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TITLE: ACTH and Cortisol

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Slide 1:

Hello, my name is **Jieli Shirley Li**. I am an **assistant professor and lab director at Ohio State University Wexner Medical Center**. Welcome to this Pearl of Laboratory Medicine on "**ACTH and Cortisol**"

Slide 2:

Hormones released by the hypothalamus regulate the anterior pituitary hormones. CRH is synthesized and released from the hypothalamus; it stimulates the synthesis and release of ACTH from the pituitary gland. ACTH is secreted in response to several factors, of which CRH is the most important. The adrenal cortex secretes cortisol in response to ACTH. When plasma cortisol increases, it suppresses the release of CRH and ACTH, which, in turn, leads to lowering of the cortisol level. Conversely, when serum cortisol reaches a decrease level, the hypothalamus and pituitary gland respond by increasing CRH and ACTH production, leading to stimulation of cortisol formation and secretion. By this mechanism, ACTH and cortisol control the concentration of each other within a very narrow range.

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Cushing's syndrome is composed of a group of clinical and metabolic disorders resulting from prolonged exposure to elevated concentrations of glucocorticoids. The excessive levels of glucocorticoids may be of endogenous origin, which is secreted by the adrenal zona fasciculata, or of exogenous origin, for example, pharmacologically administered steroids. Patients with

severe forms of the syndrome are easily recognized by the symptoms like striate rubrae, facial plethora, proximal muscle weakness, visceral fat accumulation, easy bruising and hypokalemia. Many of the signs and symptoms like obesity, diabetes, due to hypercortisolism are common medical complaints, however it is important to suspect Cushing's in certain situations as these have an increase in morbidity and mortality.

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Cushing's syndrome is categorized as ACTH dependent or ACTH independent. ACTH-dependent Cushing's syndrome represents increased ACTH produced in turn increases the cortisol production. These includes ACTH-producing pituitary adenoma, or Cushing's disease, and ectopic ACTH syndrome where ACTH is produced by other organs. In contrast, ACTH-independent Cushing's syndrome happens when endogenous and exogenous high levels of cortisol suppresses ACTH secretion and makes ACTH level low. These includes adrenocortical adenoma or carcinoma which produces very high level of cortisol. The tests for differential diagnosis will be discussed in next slides.

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The most important first step in the management of patients with suspected Cushing's syndrome is to establish the correct diagnosis. The diagnosis of Cushing syndrome is a rigorous process often requiring confirmatory tests at each step and endocrine consultation. A simplified diagnostic approach is shown in the figure. For initial screening, 1-mg overnight dexamethasone suppression test is a simple test to perform and suggested to be the preferred screening test. The diagnosis depends on the demonstration of increased cortisol production and failure to suppress cortisol secretion normally when dexamethasone is administered. In difficult cases, like obese or depressed patients, measurement of a 24-h urine free cortisol can also be used as a screening test. The 24-hour urinary free cortisol is a reflection of the unbound circulating cortisol that is freely filtered by the glomerulus. Unlike serum cortisol, it is unaffected by the level of circulating cortisol binding globulins or albumin. Urine free cortisol greater than three times of upper limit references is suggestive of Cushing's syndrome. The midnight salivary cortisol carries a high diagnostic sensitivity and specificity. The definitive diagnosis is established by failure of plasma cortisol less than 1.8 μg/dL after a standard low-dose dexamethasone suppression test, which is 0.5 mg every 6 hours for 2 days.

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Plasma ACTH levels can be useful in distinguishing the various causes of Cushing's syndrome, especially in separating ACTH-dependent from ACTH-independent causes. Generally, plasma ACTH levels are suppressed in cases of autonomous adrenal cortisol excess, as a consequence of enhanced negative feedback to the hypothalamus and pituitary. In contrast, patients with ACTH-dependent Cushing's have normal or increased plasma ACTH, with very high levels being found in some patients with ectopic ACTH syndrome. In all cases of confirmed ACTH-dependent Cushing's, further tests are required for the differential diagnosis of pituitary Cushing's disease and ectopic ACTH syndrome. These tests include residual ACTH suppression by high-dose dexamethasone and CRH responsiveness.

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Ectopic ACTH syndrome is caused by non-pituitary tumors that secrete either ACTH and/or CRH and cause bilateral adrenal hyperplasia. The rationale underlying CRH test is that cortisol hypersecretion by an adrenal tumor or the ectopic production of ACTH will suppress the hypothalamic-pituitary axis so that inhibition of pituitary ACTH release can be demonstrated. So most patients with pituitary-hypothalamic dysfunction or a microadenoma have an increase in ACTH secretion in response to CRH administration, while most patients with ectopic ACTH-producing tumors do not. Ectopic sources of ACTH are typically resistant to dexamethasone suppression as well. If these two tests show discordant results, or if there is any other reason for doubt, the differential diagnosis can be further clarified by performing bilateral inferior petrosal sinus sampling (IPSS) with concurrent blood sampling for ACTH in the right and left inferior petrosal sinus and a peripheral vein. An increased central/peripheral plasma ACTH ratio >2 at baseline and > 3 after CRH injection is indicative of Cushing's disease, with very high sensitivity and specificity.

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Adrenal insufficiency is categorized according to the key site of dysfunction within the hypothalamic-pituitary-adrenal axis: primary, which is adrenal, secondary, which is pituitary, and tertiary, which is hypothalamic. A major distinction between primary adrenal insufficiency and either of the other causes is that primary disease is associated with mineralocorticoid deficiency. In the developed countries, primary adrenal insufficiency, also known as Addison's disease, is

most commonly due to autoimmune adrenalitis; other causes include tuberculosis, which is the most common cause in developing countries, other includes granulomatous disorders, metastatic disease, adrenal hemorrhage, human immunodeficiency virus, acquired immunodeficiency syndrome, and infection.

Slide 9:

ACTH deficiency causes secondary adrenocortical insufficiency. Patients with total pituitary insufficiency may have manifestations of multiple hormone deficiencies. Patients receiving long-term steroid therapy may develop adrenal insufficiency because of prolonged pituitary-hypothalamic suppression and adrenal atrophy secondary to the loss of endogenous ACTH.

Slide 10:

Plasma ACTH is a useful tool for distinguishing primary from secondary or tertiary adrenal insufficiency. In primary adrenal insufficiency, low cortisol concentrations are found, along with increased ACTH levels. In secondary or tertiary adrenal insufficiency, both ACTH and cortisol are expected to be low.

Most patients with hypocortisolism have low serum cortisol levels, however, some patients' cortisol level falls within the reference range due to the stress, which does not exclude the diagnosis. The most convenient procedure for studying patients suspected of having hypocortisolism is the ACTH stimulation test. A normal response is a cortisol level greater than 18 to 20 µg/dL at 30 and 60 minutes post-Cosyntropin.

The CRH test has been discussed already.

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To prevent degradation of ACTH, it is best to collect the sample in a pre-chilled EDTA lavender-top tube. The specimen should be kept in an ice bath and should be processed as soon as possible. Following centrifugation in a refrigerated centrifuge, the specimen should be separated, transferred to a plastic tube, and kept frozen at -20° C until time of analysis.

Plasma ACTH is measured using a two-site immunoradiometric assay. ACTH consists of 39 amino acid residues, of which residues 1 to 24 at the amino terminal has full hormonal activity. Most polyclonal antibodies recognize and react with intact ACTH, N-terminal ACTH fragments, and ACTH precursors.

ACTH assays have been developed for automated immunoassay platforms using chemiluminescent labels. These methods are more precise than manual methods, and analytically, they are sensitive enough to distinguish between low-normal and suppressed hormone secretion.

Slide 12:

About 90% of circulating cortisol is bound to serum protein, including cortisol-binding globulin and albumin. The remaining 10% of circulating cortisol is the unbound, free hormone. Only free cortisol is active. Serum cortisol includes the total of free, cortisol binding globulin-bound and albumin-bound cortisol. The measurement of cortisol includes radioimmunoassay (RIA) and chemiluminescent techniques, as well as LC-MS, which offers the ultimate in specificity.

Only free cortisol is filtered by the kidneys. Therefore unlike serum cortisol, urine free cortisol is not affected by conditions and medications that alter cortisol binding globulins and albumin concentrations. Urine free cortisol provides an integrated profile of total cortisol secretion over a 24-hour period. LC-MS is considered the current reference method for measuring urine free cortisol. All 24-hour urine collections should include the measurement of creatinine to assay for adequacy of the collection.

Up to 30% of urinary free cortisol and dexamethasone suppression screening tests may come with an incorrect result. Cortisol binding globulin is not present in saliva, therefore midnight salivary cortisol is an alternative method. LC-MS is considered the current reference method for measuring salivary cortisol.

Slide 13: References

Slide 14: Disclosures

Slide 15: Thank You from www.TraineeCouncil.org

Thank you for joining me on this Pearl of Laboratory Medicine on "**ACTH and Cortisol**"

Question Bank

A patient's midnight salivary cortisol is found to be elevated (50 ug/dL, reference range <0.1 ug/dL) with a slightly decreased plasma ACTH level. The diagnosis of Cushing's syndrome is considered.

Which test is the most likely next diagnostic step to confirm Cushing's syndrome?

a) Urinary free cortisol
b) Plasma aldosterone
c) Low-dose dexamethasone suppression test
d) Repeat salivary cortisol in the morning

Answer: **c**

Discussion: When suspecting Cushing's syndrome, low-dose dexamethasone suppression test is useful. Lack of suppression of cortisol levels suggests Cushing's syndrome.

Source: Tietz Textbook of Clinical Chemistry and Molecular Diagnostics - 6th Edition

Difficulty: Easy

A patient was confirmed with diagnosis of Cushing's syndrome, and his ACTH level is 30 pg/mL (reference range < 15 pg/mL). His ACTH levels are 35 pg/mL and 25 pg/mL after CRH test and high-dose dexamethasone test respectively, which one of the following is expected?

a) Ectopic ACTH syndrome.
b) Cushing's disease.
c) Adrenocortical adenoma
d) Long-term steroid therapy.

Answer: **a**

Discussion: The patient's ACTH baseline levels were already elevated and they were neither increased by CRH test nor suppressed by high dose dexamethasone test, indicating the high level ACTH is not from pituitary. Ectopic ACTH syndrome is considered.

Source: Harrison's Principles of Internal Medicine, 20th Edition

Difficulty: Intermediate