

## DIAGNOSIS OF CUSHING'S SYNDROME: BIOCHEMICAL INVESTIGATIONS

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### Abstract

Cushing's syndrome remains an uncommon diagnosis with majority of non-specific and few specific clinical features suggestive of the condition. Results of biochemical investigations are often affected by confounding factors making diagnosis of Cushing's syndrome difficult and localisation of the disease even more challenging. Careful assessment of the individual patient and use of the most suitable test in that patient may allow improved outcome in diagnosing and localising the condition to devise an appropriate management plan.

**Key words:** Cushing's syndrome, hypercortisolism, dexamethasone suppression test, Pseudo-Cushing's

### Introduction

Patients with Cushing's syndrome can present with varied signs and symptoms, but most patients are referred for screening in view of weight gain and unable to lose weight. Obesity is almost invariably present in Cushing's syndrome but is of little diagnostic value due to its high prevalence in the population as a whole, particularly in women between the ages of 20 and 50 years who are the most likely age group to be suffering from Cushing's syndrome.<sup>[1]</sup> Clinical acumen, however, is insufficient to establish the diagnosis, and confirmation depends on biochemical evidence of hypercortisolism using laboratory investigations. Since obesity is increasing at an alarming rate throughout the world (estimated >300 million obese people worldwide),<sup>[2]</sup> the number of patients' referrals for Cushing's screening has also increased. Over the previous decade or so, there has been an increased awareness amongst general population about obesity-related complications and they are recognising obesity syndrome as a medical condition in itself which can adversely affect almost any system of the body. There has been a visible drive amongst obese patients and their primary care physicians who encourage them to lose weight. Cushing's syndrome remains a rare diagnosis but is a potentially treatable condition and an important diagnosis to be considered in obese patients particularly those who have noticed a sudden increase in their weight

and concentrated in central parts of their bodies and is resistant to dietary and pharmacological treatments. Although hypercortisolism can affect almost any system of the body, most patients are referred for Cushing's screening for weight-related symptoms. Majority of such patients have not got Cushing's syndrome, and screening tests are negative. Cushing's syndrome is suspected on clinical symptoms and signs which is then confirmed or refuted with biochemical investigations. Imaging studies are required to localise the abnormality to help plan the management strategy. Most patients would have only one screening test, and only if it is positive or the results are not decisive, further investigations would be carried out. Localisation investigations are generally carried out if these screening tests confirm biochemical evidence of hypercortisolism.

A screening test is expected to pick up all the cases with a particular condition. A good screening test should have a high sensitivity. The test should be simple and easy to be performed, ideally on outpatient basis, and the results should be easy to interpret.<sup>[3]</sup> There is no single test which fulfills all these criteria;<sup>[4]</sup> however, high sensitivity is probably the most important criteria so that none of the patients with that particular condition are missed by a screening test. One would like all the investigations to be simple but investigations to screen patients for a rare disease where most of the results are expected to be negative should particularly be simple to allow most patients to undergo screening. Complex and cumbersome

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investigations for screening rare conditions would not attract many patients. Cortisol level is measured in blood, urine and saliva to assess if it is being produced in excess. Overnight dexamethasone suppress test (ONDST), low-dose dexamethasone suppression test (LDDST), midnight cortisol level (MCL), 24 h urine-free cortisol (24 h Manchester Royal Infirmary) assessment and salivary cortisol levels are usually run to screen patients for cortisol hypersecretion. In most patients, one or two of these screening tests are completed and further investigations are conducted if the results of these screening tests confirm hypercortisolism. Each of these screening tests has its limitations, and one may be the preferred investigation in a given clinical scenario.

### Physiological Hypercortisolism

Cortisol is a steroid hormone synthesised from cholesterol in zona fasciculata of adrenal glands. Cortisol production in normal individuals is under the stimulatory effect of adrenocorticotropic hormone (ACTH) of the anterior pituitary gland, and ACTH production, in turn, is regulated by corticotropin-releasing hormone (CRH) of the hypothalamus. This stimulatory effect of CRH/ACTH is balanced by the inhibitory effect of cortisol by its negative feedback on CRH/ACTH production once there are sufficient levels of cortisol in blood circulation. Cortisol secretion follows a circadian rhythm with levels highest early in the morning and then continues to drop during the day. Its level is almost negligible at midnight (11 pm–2 am)<sup>[5]</sup> before it starts to rise again from early morning. Cortisol production not only fluctuates during the day but it also fluctuates in response to environmental factors and individuals' general health. Stressful situations are believed to increase cortisol levels and most patients in intensive care unit of a hospital will have high cortisol levels. This excessive production is transient and is essential for an individual to cope with the stressful situation. This state of hypercortisolism returns back to physiological range once the stimulus is over. The hypercortisolism does not need treatment in these conditions; however, the primary condition should be treated to reduce the duration of tissue exposure to high circulating level of cortisol. Ideally, cortisol level should not be assessed during these stressful conditions as a false positive rate of screening investigations is likely to be high and patients' cortisol production should be assessed once patients are no longer

in a stressful state. Once released in circulation, cortisol circulates predominantly bound to a protein called cortisol-binding globulin (CBG) and albumin. In circulation, about 90% of the cortisol circulates bound to CBG<sup>[6]</sup> and only <10% cortisol is circulating free.<sup>[4]</sup> CBG-bound cortisol serves as a reservoir and free cortisol is released to tissues locally for local metabolism.

### Pathological Hypercortisolism

Prolonged and persistent exposure of tissues to high circulating cortisol level usually secondary to the pathology of CRH/ACTH or cortisol-producing organs leading to pathological changes in tissues is termed pathological hypercortisolism. The dynamic equilibrium of CRH/ACTH and cortisol production is pathologically disturbed either by uncontrolled production of ACTH which then leads to excessive cortisol production (Cushing's disease) or ACTH independent uncontrolled production of cortisol due to adrenal gland pathology (Cushing's syndrome). For the sake of this manuscript, we will use the term Cushing's syndrome to cover all of these (Cushing's disease, ectopic CRH/ACTH production and Cushing's syndrome). The production of CRH/ACTH and cortisol in these conditions is uncontrolled as pituitary gland and adrenal glands partially or completely no longer respond to the physiological mechanisms of stimulation and negative feedback inhibition. It is important to recognise that some patients of Cushing's syndrome may partially respond and maintain negative feedback inhibition at higher cortisol levels in circulation. Majority of such patients are suffering from ACTH-dependant Cushing's of pituitary origin. In majority of patients, Cushing's syndrome is caused by pituitary tumours, adrenal tumours (benign and malignant), adrenal macronodular hyperplasia, bilateral primary pigmented nodular adrenal hyperplasia, part of McCune-Albright syndrome and Carney's complex.

Weight gain and obesity, proximal myopathy, hyperglycaemia and diabetes, hypertension, easy bruising, psychotic episodes and osteoporosis are some of the common symptoms and signs of hypercortisolism<sup>[7]</sup> as shown in Table 1.

Screening laboratory investigation for the diagnosis of Cushing's syndrome is measurement of urinary free cortisol (UFC).

**Table 1: Signs, Symptoms and conditions associated with Cushing's syndrome**

Signs	Symptoms	Associated conditions
Weight gain and central obesity	Tiredness and lethargy	Diabetes Hypertension
Moon face Buffalo hump	Generalised aches and pains	Obesity
Thin skin with easy bruising	Joint pains	Osteoarthritis
Purplish striae	Anxiety	Sleep apnea
Pigmentation	Poor concentration	Fatty liver Osteoporosis
Proximal myopathy	Psychological symptoms	Psychiatric disorders
Muscle atrophy	Migraines	Cognitive impairment
Acne	Poor memory	Menstrual disorders and Hypogonadism
Virilisation frontal balding		
Hirsutism		
Generalised edema		Growth retardation

24 h UFC used to be considered a gold standard test for hypercortisolism screening. Subsequently, some studies suggested that its sensitivity was not as good as it was thought and ONDST had better sensitivity than 24 h UFC.<sup>[8]</sup> Ceccato *et al.* conducted a study in 2015 and found that 24 h UFC achieves the best diagnostic accuracy compared with ONDST and MCL if processed properly using liquid chromatography with tandem mass spectrometry method.<sup>[9]</sup> It is a good screening test as this is an outpatient investigation and patient can collect urine at home and return the sample to laboratory once the collection is completed.

Once cortisol is released from adrenal glands in blood circulation, it circulates predominantly bound to CBG. The uncontrolled excessive production of cortisol in Cushing's syndrome patients overwhelms available CBG and it starts circulating as free biologically active cortisol in blood circulation. CBG-bound cortisol stays in circulation as globulin is a large protein and is not filtered

through glomerulus membrane during ultrafiltration, but the free cortisol (cortisol not bound to CBG) is filtered and excreted in urine. 24 h UFC is helpful in situations when CBG is high and total cortisol (CBG bound cortisol + free cortisol) is high due to high CBG-bound cortisol. 24 h UFC in these situations will show normal urinary cortisol levels as free cortisol is not high. CBG is high in patients taking oestrogen (contraceptive pill and hormone replacement therapy), drinking alcohol in excessive amounts, obesity and certain medicines.<sup>[11]</sup> Since 24 h UFC depends on renal excretion of cortisol, this test becomes less reliable when renal functions are not normal (eGFR <60 ml/min) (10(13)). In addition, high fluid intake, contamination and incomplete collection can also make the results unreliable.<sup>[10]</sup> One could use creatinine: Cortisol in such patients instead of total 24 h UFC. A high creatinine:cortisol ratio is suggestive of high circulating free cortisol levels. Creatinine level could also help to assess proper urine collection. Urine collection must be repeated if creatinine levels are <1.5g per day for men and <1g per day for women.<sup>[11]</sup> The test had a false-positive rate of 3.3% and false-negative rate of 5.6% in pooled data of chronically ill, obese and non-obese 479 individuals<sup>[12]</sup> Most laboratories use assays with normal cortisol level from 220 to 330 nmol/24 h (80–120 µg/24 h). It shows a sensitivity of 100% and specificity of 98%,<sup>[13]</sup> but a significant number of pseudo-Cushing's syndrome patients have high urinary cortisol levels posing a challenge in using this test alone to screen such patients.

Patients with 24 h UFC level more than 4 times the upper normal limit of 24 h UFC in the presence of clinical features of Cushing's syndrome would not require any more biochemical investigations to confirm the diagnosis of Cushing's syndrome. These patients should then undergo further investigations to localise the source of disease.

Cortisol secretion follows a circadian rhythm and its level is highest early in the morning and drops to minimal levels at night. Most patients with Cushing's syndrome lose this circadian rhythm and do not tend to follow this pattern of cortisol secretion. Two separate small studies assessed cortisol levels in urinary samples collected between 20:00–24:00 h and 22:00–23:00 h.<sup>[14,15]</sup> The results of these studies were encouraging, but there was a possibility of slight overlap between Cushing's syndrome patients and

very obese patients. These investigations can be of use in those patients where 24 h urine collection is not possible and other screening investigations cannot be used.

### ONDST

In many endocrine disorders, dynamic endocrine tests are run to assess hyper- or hypo-secretion of a hormone. In most cases, one would need to try to suppress a particular hormone if suspecting hypersecretion of that hormone and to stimulate a hormone secretion when suspecting hyposecretion of that hormone. 1 mg dexamethasone tablet taken at 11 pm is expected to suppress CRH/ACTH secretion, and cortisol level should be <50 nmol/l in the blood sample collected at 8 am the following morning in normal individuals. 1 mg dexamethasone is expected to sufficiently raise steroid level in blood circulation to suppress CRH/ACTH production. Dexamethasone 1 mg should be taken at 11 pm as CRH/ACTH secretion starts to rise past midnight. CRH/ACTH secretion is inhibited in normal subjects by negative feedback if there are already sufficient steroids circulating in blood. Dexamethasone is not detected by assays used by most laboratories to assess cortisol level. This is a simple test and many patients prefer this over other screening tests. The reliability of the test relies on the sufficient rise in steroid levels in circulation following ingestion of 1 mg dexamethasone tablet. Dexamethasone is metabolised in liver, but there is interperson variability in normal subjects in metabolising dexamethasone. Slow metabolisers will have higher than required circulating levels of dexamethasone, and fast metabolisers may end up lower than required circulating dexamethasone levels. Dexamethasone levels should be at least >5.6 nmol/L (0.22 µg/dl) to ensure reliable results.<sup>[16]</sup> Serum dexamethasone levels should be assessed where such a facility is available; however, the availability of such an investigation is limited to a few centres only. To overcome this potential possibility of fast metabolism in some patients, it has been suggested that ONODST can be repeated with 2 mg dexamethasone in patients with negative test results with 1 mg dexamethasone and high clinical suspicion of Cushing's syndrome. The test has a reported sensitivity of 97–100%<sup>[17]</sup> and specificity of 87.5%.<sup>[18]</sup>

### MCL

This screening test is based on loss of circadian rhythm of cortisol secretion in patients of Cushing's syndrome.

Cortisol level is assessed in a blood sample collected at midnight. This test is not usually used as first-line screening test in majority of patients as patients are required to be admitted to hospital to collect blood sample at midnight. The sample has to be collected within 5 min of patient waking up or through a previously inserted indwelling catheter in case of awake patients<sup>[19]</sup> to avoid false-positive results. This is a good test to exclude Cushing's syndrome, and if a patient achieves cortisol level <50 nmol/l on a midnight blood sample, they may not need any further investigations. The sensitivity of this test was found to be 100% in one study and in presence of clinical features may not require any further screening tests before localising investigations.<sup>[19]</sup>

### LDDST

This test can be considered as a second-line investigation. This 48-h long test is usually performed once at least one of the screening tests has confirmed high cortisol level. In a total of eight doses, each of 0.5 mg dexamethasone is to be taken every 6 h and blood sample is collected 6 h after the last dose. In normal individuals, dexamethasone will suppress CRH/ACTH secretion, and cortisol level at the end of test will be <50 nmol/l. The reliability of the test is believed to be increased by longer exposure of CRH/ACTH-producing organ to high cortisol blood levels. This test is believed to be 98% sensitive and specific for the diagnosis of Cushing's syndrome. Since this test is more specific than other screening tests, a negative result would be strongly suggestive of the absence of Cushing's syndrome in the patient. The test has a reported sensitivity and specificity of 97–100%.<sup>[17,20]</sup>

### Localising Tests

#### ACTH assessment

Once biochemical investigations confirm pathological excessive production of cortisol, further investigations are focussed to find out if it is ACTH-dependant or ACTH in-dependant Cushing's syndrome. Assessment of ACTH level in most cases is very helpful. Suppressed ACTH in the presence of high cortisol level is strongly suggestive of adrenal source; however, normal or high ACTH in the presence of high cortisol is suggestive of ACTH-dependant Cushing's syndrome. It is recommended to assess ACTH levels on more than one occasion to confirm diagnosis

as some patients of pituitary Cushing's syndrome can present with suppressed ACTH levels on some of their blood samples.<sup>[21,22]</sup> Imaging studies should be focussed on adrenal glands in patients with undetectable or suppressed ACTH levels. Normal or high ACTH level patients require further investigations to confirm the source of CRH/ACTH to plan further management.

### High-Dose Dexamethasone Suppression Test (HDDST)

This test is based on the belief that patients with pituitary Cushing's syndrome respond to higher cortisol level in blood circulation and suppress CRH/ACTH secretion of pituitary gland leading to reduction in cortisol level circulation. The original test was performed by taking 2 mg dexamethasone every 6 h over 48 h. Urinary 17 hydroxycorticosteroid (17 OHCS) or UFC in a 24 h urine sample was used to assess the suppression in cortisol production from day 1 to day 3 of the test. Over time, only UFC was suggested to be assessed in urine samples as it was found as specific and sensitive as 17 OHCS. Subsequently, plasma cortisol level was recommended for assessment in HDDST although UFC and 17 OHCS can still be used. The sensitivity of HDDST with target of 90% reduction in UFC has been reported to be as low as 64% in some studies.<sup>[23]</sup>

48 h HDDST is complex and cumbersome. An 8-mg HDDST has been developed. Patient takes 8 mg dexamethasone at 23:00 h and blood samples are collected for cortisol level before dexamethasone intake and at 08:00 h the following morning. A 50% reduction in plasma cortisol level achieved a sensitivity of 92% and sensitivity of 100%.<sup>[24]</sup> Since the test is much more convenient and provides better reliability of results it is replacing the traditional 48 h HDDST in most centres.

### Inferior Petrosal Sinus Sampling

Assessment of ACTH gradient in samples collected from bilateral inferior petrosal sinuses which drain pituitary veins (central) and plasma (peripheral vein) helps to confirm the source of ACTH. In non-stimulated (basal) state, a ratio of  $\geq 2$  between central and peripheral plasma ACTH levels is considered diagnostic for pituitary Cushing's disease.<sup>[25]</sup> Since some ectopic sources can produce a significant amount of ACTH and also pituitary

ACH production can be episodic, stimulated samples are also collected. 100 ug CRH injection is given in a peripheral vein, and both central (inferior petrosal sinuses) and peripheral (peripheral vein) samples are collected at 3, 5, 10 and 15 min. A stimulated ratio of  $\geq 3$  of ACTH levels in central and peripheral blood samples is considered diagnostic for pituitary Cushing's disease.<sup>[26]</sup> Since the procedure is invasive and requires a high level of skill, this should be performed only at specific centres where such facilities are available and laboratory support can back this up.

### Adrenal

Adrenal glands, one on top of each kidney, can produce excessive cortisol from zone fasciculata of either or both of adrenal glands with benign or malignant adrenal pathologies. Majority of cases have benign adrenal disease differentiated based on the morphology of adrenal glands on adrenal imaging. Unilateral or bilateral adrenal hyperplasia and adrenal adenomas are the benign adrenal pathologies responsible for Cushing's syndrome. There has been a significant increase in incidental detection of adrenal pathology with a rise in imaging (computed tomography [CT] and magnetic resonance imaging scans) investigations of the abdomen. About 0.6–1.3% of abdominal CT scans detect adrenal lesions.<sup>[27]</sup> Adrenal adenoma remains the more common of the two causes of Cushing's syndrome. So far, there are no risk factors or genes have been identified responsible for Cushing's syndrome. Majority of such patients produce only cortisol and its metabolites in excess but some patients, especially with malignant adrenal lesions, may cosecrete excessive amounts of other adrenal hormones including adrenal androgens and its metabolites. Detection of these substances can help to identify the source of Cushing's syndrome and plan further management accordingly.

### Pituitary

ACTH is produced by basophilic corticotrophs of the anterior pituitary gland which constitute 20% of anterior pituitary gland cells. Pituitary ACTH-dependant Cushing's constitutes up to 80% of all cases of Cushing's syndrome.<sup>[28]</sup> Malignant lesions of the anterior pituitary gland are exceptionally rare and constitute <1% of all pituitary lesions. ACTH-producing pituitary lesions are notoriously small and challenging to be treated surgically.

Like any other pituitary lesion, ACTH-producing pituitary adenomas can either cosecrete other pituitary hormones and/or reduce production of other pituitary hormones due to pressure effects of adenoma on surrounding healthy pituitary cells. The initial biochemical investigations must include assessment of all hormones of the anterior pituitary gland in all such patients. This could help to confirm the source of the ACTH/CRH and further management plan.

### Ectopic

ACTH production from sites other than pituitary gland or ACTH production from a pituitary gland under the influence of CRH produced from other than hypothalamus is termed as ectopic. In some patients, these ectopic sources produce metabolically active metabolites of ACTH which can still stimulate adrenal glands and produce Cushing's syndrome. Many such patients may not have florid signs and symptoms of Cushing's syndrome as they are usually diagnosed early on in the disease process, and usually with high levels of cortisol in circulation. Since this is not the case with all such patients, it cannot be used as a distinguishing feature of the condition. Since majority of such patients have very high cortisol level, they usually present with very low potassium. In majority of these patients, hypokalaemia is resistant and usually requires hospital admission for potassium replacement. Lung cancers are a common source of ectopic ACTH/CRH production. Ectopic CRH is often produced by carcinoid tumours. Most such patients will produce other metabolites in addition to CRH/ACTH and detection of such substances in blood could help to localise the source of CRH/ACTH production. Pro-opiomelanocortin (POMC) precursors due to its partial processing and 'large ACTH' due to its incomplete cleavage are found in patients with ectopic Cushing's, especially, in patients of lung cancer. Detection of these molecules is suggestive although not diagnostic of ectopic Cushing's as some pituitary adenomas may also have incomplete processing of POMC precursor and partial cleavage of ACTH due to their overproduction by pituitary tumour cells.<sup>[22,29]</sup>

At time, CRH/ACTH can be produced from sources other than pituitary gland (ectopic ACTH production) and can still stimulate adrenal glands to produce excessive uncontrolled amounts of cortisol, resulting in hypercortisolism.<sup>[30]</sup>

### Cyclical Cushing's

The excessive cortisol production can be continuous or cyclical. This episodic cortisol hypersecretion is often termed as cyclical Cushing's and is usually more challenging to diagnose. Such patients have episodes of variable duration of excessive cortisol production separated by the episodes of normal cortisol production.<sup>[31]</sup> Such patients require cortisol assessment more than once in the presence of clinical signs and symptoms suggestive of hypercortisolism.

The presence of this type of Cushing's syndrome necessitates an assessment of endogenous cortisol production on more than one occasion in patients suspected to have symptoms of Cushing's syndrome. A significant change in psychological symptoms and behaviour is often the clue in such patients, and investigations should be completed during this episode.

### Pseudo-Cushing's

Pseudo-Cushing's is a term used for conditions when patients have excessive endogenous production of glucocorticoids from adrenal glands in response to certain stimulating factors. The condition is potentially reversible and does not require treatment along the lines of Cushing's syndrome. Such patients require treatment of other stimulating factors to bring endogenous glucocorticoid production within normal range. These patients present with signs and symptoms of Cushing's syndrome. The severity and extent of symptoms and signs depend on the duration of tissues' exposure to high levels of glucocorticoids and duration of those stimulating factors. Since the cortisol is being produced in excess, the laboratory screening tests are likely to show excessive cortisol production. It is important to recognise these factors and manage patients accordingly.<sup>[32]</sup> These factors are often termed as pseudo-Cushing's factors or states. These include depression, obesity, physical stress, malnutrition, eating disorders, polycystic ovarian syndrome, uncontrolled diabetes, obstructive sleep apnoea and chronic alcoholism.<sup>[33]</sup> In most cases, clinicians should try to address to these factors before considering screening for Cushing's syndrome. The clinical manifestations of hypercortisolism should settle once these factors are controlled. Once these factors are well controlled and the patient still continues to experience symptoms and signs of Cushing's syndrome, clinicians should then run

screening investigations for Cushing's syndrome. Detection of alcohol in blood confirmed by blood alcohol level can help in such patients. Such patients should be admitted to ward to closely monitor for few days and MCL should drop to within normal range within 5 days in such patients.<sup>[34]</sup>

### Iatrogenic Cushing's

Patients requiring glucocorticoids for treatment of their medical conditions can develop iatrogenic Cushing's syndrome due to prolonged exposure of tissues to supraphysiologic levels of glucocorticoids in blood circulation. The diagnosis in such patients is usually obvious with a history of use of glucocorticoids in therapeutic doses. Prednisolone and dexamethasone remain the two most commonly used oral agents in such patients. A significant number of patients using steroid inhalers on long-term basis may also suffer from this condition due to the absorption of inhaled steroid into blood circulation. Surreptitious ingestion of steroids is not commonly seen. Not all steroids are detected by commonly used laboratory assays. Undetectable ACTH levels along with undetected cortisol levels are strongly suggestive of surreptitious ingestion of steroids. This scenario would be encountered in patients ingesting the type of steroids not detected by laboratory assays. A similar picture would be expected with dexamethasone intake as dexamethasone is not detected by assays commonly used by laboratories for steroids.

### Summary

Cushing's syndrome remains a rare diagnosis but requires careful and systematic assessment and laboratory investigations. The diagnosis and localisation of the disease remain challenging in most patients although availability of ACTH levels has made it a bit easier. Patients with florid signs and symptoms and clear laboratory results may have management plans completely different from those who present early in their disease and laboratory results may not be diagnostic. In majority of patients, In majority of patients, Cushing's syndrome is not caused by malignant lesions but carries high morbidity and mortality. Diagnosis must be considered in patients who may present with clinical features suggestive of the condition.

### Conflict of Interest

The authors declare that they have no conflict of interest.

### References

1. Cushing's Syndrome. Available from: <https://www.niddk.nih.gov/health-information/endocrine-diseases/cushings-syndrome>. [Last accessed on 2017 Dec 01].
2. World Health Organisation; 2006. Available from: <http://www.who.int/dietphysicalactivity/publications/factsheets/obesity/end>. [Last accessed on 2017 Dec 01].
3. Guignat L, Bertherat J. The diagnosis of Cushing's syndrome: An endocrine society clinical practice guideline: Commentary from a European perspective. *Eur J Endocrinol* 2010;163:9-13.
4. Nieman LK, Biller BM, Findling JW, *et al*. The diagnosis of Cushing's syndrome: An endocrine society clinical practice guideline. *J Clin Endocrinol Metab* 2008;93:1526-40.
5. Zhang Q, Dou J, Gu W, *et al*. Reassessing the reliability of the salivary cortisol assay for the diagnosis of Cushing syndrome. *J Int Med Res* 2013;41:1387-94.
6. Brien TG. Human corticosteroid binding globulin. *Clin Endocrinol (Oxf)* 1981;14:193-212.
7. Nieman LK. Cushing's syndrome: Update on signs, symptoms and biochemical screening. *Eur J Endocrinol* 2015; 173:M33-8.
8. Holleman F, Endert E, Prummel MF, *et al*. Evaluation of endocrine tests. B: Screening for hypercortisolism. *Neth J Med* 2005;63:348-53.
9. Ceccato F, Barbot M, Zilio M, *et al*. Screening tests for Cushing's syndrome: Urinary free cortisol role measured by LC-MS/MS. *J Clin Endocrinol Metab* 2015;100:3856-61.
10. Raff H, Auchus RJ, Findling JW, *et al*. Urine free cortisol in the diagnosis of Cushing's syndrome: Is it worth doing and, if so, how? *J Clin Endocrinol Metab* 2015;100:395-7.
11. Loriaux DL. Diagnosis and differential diagnosis of Cushing's syndrome. *N Engl J Med* 2017;377:e3.
12. Crapo L. Cushing's syndrome: A review of diagnostic tests. *Metabolism* 1979;28:955-77.
13. Mengden T, Hubmann P, Müller J, *et al*. Urinary free cortisol versus 17-hydroxycorticosteroids: A comparative study of their diagnostic value in Cushing's syndrome. *Clin Investig* 1992; 70:545-8.
14. Laudat MH, Billaud L, Thomopoulos P, *et al*. Evening urinary free corticoids: A screening test in Cushing's syndrome and incidentally discovered adrenal tumours. *Acta Endocrinol (Copenh)* 1988;119:459-64.
15. Contreras LN, Hane S, Tyrrell JB. Urinary cortisol in the assessment of pituitary-adrenal function: Utility of 24-hour and spot determinations. *J Clin Endocrinol Metab* 1986; 62:965-9.
16. Meikle AW. Dexamethasone suppression tests: Usefulness of simultaneous measurement of plasma cortisol and dexamethasone. *Clin Endocrinol (Oxf)* 1982;16:401-8.
17. Yanovski JA, Cutler GB Jr., Chrousos GP, *et al*. Corticotropin-releasing hormone stimulation following low-dose dexamethasone administration. A new test to distinguish Cushing's syndrome from pseudo-Cushing's

- states. *JAMA* 1993;269:2232-8.
18. Cronin C, Igoe D, Duffy MJ, *et al.* The overnight dexamethasone test is a worthwhile screening procedure. *Clin Endocrinol (Oxf)* 1990;33:27-33.
  19. Newell-Price J, Trainer P, Perry L, *et al.* A single sleeping midnight cortisol has 100% sensitivity for the diagnosis of Cushing's syndrome. *Clin Endocrinol (Oxf)* 1995;43:545-50.
  20. Kennedy L, Atkinson AB, Johnston H, *et al.* Serum cortisol concentrations during low dose dexamethasone suppression test to screen for Cushing's syndrome. *Br Med J (Clin Res Ed)* 1984;289:1188-91.
  21. Lytras N, Grossman A, Perry L, *et al.* Corticotrophin releasing factor: Responses in normal subjects and patients with disorders of the hypothalamus and pituitary. *Clin Endocrinol (Oxf)* 1984;20:71-84.
  22. Newell-Price J, Trainer P, Besser M, *et al.* The diagnosis and differential diagnosis of Cushing's syndrome and pseudo-Cushing's states. *Endocr Rev* 1998;19:647-72.
  23. Aytug S, Laws ER Jr, Vance ML. Assessment of the utility of the high-dose dexamethasone suppression test in confirming the diagnosis of Cushing disease. *Endocr Pract* 2012;18:152-7.
  24. Tyrrell JB, Findling JW, Aron DC, *et al.* An overnight high-dose dexamethasone suppression test for rapid differential diagnosis of Cushing's syndrome. *Ann Intern Med* 1986;104:180-6.
  25. Trainer PJ, Grossman A. The diagnosis and differential diagnosis of Cushing's syndrome. *Clin Endocrinol (Oxf)* 1991; 34:317-30.
  26. Weber A, Trainer PJ, Grossman AB, *et al.* Investigation, management and therapeutic outcome in 12 cases of childhood and adolescent Cushing's syndrome. *Clin Endocrinol (Oxf)* 1995;43:19-28.
  27. Delgrange E, Goethals P, Laka A, *et al.* An unusual case of bilateral virilizing adrenal adenoma co-secreting androgens and cortisol. *J Endocrinol Invest* 1996;19:377-81.
  28. Sharma ST, Nieman LK, Feelders RA. Cushing's syndrome: Epidemiology and developments in disease management. *Clin Epidemiol* 2015;7:281-93.
  29. Fuller PJ, Lim AT, Barlow JW, *et al.* A pituitary tumor producing high molecular weight adrenocorticotropin-related peptides: Clinical and cell culture studies. *J Clin Endocrinol Metab* 1984;58:134-42.
  30. Kirk LF Jr, Hash RB, Katner HP, *et al.* Cushing's disease: Clinical manifestations and diagnostic evaluation. *Am Fam Physician* 2000;62:1119-27, 1133-4.
  31. Meinardi JR, Wolffenbuttel BH, Dullaart RP. Cyclic Cushing's syndrome: A clinical challenge. *Eur J Endocrinol* 2007;157:245-54.
  32. Tirabassi G, Boscaro M, Arnaldi G. Harmful effects of functional hypercortisolism: A working hypothesis. *Endocrine* 2014;46:370-86.
  33. Besemer F, Pereira AM, Smit JW. Alcohol-induced Cushing syndrome. Hypercortisolism caused by alcohol abuse. *Neth J Med* 2011;69:318-23.
  34. Rees LH, Besser GM, Jeffcoate WJ, *et al.* Alcohol-induced pseudo-Cushing's syndrome. *Lancet* 1977;1:726-8.