endocrine disorders. These could broadly be classified as either stimulation or suppression tests. Stimulation tests employ an exogenous agent to stimulate hormonal reserve and are primarily used to evaluate the hypofunction of an endocrine gland. On the other hand, suppression tests involve the use of an exogenous agent to assess autonomous hormone secretion, and endocrine hyperfunction.^[1]

Notably, there is poor uniformity in terms of the indications for test usage, method of performing the test, and interpretation of its results across clinicians.^[2] Herein, we describe various dynamic tests for evaluation of adrenal and gonadal function in pediatric and adult endocrinology as used in our department for last many years. We understand that their methodology and interpretation may vary from one center to another. However, the sole purpose of this review is to provide pediatricians and

endocrinologists with standard test protocols that could be useful in their routine clinical practice.

Evolution of immunoassay technology and implications in test interpretation

Immunoassays work on the principle of high-affinity binding between an antigen and antibody. The field of immunoassays began with the development of a radioimmunoassay for insulin by Solomon Berson and Rosalyn S. Yalow in 1959.^[3] With the development of specific monoclonal antibodies and various highly-sensitive labels (such as chemiluminescent and electrochemiluminescent tags), the sensitivity and specificity of modern-day immunoassays have improved remarkably.^[4] There are two basic types of immunoassays, namely, competitive binding assays and immunometric or sandwich assays. Competitive immunoassays are used for measuring small analytes, for which matched pair of antibodies to two different epitopes do not exist (such as cortisol, testosterone, estradiol, 17-hydroxyprogesterone, aldosterone, and thyroxine). In these assays, the analyte in specimen competes with a labeled

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reagent analyte for a limited number of antibody binding sites. Immunometric assays are used for larger analytes which contain multiple epitopes for antibody binding (peptide hormones such as luteinizing hormone, follicle-stimulating hormone, adrenocorticotrophic hormone, renin, thyroid-stimulating hormone, growth hormone, and prolactin). In these assays, the analyte in the specimen is sandwiched between a solid phase monoclonal antibody (capture antibody) and labeled monoclonal antibody (detection antibody). Unlike competitive immunoassays, the dose-response curve generated using these assays is directly proportional to the analyte concentration (i.e. higher the signal, higher the analyte concentration). Nowadays, both types of immunoassays are widely available on automated platforms, providing quick results.^[5]

When interpreting the results of a study involving the measurement of an analyte, it is important to understand the assay methodology and its sensitivity, specificity, precision, and accuracy. The cutoffs for dynamic tests presented here have been derived taking into account various studies done in different time points and using different assay methodologies. Therefore, rather than a specific cutoff, a range has been provided for certain tests depending upon the assay used.

Dynamic tests of adrenal and gonadal function

The following dynamic tests will be discussed in this review [Table 1]:

1. Human chorionic gonadotropin (HCG) stimulation test
2. Gonadotropin-releasing hormone (GnRH) agonist stimulation test
3. Stanozolol sex hormone-binding globulin (SHBG) test (androgen sensitivity test)
4. Adrenocorticotrophic hormone (ACTH) stimulation test
5. Dexamethasone suppression test (low-dose) (LDDST)
6. Gonadotropin-releasing hormone (GnRH) agonist suppression test

Human Chorionic Gonadotropin (HCG) stimulation test

This test is used to evaluate Leydig cell function in prepubertal boys. It is traditionally performed using the 3-day stimulation protocol.^[6-8] However, single-dose and prolonged stimulation protocols have also been described in the literature.^[9,10]

Indications

1. To detect functioning testicular tissue in patients with cryptorchidism
2. Evaluation of patients with testosterone biosynthetic defects
3. This test can also be used to differentiate between the constitutional delay of growth and puberty (CDGP) and hypogonadotropic hypogonadism (HH).^[11] However, the GnRH agonist stimulation test is more commonly used for this indication.

Protocol

Three-day stimulation test

- <1 year: 500 IU intramuscular HCG for three consecutive days (days 1–3)

- 1–10 year: 1000 IU intramuscular HCG for three consecutive days (days 1–3)
- >10 years: 1500 IU intramuscular HCG for three consecutive days (days 1–3).

Obtain serum testosterone (T) at baseline (before the first dose of HCG) and 24 h after the last injection (day 4). In addition, while evaluating for testosterone biosynthetic defects, blood may be sent for dihydrotestosterone (DHT) and androstenedione (A) measurement.

Interpretation

In normal individuals, peak serum testosterone level of 2.3–2.8 ng/mL (8–10 nmol/L) is expected. However, the cutoff for determining an abnormal response varies. In a study by Davenport *et al.*, 31 boys with undescended testis were evaluated using the test protocol mentioned above.^[6] For the diagnosis of anorchia, failure of basal testosterone (measured using radioimmunoassay) to double following HCG stimulation had 100% sensitivity and 96% specificity, while stimulated testosterone <1.4 ng/mL (5 nmol/L) had 100% sensitivity and 65% specificity. Ishii *et al.* retrospectively reported HCG-stimulated serum testosterone (measured using radioimmunoassay) of 50 prepubertal children with micropenis [using the three days HCG stimulation test (3000 IU/m²/day)].^[7] The median stimulated serum testosterone was significantly higher in group 1 ($n = 34$, entered puberty spontaneously; 2.4 ng/mL) compared to group 2 ($n = 16$, require pubertal induction subsequently; 0.24 ng/mL). A cutoff serum testosterone level of <1.1 ng/mL (3.8 nmol/L) predicted the future requirement of hormone replacement therapy with 100% sensitivity and 94% specificity. Similarly, Segal *et al.* performed a retrospective analysis of 43 children with delayed puberty who underwent GnRH stimulation test (2.5 µg/kg intravenous), followed by short (3 day, 1500 IU intramuscular days 1–3; $n = 38$) or extended (19 day, 1500 IU intramuscular day 1, 8, 11, 15 and 18; $n = 31$) HCG stimulation test or both ($n = 27$).^[8] Of these, 29 were diagnosed with CDGP and 14 with HH. Day-4 and day-19 serum testosterone (measured using chemiluminescent microparticle immunoassay [CMIA]) cutoff <1.04 ng/mL (3.6 nmol/L) and <2.75 ng/mL (9.5 nmol/L), respectively, predicted HH with a high sensitivity and specificity (92% and 92% for the short stimulation test; 92% and 95% for the extended stimulation test).

HCG stimulation test is also useful in the evaluation of prepubertal subjects with testosterone biosynthetic defects. The utility of stimulated T/DHT ratio for the diagnosis of steroid 5-alpha-reductase type 2 deficiency was evaluated in two studies involving 34 families from the Dominican Republic and Brazil.^[12,13] The stimulated ratio varied from 35 to 162 in the affected subjects. Similarly, in published data from our center, HCG stimulated T/DHT ratio varied from 34 to 50 in five subjects with this condition.^[14] While a T: DHT ratio >30 confer a high specificity (99%) but poor sensitivity (11%), a cutoff value of >10 is associated with moderate specificity (72%) and

Table 1: Dynamic tests for evaluation of adrenal and gonadal function in pediatric and adult endocrinology

Dynamic test	Indication(s)	Protocol	Interpretation
HCG stimulation test	Detection of functioning testicular tissue in patients with cryptorchidism Evaluation of testosterone biosynthetic defects Differentiation between CDGP and HH	HCG (IM) for three consecutive days (days 1-3) <1 year: 500 IU/d; 1-10 year: 1000 IU/d; >10 years: 1500 IU/d Serum T/A/DHT at baseline and on day 4.	Stimulated T <1.0-1.4 ng/mL (3.6-5.0 nmol/L) considered abnormal. Stimulated T/DHT ratio >20 suggest steroid 5-alpha-reductase type 2 deficiency. Stimulated T/A ratio <0.8 suggests 17β-HSD3 deficiency.
GnRH agonist stimulation test	Differentiate CPP from precocious pseudopuberty Differentiation between CDGP and HH	100 µg/m ² triptorelin (maximum 100 µg) SC OR Leuprolide (20 µg/kg) SC Serum LH at 0, 1, 2, and 4 h	Precocious puberty: Stimulated LH ≥5-8 IU/L suggestive of CPP. Delayed puberty: Stimulated LH <8 IU/L suggestive of HH.
Stanozolol SHBG test	Support the diagnosis of AIS	Stanozolol (0.2 mg/kg/day) orally for 3 consecutive days in a single evening dose (days 1-3). Serum SHBG at baseline and on days 5, 6, 7, and 8 (between 2-6 pm) Calculate the ratio of nadir serum SHBG to baseline serum SHBG.	Limited literature SHBG decline proportional to the degree of androgen responsiveness Nadir SHBG ≤63.4% of the baseline level considered normal. 73-89% of the baseline in PAIS 93-97% of the baseline in CAIS
ACTH stimulation test	Confirm the diagnosis of primary and secondary long-standing AI Confirm the diagnosis of CAH and assess the glucocorticoid reserve at baseline.	Synacthen (IM or IV) <1 year: 15 µg/kg; 1-2 year: 125 µg; >2 years: 250 µg Serum cortisol, 17-OHP at 30 and 60 min after injection	Peak cortisol <18 µg/dL (500 nmol/L) indicates AI Peak 17-OHP >10 ng/mL (30.3 nmol/L) indicates CAH due to 21-hydroxylase deficiency.
LDDST	Diagnosis of endogenous CS Differentiation between tumorous and non-tumorous hyperandrogenism	Dexamethasone (oral) <40 kg: 30 µg/kg/day in four divided doses q 6 hourly for 48 h; ≥40 kg: 0.5 mg q 6 hourly for 48 h Blood for cortisol (and testosterone) at 6 h (9-3-9-3 schedules) or 3 h (12-6-12-6 schedules) following the last dose	Serum cortisol >1.8 µg/dL (50 nmol/L) suggests endogenous CS. Normalization or reduction of serum testosterone by >40% suggests non-tumorous hyperandrogenism.
GnRH agonist suppression test	Differentiation between LH-dependent and LH-independent hyperandrogenism	Leuprolide/Triptorelin depot 3.75 mg IM Serum LH and testosterone at baseline and 4 weeks after the injection	Reduction of serum testosterone by >50% indicates LH-dependent hyperandrogenism

A: Androstenedione, ACTH: Adrenocorticotropic hormone, AI: Adrenal insufficiency, AIS: Androgen insensitivity syndrome, CAH: Congenital adrenal hyperplasia, CAIS: Complete androgen insensitivity syndrome, CDGP: Constitutional delay of growth and puberty, CPP: Central precocious puberty, CS: Cushing syndrome, DHT: Dihydrotestosterone, GnRH: Gonadotropin-releasing hormone, HCG: Human chorionic gonadotropin, HH: Hypogonadotropic hypogonadism, IM: Intramuscular, IV: Intravenous, LDDST: Low dose dexamethasone suppression test, LH: Luteinizing hormone, PAIS: Partial androgen insensitivity syndrome, SC: Subcutaneous, SHBG: Sex hormone-binding globulin, T: Testosterone, 17-βHSD3: 17β-hydroxysteroid dehydrogenase type 3, 17-OHP: 17-hydroxyprogesterone

sensitivity (78%).^[15] An intermediate cutoff value of >20 has also been proposed.^[16] For the diagnosis of 17β-HSD3 deficiency, stimulated T/A ratio <0.8 has been suggested.^[17,18] This cutoff was derived from a study by Boehmer *et al.*, where mean stimulated T: A ratio in cases with 17β-HSD3 deficiency was 0.4 (SD = 0.2).^[18] The sensitivity and specificity using this cutoff are 79% and 94%, respectively.^[15] However, it is important to note that the biochemical findings can be variable and genetic testing is required for the confirmation of the diagnosis in both conditions.

Adverse effects

The concern for adverse effects described with the use of long-term HCG therapy (such as behavioral changes, virilization, bone age advancement, and germ cell apoptosis) is mainly theoretical when employing the short (three-day) stimulation protocol.^[11,19,20]

Summary

- Stimulated testosterone <1.0–1.4 ng/mL (3.6–5.0 nmol/L) on a three-day stimulation test should be considered as an abnormal response. Based on the available literature, a cutoff value of 1.4 ng/mL (5.0 nmol/L) may be used during the evaluation of cryptorchidism, and 1.0 ng/mL (3.6 nmol/L) for differentiation between CDGP and HH.
- A stimulated T/DHT ratio >20 indicates steroid 5-alpha-reductase type 2 deficiency, while a T/A ratio <0.8 is suggestive of 17β-HSD3 deficiency.

Gonadotropin-releasing hormone (GnRH) agonist stimulation test

This test is useful for the evaluation of children with both delayed and precocious puberty. The original test employed the use of an intravenous bolus of GnRH (100 µg) and serial

blood sampling for luteinizing hormone (LH) until 120 min after the injection.^[21] However, due to limited availability of GnRH, these days the test is commonly performed using a subcutaneous injection of GnRH agonist (triptorelin or leuprolide).^[22]

Indications

1. To differentiate CPP from precocious pseudopuberty
2. To differentiate between CDGP and HH.

Protocol

Inject 100 µg/m² triptorelin (maximum 100 µg) subcutaneously.^[23,24] Alternatively, leuprolide (20 µg/kg body weight) may be injected subcutaneously.^[25,26] Measure serum LH at baseline (before injection), and at 1, 2, and 4 h interval.

Interpretation

In children evaluated for precocious pubertal development, a peak serum LH ≥5-8 IU/L following GnRH agonist stimulation test is suggestive of CPP. The threshold has been derived using highly sensitive immunoassays such as electrochemiluminescence immunoassay (ECLIA), chemiluminescence immunoassay (CLIA), and immunofluorometric assay (IFMA) in various studies [Table 2].^[23-26] Depending upon the assay employed at a given center, a peak LH cutoff ≥6 IU/L (CLIA), ≥7 IU/L (IFMA), or ≥8 IU/L (ECLIA) may be utilized.

Single basal gonadotropin (LH) measurement has also been used to predict the onset of puberty and diagnose CPP. In our own experience, serum LH (measured by ECLIA) of 0.88 IU/L predicted the onset of puberty in girls with a sensitivity of 69% and specificity of 69%, while in boys a cutoff level of

1.02 IU/L predicted the same with a sensitivity of 60% and specificity of 59%.^[27,28] In a study by Pasternak *et al.*, 80 girls with precocious breast development were evaluated. Basal serum LH (measured by CLIA) >0.1 IU/L diagnosed CPP with a sensitivity of 64% and specificity of 94%.^[29] Similarly, Lee *et al.* (girls, *n* = 336) reported that basal serum LH (measured by ECLIA) >0.1 IU/L predicted CPP with a sensitivity of 56% and specificity of 88%.^[30] The authors also reported that about 56% of girls with low basal LH (<0.1 IU/L) had a positive response on the GnRH stimulation test (suggestive of CPP). Notably, basal serum LH (measured by CLIA) >0.3 IU/L diagnosed CPP with a sensitivity of 42% and specificity of 88% in a study by Catli *et al.*^[31] Based on these observations, it may be concluded that a substantial proportion of subjects with precocious pubertal development and low basal LH may have CPP, requiring GnRH stimulation testing for confirmation.

It is difficult to differentiate between CDGP and HH on clinical grounds at the initial presentation. The presence or absence of spontaneous pubertal onset by the age of 18 years remains the hallmark for differentiation between the two conditions. However, an earlier diagnosis has the potential to reduce psychological stress associated with uncertainty in diagnosis. GnRH stimulation test works on the premise that dormant but primed gonadotrophs in patients with CDGP show a positive response to stimulation, as opposed to HH.^[11] However, a significant overlap in LH response between the two groups has been reported while using intravenous GnRH.^[32,33] Notably, owing to increased potency and half-life associated with GnRH agonists, the distinction between two conditions is much better when using these agents.^[34,35] In a retrospective evaluation of 23 prepubertal children who underwent

Table 2: Studies on the use of GnRH agonist stimulation test for the diagnosis of CPP

Author, Year, reference	Subject characteristics, sample size	CPP definition	Stimulating agent, dose	Immunoassay	Result
Poomthavom P <i>et al.</i> , 2009 ^[23]	101 girls with premature breast development (55-CPP, 46-PT)	Clinical and radiological*	Triptorelin (100 µg SC)	CLIA	Peak LH ≥6 IU/L Sensitivity: 89.1% Specificity: 93.1%
Sathasivam A <i>et al.</i> , 2010 ^[25]	39 girls with premature breast development (23-CPP, 16- nonprogressive)	Clinical and radiological*	Leuprolide (20 µg/kg SC)	CLIA	Peak LH ≥5 IU/L Sensitivity: 78% Specificity: 100%
Yazdani P <i>et al.</i> , 2012 ^[26]	107 boys and girls with precocious puberty (71- CPP, 36-nonprogressive)	Clinical and radiological*	Leuprolide (20 µg/kg SC)	CLIA	LH at 1 h ≥5 IU/L Sensitivity: 73% Specificity: 100% LH at 3 h ≥5 IU/L Sensitivity: 83% Specificity: 97%
Freire AV <i>et al.</i> , 2013 ^[24]	46 girls with premature breast development (33-CPP, 13-PT)	Peak LH (IFMA) by i.v GnRH ≥6 IU/L, plus clinical assessment	Triptorelin (100 µg/m ² , maximum 100 µg SC)	ECLIA IFMA	Peak LH ≥8 IU/L Sensitivity: 76% Specificity: 100% Peak LH ≥7 IU/L Sensitivity: 76% Specificity: 100%

CPP: Central precocious puberty, PT: Premature thelarche, LH: Luteinizing hormone, CLIA: Chemiluminescence immunoassay, ECLIA: Electrochemiluminescence immunoassay, IFMA: Immunofluorometric assay; SC: Subcutaneous, IV: Intravenous. *Growth acceleration, the progression of secondary sexual characteristics and/or advancement of bone age on follow-up

stimulation with triptorelin, serum LH (4 h) was significantly different (mean 33.2 ± 9.3 IU/L versus 3.3 ± 2.6 IU/L) between subjects with CDGP and HH without any overlap.^[36] Similarly, Ghai *et al.* and Zamboni *et al.* found that LH response following a single dose of GnRH agonist (nafarelin and triptorelin, respectively) allowed a clear distinction between adolescents belonging to the two groups.^[37,38] In a study by Kauschansky *et al.*, 32 prepubertal males underwent stimulation with triptorelin and were prospectively followed for spontaneous pubertal development ($n = 13$, spontaneous progression [CDGP]; $n = 19$, no progression [HH]).^[39] The peak LH response was significantly different between the two groups (20.4 ± 7.5 IU/L versus 2.4 ± 2.0 IU/L) without any overlap and a cutoff value of 8 IU/L was recommended to differentiate between the two conditions [Table 3].

Summary

- Basal LH alone cannot exclude CPP, and the GnRH agonist stimulation test is invariably required in patients with precocious pubertal development and a low basal value.
- Using sensitive immunoassays, GnRH agonist stimulated serum LH ≥ 6 IU/L (CLIA) or ≥ 8 IU/L (ECLIA) is suggestive of CPP.
- GnRH agonist testing in precocious puberty has to be interpreted on the background of the clinical picture pertaining to skeletal age advancement, height advancement and rate of progression of secondary sexual characteristics.
- GnRH agonist stimulation test is helpful in distinguishing between CDGP and HH.
- Using sensitive immunoassays, GnRH agonist stimulated serum LH < 8 IU/L is suggestive of HH.

Stanozolol Sex Hormone-binding Globulin (SHBG) test (Androgen sensitivity test)

This test is helpful to differentiate patients with androgen insensitivity syndrome from those with other testosterone biosynthetic defects. Androgen insensitivity syndrome is a rare X-linked recessive disorder that results from end-organ resistance to androgen action due to a mutation in the androgen receptor (*AR*) gene. The androgen sensitivity test is based

on the principle that androgens reduce SHBG in normal individuals, and the decline in SHBG is correlated with the degree of androgen responsiveness. The test is performed using a non-aromatizable anabolic steroid, stanozolol. With the increasing availability of genetic diagnosis, this test has become less popular and is used less often. However, it could still be employed to support the diagnosis of androgen insensitivity syndrome in a scenario when a genetic diagnosis is not available due to financial or technical reasons.

Indication

1. To support the diagnosis of androgen insensitivity syndrome.

Protocol

The prerequisites for this test are that baseline serum SHBG should be within normal range and the patient should not be receiving hormone replacement therapy.

Administer stanozolol (e.g., menabol, tanzol, neurobol) orally (0.2 mg/kg/day) for 3 consecutive days in a single evening dose (days 1–3).

Obtain serum SHBG at baseline (before the first dose) and on days 5, 6, 7, and 8. All samples should be taken between 2 pm–6 pm. The peculiar time has been suggested because SHBG shows a diurnal variation with the peak levels reported in the afternoon hours.^[40]

The lowest serum SHBG measured on days 5–8 represents the nadir and is representative of the largest response to stanozolol. Calculate the ratio of nadir serum SHBG to baseline serum SHBG.

Interpretation

The test interpretation is based on a study by Sinnecker *et al.* which evaluated 25 healthy controls, 4 subjects with partial androgen insensitivity syndrome (PAIS), and 3 subjects with complete androgen insensitivity syndrome (CAIS).^[41] The nadir serum SHBG was 51.6% of the basal value in the control group, while it ranged between 73–89% and 93–97% of the baseline in subjects with PAIS and CAIS, respectively. Thus, there was a graded serum SHBG decline depending upon the responsiveness at the androgen receptor. A normal response

Table 3: Studies on the use of GnRH agonist stimulation test for differentiating CDGP and HH

Author, Year, Reference	Subject characteristics, sample size	Stimulating agent, route	Result
Ghai K, <i>et al.</i> , 1995 ^[37]	21 prepubertal boys (11: CDGP, 10: HH)	Nafarelin, SC	Peak LH (RIA) < 7.2 IU/L Sensitivity: 90%; Specificity: 100%
Zamboni G, <i>et al.</i> , 1995 ^[38]	28 prepubertal boys (18: CDGP, 10: HH)	Triptorelin, SC	Peak LH (IEFA): 22.8 ± 7.8 IU/L in CDGP versus 4.0 ± 2.6 IU/L in HH
Ozkan B <i>et al.</i> , 2001 ^[36]	23 prepubertal boys (16: CDGP, 7: HH)	Triptorelin, SC	Peak LH: 33.2 ± 9.3 IU/L in CDGP versus 3.3 ± 2.6 IU/L in HH
Kauschansky A <i>et al.</i> , 2002 ^[39]	32 prepubertal boys (13: CDGP, 19: HH)	Triptorelin, SC	Peak LH (CLIA): 20.4 ± 7.5 IU/L in CDGP versus 2.4 ± 2.0 IU/L in HH

CDGP: Constitutional delay of growth and puberty, HH: Hypogonadotropic hypogonadism, SC: Subcutaneous, RIA: Radioimmunoassay, IEFA: Immunoenzymefluorometric assay, CLIA: Chemiluminescence immunoassay

was defined as $\leq 63.4\%$ of the baseline level on the basis of this study. In another study by Krause *et al.*, the utility of SHBG measurement in the differential diagnosis of patients with 46 XY DSD, ovotesticular DSD, and androgen insensitivity syndrome was studied.^[42] The mean nadir SHBG level was 51.6% in group 1 (46 XY DSD and ovotesticular DSD not on hormone replacement, n=11), while it varied between 80.1–80.7% in group 2 (androgen insensitivity syndrome, n=2). Notably, there was a significant SHBG decline (to 80.1% of baseline) in a subject with clinically apparent and genetically proven CAIS.

The literature on this test is sparse, and more studies are needed in the future to accurately define the thresholds for its interpretation. It is important to note that the test only supports the diagnosis of androgen insensitivity syndrome, which needs to be confirmed using a genetic test.

Summary

- Stanozolol SHBG test is used to support a diagnosis of androgen insensitivity syndrome
- A nadir SHBG $\leq 63.4\%$ of the baseline level is considered normal. The nadir SHBG levels range between 73 to 89% and 93 to 97% of the baseline in subjects with PAIS and CAIS, respectively.

Adrenocorticotrophic hormone (ACTH) stimulation test

This test is performed using synthetic ACTH 1–24 (Synacthen, Novartis). It is used to confirm the diagnosis of adrenal insufficiency and congenital adrenal hyperplasia (CAH). The low-dose stimulation test (using 1 μg ACTH) has been proposed to improve sensitivity for the diagnosis of secondary adrenal insufficiency.^[43] However, the 1 μg preparation is not commercially available and needs to be freshly prepared using 250 μg vial and normal saline (as a diluent), making the process cumbersome.^[44,45] Therefore, the use of a standard-dose test (250 μg for age >2 years, 125 μg for age 1–2 years and 15 $\mu\text{g}/\text{kg}$ during infancy) is preferred over the low-dose test in the clinical setting.^[46]

For primary adrenal insufficiency, in presence of unequivocally low serum cortisol ($<5 \mu\text{g}/\text{dL}$) and elevated ACTH (>2 -fold the upper limit of the reference range), the test can be skipped.^[46] For secondary adrenal insufficiency, this test should only be employed in patients with long-standing disease. If secondary adrenal insufficiency is of recent onset (<3 months), adrenal glands (not yet atrophied) would be capable of responding to standard ACTH stimulation, causing a false reassuring result. In such cases, the insulin tolerance test remains the gold standard for establishing the diagnosis. In patients with CAH, the ACTH stimulation test not only confirms the diagnosis (17-hydroxyprogesterone elevation) but also helps in the assessment of glucocorticoid reserve at baseline.^[47]

Indications

1. To confirm the diagnosis of adrenal insufficiency (primary and long-standing secondary insufficiency)

2. To confirm the diagnosis of CAH and assess the glucocorticoid reserve at baseline.

Protocol

Fasting is not required and the test can be performed irrespective of the time of the day.

Synacthen dose: age: <1 year: 15 $\mu\text{g}/\text{kg}$, 1–2 year: 125 μg , >2 year: 250 μg .

Administration: Intramuscular or intravenous (same intravenous catheter could be used for drug administration and blood sample collection).

Collect blood at 30 min and 60 min after the injection for measurement of cortisol (and 17-OH progesterone, if needed).

Interpretation

A peak serum cortisol below 18 $\mu\text{g}/\text{dL}$ (500 nmol/L) at 30 or 60 min indicates adrenal insufficiency.^[46] A stimulated (30 or 60 min) serum 17-hydroxyprogesterone $>10 \text{ ng}/\text{mL}$ (30.3 nmol/L) indicates CAH.^[47,48] Generally, the levels are $>100 \text{ ng}/\text{mL}$ (302.6 nmol/L) in patients with classical form, and between 10–100 ng/mL (30.3–302.6 nmol/L) in those with nonclassical form.^[49,50] The diagnosis of 21-hydroxylase deficiency CAH is mainly based on biochemical assessment and genotyping is suggested only when the biochemical results are equivocal or for genetic counseling.^[47]

It should be remembered that serum 17-hydroxyprogesterone elevation is not specific for 21-hydroxylase deficiency CAH. It may be elevated in patients with other forms of CAH (11- β -hydroxylase defect, 3 β -hydroxysteroid dehydrogenase deficiency and P450 oxidoreductase defect) and adrenocortical carcinoma.^[51–53] The elevated levels should, therefore, be interpreted in the background of clinical presentation (presence or absence of hypertension), biochemistry (other adrenocortical hormones), and radiology (mass lesion or bilateral adrenal hyperplasia).

Summary

- ACTH-stimulated serum cortisol $<18 \mu\text{g}/\text{dL}$ (500 nmol/L) indicates adrenal insufficiency
- ACTH-stimulated serum 17-OHP $>10 \text{ ng}/\text{mL}$ (30.3 nmol/L) indicates CAH due to 21-hydroxylase deficiency. Generally, the levels are between 10–100 ng/mL (30.3–302.6 nmol/L) in nonclassical CAH and $>100 \text{ ng}/\text{mL}$ (302.6 nmol/L) in classical CAH.

Dexamethasone suppression test (low-dose) (LDDST)

Dexamethasone is a potent glucocorticoid (about 30–40 times more potent than hydrocortisone), which is used to assess the responsiveness of pituitary corticotrophs to glucocorticoid negative feedback inhibition. It is the preferred agent for this purpose due to the lack of cross-reactivity with cortisol in the routinely employed assays.

Most patients with endogenous Cushing syndrome fail to suppress cortisol production in response to LDDST, making

it a useful diagnostic test. In addition, LDDST finds its use in the evaluation of patients with hyperandrogenism. Suppression of androgens in response to LDDST suggests non-tumorous hyperandrogenism (such as CAH).

Indications

1. Diagnosis of endogenous Cushing syndrome
2. To differentiate between non-tumorous and tumorous hyperandrogenism.

Protocol

Dexamethasone dose: weight <40 kg: 30 µg/kg/day in four divided doses q 6 hourly for 48 h; ≥40 kg: 0.5 mg q 6 hourly for 48 h.^[54]

Administer dexamethasone beginning at 9 am (9–3–9–3 schedules) or 12 pm (12–6–12–6 schedule) every 6 h for 48 h and obtain a sample for serum cortisol (and serum testosterone, if required) at 6 h (9–3–9–3 schedule) or 3 h (12–6–12–6 schedule) following the last dose.

Interpretation

Unsuppressed serum cortisol >1.8 µg/dL (50 nmol/L) on a 48-h LDDST is suggestive of endogenous Cushing syndrome.^[54] The 48-h LDDST is associated with improved specificity compared to the 1 mg overnight dexamethasone suppression test (ONDST). One should be wary of common sources of error in dexamethasone suppression tests-inadequate compliance, increased dexamethasone metabolism due to concomitant use of CYP3A4 inducing drugs and increased corticosteroid-binding globulin due to use of exogenous estrogen may all lead to false-positive results.^[55]

LDDST is also useful in the evaluation of patients with hyperandrogenism. Normalization or reduction of serum testosterone by >40% (compared to the baseline) suggests non-tumorous hyperandrogenism.^[56,57] This threshold was derived based on the study by Kaltsas *et al.* which evaluated serum testosterone response during the 48-h LDDST in 211 women with non-tumorous hyperandrogenism and 11 women with adrenal and ovarian androgen-secreting tumors.^[56] The median (range) percentage change in serum testosterone was -43 (-54 to +16) in the non-tumorous group and -17 (-32 to +23) in the tumorous group. None of the women in the tumorous group showed normalization of serum testosterone or >40% reduction from the baseline, while 88% of the women in the non-tumorous group fulfilled this criterion. Thus, lack of testosterone suppression was associated with 100% sensitivity and 88% specificity for the diagnosis of androgen-secreting tumors.

Summary

- Post-LDDST serum cortisol >1.8 µg/dL (50 nmol/L) is suggestive of endogenous Cushing syndrome.
- Normalization or reduction of serum testosterone by >40% (compared to the baseline) following LDDST is suggestive of non-tumorous hyperandrogenism.

GnRH agonist suppression test

This test (similar to LDDST) is helpful in the evaluation of patients with hyperandrogenism. Suppression of serum testosterone in response to GnRH agonists suggests LH-dependent ovarian hyperandrogenism (such as ovarian hyperthecosis).^[58] In patients with coexisting adrenal incidentaloma, this test may be especially helpful to direct the appropriate diagnostic workup and management (towards ovarian etiology).

Indication

1. To differentiate between LH-dependent and LH-independent hyperandrogenism.

Protocol

Injection leuprolide/triptorelin depot 3.75 mg intramuscular.

Obtain serum LH and testosterone at baseline and 4 weeks after the injection.

Interpretation

The reduction of serum testosterone by >50% (compared to the baseline) is indicative of LH-dependent ovarian hyperandrogenism.^[57-59] Virilizing ovarian neoplasms are expected to show an unsuppressed response to GnRH agonists (LH-independent hyperandrogenism). However, numerous cases of semi-autonomous virilizing ovarian neoplasm which continue to retain gonadotropin receptors, and show suppression in response to GnRH agonists have been described in the literature.^[59-62] In a study by Pascale *et al.*, five women with clinical features of virilization, serum testosterone >2 ng/mL (7 nmol/L) and normal serum DHEAS were evaluated with GnRH agonist suppression test.^[60] In each of these patients, the administration of GnRH agonist (Triptorelin 3.75 mg) was associated with suppression of gonadotropins and normalization of serum testosterone levels (at 3 weeks following the injection). Exploratory laparotomy was performed in all, and on histopathology, a diagnosis of virilizing ovarian neoplasm ($n = 3$) and bilateral hyperthecosis ($n = 2$) was established. The authors, thus, concluded that both ovarian virilizing neoplasms and hyperthecosis result in nonautonomous gonadotropin-dependent hyperandrogenism and cannot be differentiated using the GnRH agonist suppression test.

Summary

- Suppression of serum testosterone by >50% in response to the GnRH agonist suppression test is suggestive of LH-dependent ovarian hyperandrogenism
- This test is not reliable to differentiate between neoplastic and non-neoplastic causes of ovarian hyperandrogenism since many virilizing neoplasms are gonadotropin-dependent.

CONCLUSION

Dynamic testing is fundamental to the evaluation of adrenal and gonadal function in children and adults. These tests play

an important role in clinical decision-making in various endocrine disorders, especially disorders of sex development and disorders of growth and pubertal maturation. This article reviews various dynamic test protocols for the evaluation of adrenal and gonadal dysfunction, along with their clinical application and interpretation.

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Conflicts of interest

There are no conflicts of interest.

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