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Review Article

A Practical Approach to Cushings Syndrome

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Abstract

Cushings syndrome is one of the common endocrine disorder. Diagnosis of Cushing's syndrome (CS) and identification of the aetiology of hypercortisolism can be challenging. The Endocrine Society clinical practice guidelines recommends one of the four tests for initial screening of CS, namely, urinary-free cortisol, late night salivary cortisol, overnight dexamethasone suppression test or a longer low-dose dexamethasone suppression test, for 48 hours. Confirmation and localisation of CS requires additional biochemical and radiological tests. Radiological evaluation involves different imaging modalities including MRI with or without different radio-nuclear imaging techniques. Invasive testing such as bilateral inferior petrosal sinus sampling may be necessary in some patients for accurate localisation of the cause for hypercortisolism. This best practice review discusses a practical approach for the diagnostic evaluation of CS with a brief discussion on differential diagnoses, and cyclical CS, to enhance the skills of clinicians and laboratory personnel.

Keywords: *Cushings Syndrome; Hypercortisolism; Glucocorticoid*

Introduction

Harvey Cushing introduced the term "pluriglandular syndrome" on September 6, 1911 but later described seven patients in 1932 with basophilic adenomas and described it as clinical syndrome of glucocorticoid excess [1].

The incidence of Cushing's syndrome is 2 to 3 cases per million population per year [2] with female to male ratio from 3:1 to 10:1 [3] and 75 cases per 1 million population in undiagnosed Cushing's syndrome [4]. Whereas in poorly controlled hypertension and diabetes prevalence of hypercortisolism is 2 - 5% [5]. Iatrogenic Cushing's is more common than the endogenous Cushing's in our part of the world.

Cushing's syndrome (CS) results from chronic exposure of endogenous or exogenous glucocorticoid excess and associated with increased morbidity and mortality from musculoskeletal, metabolic, thrombotic, infectious and cardiovascular complications [4].

Etiology and pathophysiology

The etiology of Cushing's syndrome can be divided into those that are adrenocorticotropic hormone (ACTH) dependent and those that are ACTH independent (Table 1). The ACTH-dependent forms are characterized by excessive ACTH production from a corticotroph adenoma (known as pituitary-dependent Cushing's syndrome or Cushing's disease), from an ectopic tumoral source (ectopic ACTH syndrome), or (rarely) from normal corticotrophs under the influence of excessive corticotrophin releasing hormone (CRH) production (ectopic CRH secretion) [6].

Adrenocorticotropic hormone (ACTH)-dependent causes: • Cushing's disease (pituitary dependent) 70% • Ectopic ACTH syndrome 5 - 10% • Ectopic corticotropin releasing hormone (CRH) syndrome (rare) • Macronodular adrenal hyperplasia < 5% • Iatrogenic (treatment with 1 - 24 ACTH)
ACTH-Independent causes: • Adrenal adenoma (10%) and carcinoma (5 - 10%) • PPNAD* and Carney's syndrome < 5% • McCune Albright syndrome (rare) • Aberrant receptor expression [GIP, IL-1b (rare)] • Iatrogenic (cortisone therapy)
Pseudo-Cushing's syndromes: • Alcoholism • Depression • Obesity • PCOS

Table 1: *Classification of causes of Cushing's syndrome.*
** PPNAD: Primary Pigmented Nodular Adrenal Hyperplasia.*
Abbreviations: GIP: Gastric Inhibitory Polypeptide; PCOS: Polycystic Ovary Syndrome.

ACTH-independent forms include unilateral disease (adenoma and carcinoma), bilateral disease (primary pigmented nodular adrenal disease, McCune-Albright syndrome, and macronodular adrenal disease related to aberrations of the cyclic AMP signaling pathway, or caused by ectopic expression of G-protein-coupled receptors), and hyperfunction of adrenal rest tissue.

Phenotypic features of CS may occur from overactivity (physiological) of the hypothalamic-pituitary-adrenal (HPA) axis in conditions such as chronic alcoholism, psychiatric disorders, severe obesity, poorly controlled diabetes and PCOS, known as pseudo-Cushing's syndrome (PCS) [7].

Clinical features we are aware of that the clinical features may be sensitive for CS diagnosis but not specific for the disease. Some patients may present with fluctuating symptoms and signs due to a rhythmic variation in cortisol secretion those results in a state of cyclical CS [8].

The classic clinic features of CS are as follows [9]:

- **Obesity and weight gain:** Due to stimulation of adipogenesis through transcriptional activation of adipocyte differentiation gene like lipoprotein lipase, glycerol 3-phosphate dehydrogenase and leptin. And also by reducing CRH (which normally has anorexic effect).

- **Gonadal dysfunction:** Like menstrual irregularities, loss of libido, hypogonadotropic hypogonadism - due to direct inhibitory effect of cortisol on GnRH pulsatility and LH/FSH secretion, and it is reversible on correction of the hypercortisolism.

- **Hirsutism:** Hirsutism is frequently found in female patients because of ACTH-mediated adrenal androgen excess.
- **Psychiatric abnormalities:** Like depression, paranoia and overt psychosis occurs in 50% of patients.
- **Lost height:** Due to osteoporotic vertebral collapse which is assessed by measuring the patient's sitting height or comparing the height with arm span (in normal subjects, height and arm span should be equal). Unexplained osteoporosis with increased tendency for fractures is another sign of protein wasting.
- **Thinning of the skin:** Easy bruising and plethoric appearance (due to hypercortisolaemia) and hyperpigmentation (due to over-stimulation of melanocyte receptors by POMC- derived peptides) is a strong clinical indicator to suspect ACTH-dependent CS.
- **Hypertension:** Is another prominent feature, occurring in up to 75% of cases - due to Increased cardiac output, activation of renin angiotensin system (by increasing hepatic production of angiotensinogen).
- **Infections:** Are more common in patients with CS - due to suppression of the normal inflammatory response.
- **Metabolic and endocrine features:** Glucose intolerance occurs, and overt diabetes mellitus is present in up to one third of patients - due to stimulation of hepatic lipoprotein synthesis.

The classic clinical features that discriminate Cushing's syndrome are the easy bruising, facial plethora, proximal myopathy and the purple striae of more than 1 cm width [10]. Predominantly, these are all the signs of protein wasting which are very specific for Cushing's syndrome. Weight gain and centripetal obesity are the most common signs of Cushing's syndrome centripetal obesity, fat deposition occurs in areas like thoracocervical spine, supraclavicular region, cheeks and the temporal regions, giving rise to the rounded moon facies. As most patients with obesity will have the more non-specific CS signs such as central obesity, fatigue, hyper- tension, and decreased libido [11].

Clinical features which are more common in exogenous Cushing's syndrome are glaucoma, cataract, benign intracranial hypertension, pancreatitis and avascular necrosis of the head of the femur (Table 2).

Broder., *et al.* [12] recently demonstrated 10 key conditions observed in patients with CS that may help easy identification of patients with CD from those without the disease. The relative risk (RR) of any one of these conditions like localised adiposity, hirsutism, facial plethora, polycystic ovary syndrome, abnormal weight gain, hypokalaemia, deep venous thrombosis, muscle weakness, female balding and osteoporosis, was ≥ 5 times in CD cases compared with non-CD cases.

Subclinical Cushing's syndrome: (SCS) is due to alteration in the HPA axis without overt signs or symptoms of hypercortisolism. SCS presents with biochemical evidence of cortisol excess without the classical phenotypic abnormalities [13].

Investigation of patients with suspected Cushing's syndrome

Patients with suspected Cushing's syndrome should be screened with one of the following tests [14]. Urine free cortisol (UFC; at least two measurements), late night salivary cortisol (two measurements), 1-mg overnight dexamethasone suppression test, and long lower-dose dexamethasone suppression test. In case of an abnormal test result, a repeat test (any other than the one performed during screening should be done to confirm the diagnosis [14]. A concordant result confirms the diagnosis of Cushing's disease [15].

As exogenous Cushing's syndrome is more common than the endogenous one, exclusion of this condition is very important. Apart from the history, basal 8:00 AM cortisol value will differentiate the exogenous from the endogenous Cushing's syndrome. A suppressed cortisol value with Cushingoid features suggests exogenous cause except for cyclical Cushing's syndrome which can be suppressed sometimes. Following are the various tests for screening (Table 3).

A. Features that best discriminate Cushing's syndrome		
Easy bruising		
Proximal myopathy		
Facial plethora		
Red purple striae (> 1 cm)		
In children, weight gain with decreasing growth velocity		
B. Clinical features that are common and/or less discriminatory		
Symptoms	**Signs**	**Overlapping conditions**
Depression	Dorsocervical fat pad	Hypertension
Fatigue	Facial fullness	Incidental adrenal mass
Weight gain	Supraclavicular fullness	Vertebral osteoporosis
Back pain	Thin skin	PCOS
Changes in appetite	Peripheral edema	Type 2 DM
Less concentration	Acne	Hypokalaemia
Decreased libido	Hirsutism	Kidney stones
Impaired memory	Poor healing	Unusual infections
Insomnia		
Irritability		
Children:		
Slow growth		
Abnormal virilization		
Short stature		
Delayed precious puberty		

Table 2: *Overlapping conditions and clinical features of Cushing's syndrome.*

Test	Method	Value	Sensitivity	Specificity	Comments
ONDST	1 mg dexamethasone the night before collection	> 1.8 ug/dl	98 - 100%	88%	Easy to perform on OP
UFC	24 hr urine collection	> 100 ug/24hrs	95 - 100%	90 - 95%	More than 4 times Upper limit suggests
Salivary cortisol	Midnight saliva	> 0.27 ug/dl	100%	96%	Convenient and non stressful
Midnight cortisol	Midnight serum	> 7.5 ug/dl (midnight)	94%	100%	Stress free sample -problem
LDSST	0.5 mg dexamethasone for 48hrs	> 1.8 ug/dl (awake)	98 - 100%	97 - 100%	Used to confirm Cushing

Table 3: *Screening tests.*

Abbreviation: LDSST: Low Dose Dexamethasone Suppression Test; UFC: Urinary Free Cortisol; ONDST: Overnight Dexamethasone Suppression Test; Two screening tests are required while confirming hypercortisolemia taking into consideration of their availability.

The serum cortisol level measured the morning after a 1-mg dexamethasone dose given the night before is generally < 1.8 mg/dl in healthy subjects [5]. Although a post dexamethasone level of > 5 mg/dl is clearly suggestive of Cushing's syndrome, intermediate values require additional testing. The serum cortisol level in approximately 5% - 8% of patients with Cushing's syndrome is suppressed to < 1.8 mg/dl, and additional testing is required to confirm the diagnosis.

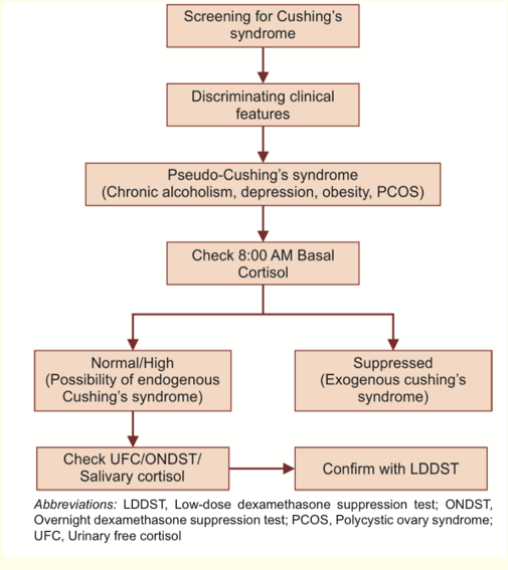
Even though 24 hr UFC may be useful to confirm Cushing's syndrome, its sensitivity and specificity are not optimal as an initial screening test and it cannot be considered as a universal single screening test for the detection of CS [16].

Several studies have validated late-night salivary cortisol as a useful screening test for hypercortisolism [17] because cortisol in saliva is in equilibrium with free plasma cortisol, is independent of salivary flow rate, stable at room or refrigerator temperatures and easy to perform at home. Sample collection of saliva for LNSC is usually performed between 23:00 and 00:00 by asking the patient to chew a cylindrical cotton swab (salivette) for 2 - 3 min and keeping the sample in a plastic tube at 2 - 8°C overnight in order to be processed the following morning. Clinicians should carefully explain the procedure to the patient and should ensure timing of sample collection has been accurate before interpreting the result.

LDDST was earlier known as Liddle test with a cut-off serum cortisol level of 1.8 mg/dl (50 nmol/L) measured on the 2nd day of the test. Most of the reports have shown a diagnostic accuracy similar to or slightly lower than that of the conventional overnight 1-mg dexamethasone suppression test [18].

Physician ordering the above tests must be aware of the collection methods and various assays that are available. The interpretation of the results depends upon the assay used and the local laboratory calibration [19] because drugs (estrogen containing pills, increase cortisol binding globulin cause false positive results) can interfere with the measurement of cortisol [7]. Sensitivity and specificity of the various tests depend upon the cut off value that was taken. In case of any equivocal results, rescreening is required after 6 months.

Pseudo-Cushing's syndrome and exogenous Cushing's syndrome need to be ruled out before the confirmation of true Cushing's syndrome. Discriminatory features help to differentiate pseudo- Cushing's syndrome clinically, and biochemically by the dexamethasone-CRH test (Flow chart 1).



Flow Chart 1: Algorithm for screening for Cushing's syndrome.

LDSST: Low Dose Dexamethasone Suppression Test; ONDST: Overnight Dexamethasone Suppression Test; PCOS: Polycystic Ovary Syndrome; UFC: Urinary Free Cortisol.

Investigating the cause of cushing's syndrome

The next step is to distinguish between ACTH-dependent and ACTH-independent causes of Cushing's syndrome.

Measurement of ACTH

Adrenocorticotropic hormone measurement will help us to differentiate ACTH from the non-ACTH-dependent Cushing's syndrome. Sample should be collected with adequate precautions in the morning at 9:00 AM and measured because ACTH is rapidly degraded by the plasma proteases. Hence, it should be collected in a pre-chilled ethylene diamine tetraacetic acid (EDTA) tube to avoid falsely low values.

Patients with suppressed morning plasma ACTH (< 5 pg/ml) need adrenal imaging. Normal or elevated ACTH (> 15 pg/ml) is suggestive of ACTH-dependent CS. Plasma ACTH values between 5 and 15 pg/ml are equivocal [5].

Other tests that are available to differentiate the cause of Cushing's syndrome are given below in table 4.

Test	Method	Value	Sensitivity	Specificity	Comments
CRH test	1 ug ovine or Human CRH	ACTH > 35% Cortisol > 20%	93%	100%	Used to diagnose Cushing's disease
HDSST	2 mg Dexamethasone for 48 hrs	8:00 am cortisol > 90% suppression of basal	60 - 70%	100%	Not much used now unreliable
BIPSS	petrosal sinus Sampling	Basal central: peripheral 2:1 CRH stimulated > 3:1	95%	100%	Used to differentiate pituitary and ectopic

Table 4: *Tests to diagnose cause of Cushing's syndrome.*

Abbreviation: CRH: Corticotropin Releasing Hormone; HDSST: High-dose Dexamethasone Suppression Test; BIPSS: Bilateral Inferior Petrosal Sinus Sampling.

Patients with equivocal ACTH values may be subjected to CRH stimulation test and the increment of the ACTH and cortisol response are suggestive of Cushing's disease [20], but the clinical clues like pigmentation and imaging has to be considered before coming to a conclusion.

High-dose dexamethasone suppression test (HDDST) though used to differentiate the pituitary from ectopic ACTH-dependent Cushing's syndrome is not commonly used now-a-days because the sensitivity and the specificity of this test depend on the amount of cortisol suppression and can be suppressed in carcinoid syndrome producing ACTH. High-dose dexamethasone suppression test shows suppression by only less than 50% of the basal cortisol value in about 80% of the patients with Cushing's disease. And there are high numbers of false positive tests (10 - 30%) in ectopic Cushing's syndrome [20].

Bilateral Inferior Petrosal Sinus Sampling is done when ACTH-dependent Cushing's syndrome and the pituitary imaging are normal or having a lesion less than 6 mm and to lateralize the microadenoma within the pituitary. A basal central, peripheral ratio of 2:1 or CRH-stimulated ratio of more than 3:1 is suggestive of the Cushing's disease. But this test requires expertise to do the test and to catheterize into the veins [21].

Imaging in cushing's syndrome

Once the diagnosis of ACTH or non-ACTH-dependent Cushing's syndrome is made appropriate imaging is done.

In non-ACTH- dependent Cushing's CT/MRI of the adrenal gland is done but CT gives the better resolution of the adrenal anatomy. Given the high incidence of both pituitary and adrenal incidentalomas imaging should be done only after confirming the hypercortisolism and ACTH values [22].

In ACTH-dependent Cushing's syndrome, the first step is to do MRI of the pituitary with gadolinium enhancement. Use of dynamic MRI (with IV gadolinium) with spoiled gradient sequences increases the sensitivity of detection [22]. But caution has to take in diagnosing microadenomas as 10% of general population can have pituitary incidentalomas [22].

If the pituitary imaging is negative, then imaging of the head and neck, thorax and abdomen are done to find the ectopic source of production of the ACTH [mostly thymoma, carcinoid, pheochromocytoma, medullary thyroid carcinoma (MTC) or malignancy]. In patients with suspected ectopic ACTH and not localized, special imaging like In-pentetreotide scintigraphy/ [23] F-uorodeoxyglucose positron emission tomography (FDG-PET) is done.

The clinical clues to differentiate ectopic from the Cushing's disease are male sex, atypical presentation, fast progression, very high cortisol/ACTH values and severe hypokalemia.

Management of cushing's syndrome

ACTH-dependent cushing's syndrome

Excision of pituitary microadenoma by an expert surgeon leads to postoperative cure rate of 65 - 90%. Rates of cure are better in patients with well-localized tumor. Rates of cure are < 65% in patients with pituitary macroadenoma. Postoperative levels of cortisol secretion provide good prognostic information regarding the outcome in patients with Cushing's disease. Patients whose cortisol levels decrease to less than 55.2 - 82.8 nmol/l (preferably undetectable) within 24 - 72 h after surgery usually have a clinical and biochemical remission [5].

If the ectopic ACTH-secreting tumor is benign and amenable to surgical excision, such as in a lobectomy for a bronchial carcinoid tumor, the chance of cure of Cushing's syndrome is high. However, if significant metastatic disease is present, surgery is not curative although it may still be of benefit in selected cases. In this situation, bilateral adrenalectomy may be an option if medical management fails.

Non-ACTH-dependent cushing's syndrome

Patients with persistence of hypercortisolism postoperatively can be subjected to repeat surgery, radiotherapy or bilateral adrenalectomy [24].

Pituitary radiation (conventional or gamma knife) has been recommended as a means to treat Cushing's disease when surgery fails. Although remission rates of 53 - 100% have been reported with conventional radiotherapy and rates as high as 76% have been reported in patients using gamma knife radiotherapy, the normalization of cortisol secretion takes 12 - 36 months. Bilateral adrenalectomy is a definitive cure for patients with persistent hypercortisolism. It is indicated when enzyme inhibitors are unable to achieve eucortisolemia. Patients require to be put on lifelong glucocorticoid and mineralocorticoid replacement. They require monitoring of ACTH and MRI of pituitary due to risk of Nelson's syndrome.

Medical management

Following are the indications for medical management in Cushing's syndrome (Table 5):

- Preoperatively to control cortisol levels
- Patients in whom surgery is contraindicated
- Patients in whom surgery/radiotherapy is failed.

Inhibits steroidogenesis	Metyrapone Ketoconazole	Days to weeks	Cause GI side effects and ketoconazole cause insufficiency
Inhibits glucocorticoid receptor	Mifepristone Etomidate	Days	Etomidate can be used for acute control
Modulate ACTH release	Cabergoline Octreotide SOM-320 Pasireoride	Months	Effect may be variable and slow
Adrenolytic	Op'DDD (mitotane)	Days to weeks	Effect may be permanent

Table 5: *Medical therapy of Cushing's syndrome.*

Ketoconazole, metyrapone and mitotane inhibit steroidogenesis. Metyrapone and ketoconazole are the ones who have rapid onset of action. Ketoconazole has been used in long-term treatment, with the dose ranging from 200 to 1,200 mg per day Ketoconazole and metyrapone lose their efficacy as elevated ACTH secretion leads to enhanced synthesis of steroids and triggers escape. Mitotane is an adrenolytic drug and has a prolonged effect may develop hypocortisolemia. Overdosing with the drugs can lead to adrenal insufficiency.

Mifepristone is the first potent glucocorticoid receptor antagonist with long half-life and lacks biochemical markers to monitor response and may be associated with significant adrenal insufficiency [24].

Dopamine agonists (cabergoline and bromocryptine) can be used in tumors which secrete prolactin. A new multi ligand somatostatin analogue (SOM 230; Pasireotide) has been shown to be effective in Phase II trials.

Current concepts

Recently newer imaging techniques such as MET-PET, FDG-PET and Gallium-SSTR-PET/CT are emerging as useful tools in localisation studies. Genetic testing may be considered if clinical picture suggests an inherited abnormality causing the disease [25]. There are case report [26] mentioning somatic USP8 mutations are common in adenomas causing CD.

Conclusion

Clinical decision making for patients with suspect hypercortisolism involves a complex diagnostic assessment. Cushing's syndrome remains one of the most challenging endocrine pathologies. The management of Cushing's syndrome depends on the exact knowledge of its various causes, paying attention to the many potential diagnostic pitfalls. The choice of test, the modality of specimen collection (blood, urine, and saliva), the quality of measurement (assay methodology and standardization) and close dialogue among endocrinologists, chemical pathologists, and neuroradiologists are key factors for optimal care of patients.

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