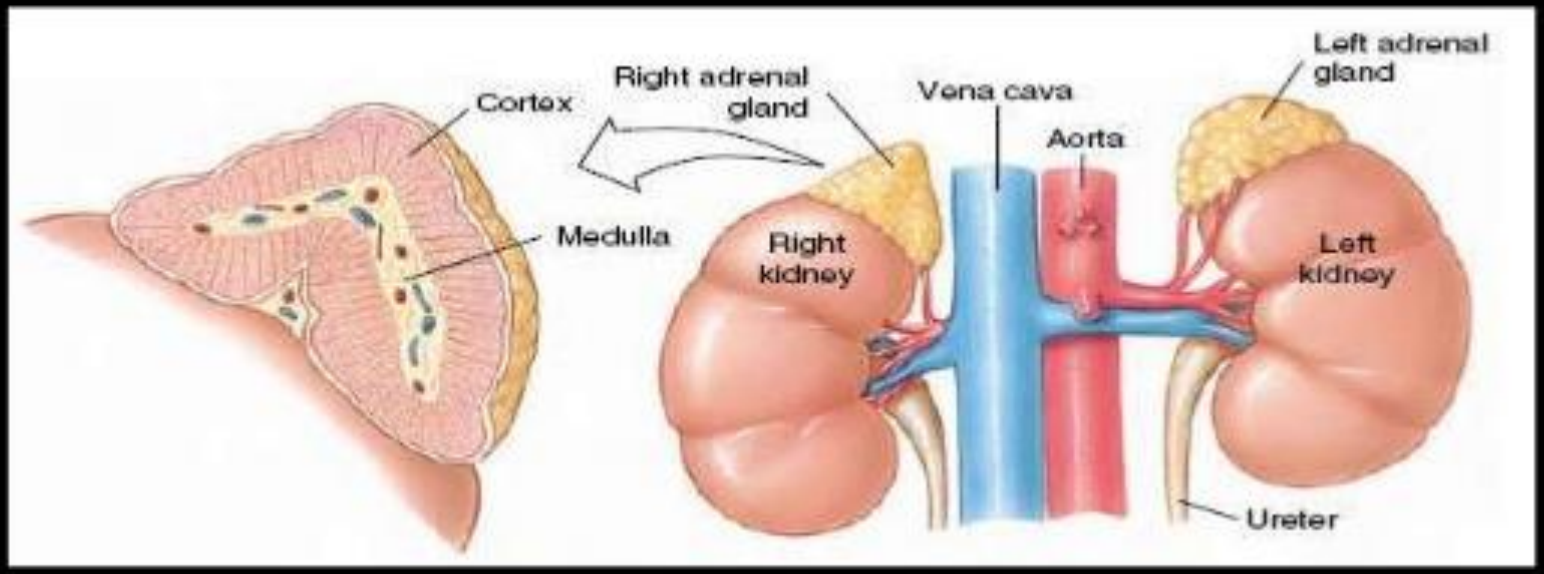
- ADRENAL FUNCTION TESTS
- Sangeeta Trimbake

INTRODUCTION

- The two adrenal glands, each of which weighs about 4 grams, lie at the superior poles of the two kidneys.
- Each gland is composed two distinct parts: the inner medulla and the outer cortex.



Adrenal glands

- Each gland is divided into tow parts:
  - **Cortex** – outer part of gland
    - Part of hypothalamus – pituitary – adrenal axis
    - Secrete a variety of steroid hormones
  - **Medulla** – inner part of gland, (20% of gland)
    - Part of sympathetic nervous system
    - Secrete catecholamines
  - Both parts are structurally and functionally different

Adrenal cortex

- The large cortical cells are arranged into three layers or zones :
  - The **zona glomerulosa**,
    - The thin outermost layer
    - Constitute about 15% of cortex
  - The **zona fasciculata**,
    - The middle and largest portion
    - Constitute about 75% of cortex.
  - The **zona reticularis**,
    - The innermost zone.

ADRENAL CORTEX:

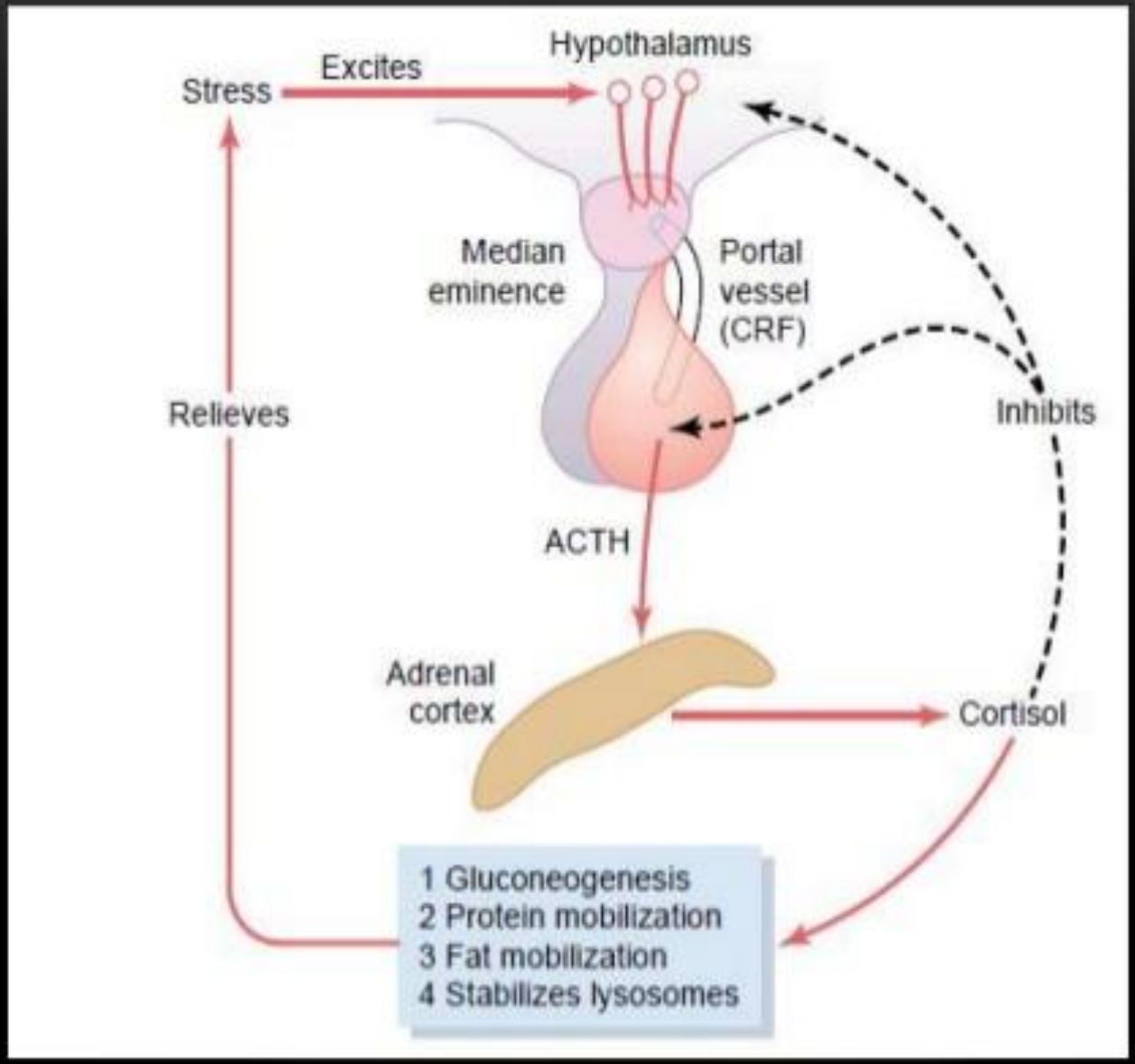
- Secretes corticosteroids- mineralo-corticosteroids, gluco-corticosteroids and androgenic hormones.
- *Mineralo-corticosteroids*- affect electrolytes of extracellular fluids: sodium and potassium.
- *Gluco-corticosteroids*- increase the blood glucose level.
- *Androgenic hormones*- exhibit the same effects as the male sex hormones.

- The cortisol has two basic anti-inflammatory effects:

(1) It can block the early stages of the inflammation process before inflammation even begins.

(2) If inflammation has already begun, it causes rapid resolution of the inflammation and increased rapidity of healing.

- *Cortisol reduces lymphocyte production and hence, suppresses immunity.*
- Cortisol Blocks the Inflammatory Response to Allergic Reactions.


Hypothalamus
Excites
Stress
Median eminence
Portal vessel (CRF)
Relieves
Inhibits
ACTH
Adrenal cortex
Cortisol
1 Gluconeogenesis
2 Protein mobilization
3 Fat mobilization
4 Stabilizes lysosomes

ADRENAL INSUFFICIENCY: CAUSES

20.44 Causes of adrenocortical insufficiency

Secondary (↓ACTH)

- Withdrawal of suppressive glucocorticoid therapy
- Hypothalamic or pituitary disease

Primary (↑ACTH)

Addison's disease

Common causes

- Autoimmune
  - Sporadic
  - Polyglandular syndromes (p. 794)
- Tuberculosis
- HIV/AIDS
- Metastatic carcinoma
- Bilateral adrenalectomy

Rare causes

- Lymphoma
- Intra-adrenal haemorrhage (Waterhouse–Friedrichsen syndrome following meningococcal septicaemia)
- Amyloidosis
- Haemochromatosis

Corticosteroid biosynthetic enzyme defects

- Congenital adrenal hyperplasias
- Drugs
  - Metyrapone, ketoconazole, etomidate

Disorders of adrenal cortex

- Patient with adrenal disorders can present with features related to:
  - **HYPOFUNCTION OF THE GLAND**
  - **HYPERFUNCTION OF THE GLAND**

Adrenal Hypofunction

- Adrenal insufficiency leads to a reduction in the output of adrenal hormones
  - glucocorticoids and/or mineralocorticoids

- Two types of adrenal insufficiency
  - **Primary insufficiency**
    - inability of the adrenal glands to produce enough steroid hormones
  - **Secondary insufficiency**
    - inadequate pituitary or hypothalamic stimulation of the adrenal glands

Adrenal Hypofunction

- **Causes**
  - **Glucocorticoid treatment**
  - **Autoimmune adrenalitis**
  - **Tuberculosis**

  } Common

  - Adrenalectomy
  - Secondary tumor deposits
  - Amyloidosis
  - Haemochromatosis
  - Histoplasmosis, tuberculosis, CMV, AIDS
  - adrenal haemorrhage

Adrenal Hypofunction

- **Causes**
  - Metabolic failure in hormone production
    - Congenital adrenal hyperplasia e.g. 21-hydroxylase deficiency, 3-β-hydroxysteroid dehydrogenase deficiency
    - Enzyme inhibition e.g. ketoconazole
    - Accelerated hepatic metabolism of cortisol e.g. phenytoin, barbiturates, rifampicin

Adrenal Hypofunction

Addison's disease
Primary hypoaldosteronism

Addison's disease: pathogenesis

- **Progressive destruction of entire adrenal cortex** , This is usually **autoimmune** based.

- Most likely the result of **cytotoxic T lymphocytes**, although 50% of patients have circulating adrenal **antibodies**.

Addison's disease: Clinical features

Common	Less common
Tiredness, generalized weakness, lethargy	Hypoglycemia
Anorexia, nausea, vomiting	Depression
Hyponatremia Hyperkalemia ,Hypercalcemia Convulsions	
Dizziness and postural hypotension	
Pigmentation	
Loss of body hair (woman)	

ADRENAL CRISIS

- **Causes :**
  - Precipitated by stress
    - infection, trauma or surgery in patients with incipient adrenal failure/treated with glucocorticoids if dosage is not increase
  - Adrenal haemorrhage
    - due to cx of anticoagulant treatment
  - Meningococcal septicaemia

INVESTIGATIONS (HORMONAL)

- **Plasma cortisol concentration**
  - <50nmol/L at 0900H → effectively diagnostic
  - >550nmol/L excludes the Dx
- **ACTH stimulation test / Synacthen test**
- Measurement of **plasma ACTH**
- Metyrapone test
- CRH stimulation test
- Plasma **renin** and **aldosterone** levels

Plasma cortisol

- The initial definitive test
- Cortisol level in blood show diurnal variation
- The levels high in morning (8.00 am)than in evening.
- Usual morning 8.00 am levels range between 8-26 ug/dl
- Loss of diurnalrythm is an indication of adrenal cortex function.

Plasma cortisol

- Levels are higher in hyperadrenocorticism and lower in hypoadrenocorticism.

PLASMA ACTH MEASUREMENT

- To differentiate between primary and secondary adrenal failure
  - Primary insufficiency - ACTH increased
  - Secondary insufficiency - ACTH decreased

Stimulation test for Hypofunction

INVESTIGATIONS (HORMONAL)

- **ACTH stimulation test / Synacthen test**

SHORT TEST	LONG TEST
➢ Take **blood sample** at **0900H** for measurement **of cortisol**	➢ **Day 1** : inject 1 mg depot ACTH IM im
➢ Inject **250µg ACTH im or iv**	➢ **Days 2 and 3** : repeat
➢ Take **further blood sample after 30 and 60 min for cortisol** measurement	➢ **Day 4** : perform short ACTH test

Stimulation test for Hypofunction

- Rapid ACTH stimulation tests
- 25 units or 250 µg of ACTH administration intravenously or intramuscularly is coupled with basal and post ACTH administration cortisol measurement at 30 and 60 minutes in plasma.
- There should be a minimum rise of more than 7 µg/dl .over the basal level or plasma level should be more than 18 µg/dl

Stimulation test for Hypofunction

- Rapid ACTHstimulation tests

- Lower results show hypofunction.
- Test may not show the rise in severely ill patients.

Stimulation test for Hypofunction

- Long ACTH stimulation test
- Lower dose of 1 µg/day of ACTH is administered for several days to effectively stimulates adrenals than single dose 250 µg.
- This successfully assesses the adrenal insufficiency.

METYRAPONE TEST

- **Measures the ability of the pituitary gland to release ACTH** in response to decreased blood cortisol levels.

- Metyrapone inhibits cortisol production by blocking the conversion of 11-deoxycortisol to cortisol by 11-beta-hydroxylase

Metapyrone stimulation test

- 750 mg of Metapyrone is given mouth every 4 hours for 24 hours. Basal and post Metapyroneadministration plasma levels of 11- deoxycortisol , cortisol and ACTH are measured .
- In normal persons following results are seen
- Plama 11- deoxycortisol should be > 7µg/dl or 200 nmol/L.

Metapyrone stimulation test

- Plasma ACTH > 75 pgm /ml or 17 pmol/L.
- These response would indicate an normal pitutary .no response would indicate an ectopic ACTH or adrenal tumor because they are not under the pitutary adrenal feedback loop.

CRH STIMULATION TEST

- To differentiate between secondary adrenal insufficiency dt pituitary or hypothalamic dis.

- Results :
  - Pituitary disease – blunted or nil response
  - Hypothalamic lesions – positive response

Tests for mineralocorticoid activity.

PLASMA RENIN AND ALDOSTERONE

- Give an indication of mineralocorticoid activity.

- Adrenal insufficiency
  - Low aldosterone level with high renin

Tests for mineralocorticoid activity.

- Plasma aldosterone
- Levels of Plasma aldosterone are more in morning than evening .potassium intake and sodium restriction indiet also increases aldosterone levels.
- Morning blood levels in supine position are 6-22 µg/dl in males and 5-33 µg/dl in females.
- Levels increase in hyperaldosteronism and decrease in hypoaldosteronism.

Management

- **Hormone replacement**
- Life-long replacement therapy
  - Hydrocortisone and 9α-fludrocortisone
- **Secondary adrenocortical insufficiency**
  - Hormone replacement
  - may also require more definitive treatment e.g. surgical removal of a pituitary tumour.

Disorders of adrenal cortex

ADRENAL HYPERFUNCTION

Primary aldosteronism

CONN'S SYNDROME

- Characterized by **autonomous** excessive production of **aldosterone** by **adrenal glands**

- Presents with HPT, hypokalaemic alkalosis and renal K+ wasting

Conn's Syndrome

- Causes:
  - Adrenal adenoma
  - Bilateral hypertrophy of zona glomerulosa cells
  - Adrenal carcinoma
    - Rare cause

Secondary aldosteronism

- Is increased adrenal production of aldosterone in response to non-pituitary, extra-adrenal stimuli

- Increase renin secretion
  - (hyperreninemic hyperaldosteronism)
- Commoner than primary aldosteronism

Conn's syndrome

- **Clinical features:**
  - Hypertension : *aldosterone induced Na retention with increase in ECF volume*
  - Muscle weakness: *Due to decrease K+*
  - Muscle paralysis: *severe hypokalaemia*
  - Latent tetany and paraesthesiae
  - Polydipsia, polyuria and nocturia: *due to hypokalaemic nephropathy*

INVESTIGATION

- **Electrolyte & blood gasses:**
  - Hypernatraemia
  - Hypokalaemica
  - Alkalosis
  - Urinary potassium loss, *level > 30 mmol daily during hypokalaemia*

INVESTIGATION

- **Plasma aldosterone : renin activity ratio**
  - Sensitive screening test
  - No need to standardize posture

Ratio	Interpretation	Action
<800	Diagnosis excluded	Seek other cause
>1000,<2000	Diagnosis possible	Confirmatory test
>2000	Diagnosis very likely	Establish cause

Diagnosis

- **Perform saline infusion test (sodium loading)**
  - Method :

    infusion of 1.25L of 0.9%saline over 2 hrs
  - Result:

    plasma aldosterone remains >240 pmol/l confirm Conn's syndrome

Establish cause

- **Plasma Aldosterone level**
- Method:
  - Morning blood sample (pt stayed recumbent since waking)
  - Second sample after 4 hrs stayed ambulant

**Standing ↓ renal blood flow → stim renin sec → ↑ aldosterone level

Establish cause

- Imaging techniques
  - CT scan
  - MRI

  - Can differentiate adenoma from hyperplasia

CUSHING'S SYNDROME

Adrenal cortex hyperfunction

- Any condition resulting from overproduction of **primarily glucocorticoid (cortisol)**

- Mineralocorticoid and androgen may also be excessive

Pseudo-Cushing's syndrome

- Appear cushingoid and have some biochemical abnormalities of true Cushing's disease
- Causes
  - Severe depression
  - Alcoholism
  - Obesity
  - Polycystic ovarian syndrome

Etiology

- **Excessive cortisol (ACTH dependent)~75%**
  - Pituitary disease
    - Adenoma (90%)
    - Hyperplasia (10 %)
  - Ectopic ACTH syndrome
    - Malignancy - (bronchus, thymus, pancreas, ovary)
  - Ectopic CRH syndrome
  - Exogenous ACTH administration

Laboratory investigations

- There are two diagnostic steps in the investigation of patient suspected of having Cushing's syndrome

  - Screening test

    for identification of Cushing's syndrome.

    the demonstration of high plasma cortisol level

  - Identification of cause

1. Demonstration of increased cortisol

- Assessment of circadian rhythm in cortisol secretion
- 24-Hour urinary free cortisol excretion
- Overnight / low dose dexamethasone suppression test

Laboratory investigations

1. **Assessment of circadian rhythm in cortisol secretion.**

- Measure 8 am and 11 pm serum cortisol level

  - Normal : Serum value @ midnight is 50% less than value @ 8 am
  - Cushing's syndrome : rhythum is loss
  - Pseudo-Cushing : normal circadian.

Urinary free cortisol

- Urinary cortisol secretion is higher in day time (7.00 am to 7.00 pm) than night (7.00 pm to 7.00 am) hence a properly collected 24 hours urine sample is required.
- Urine cortisol levels range from 10-100 µg/day.
- Levels of urine cortisol increase in hyperadrenalism and decrease in hypoadrenalism.

Laboratory investigations

2. Measuring 24-hour urinary free cortisol

Level (umol/ 24 h)	Interpretation
< 300	Normal
300 - 700	Severe depression Stress
> 700	Diagnostic of Cushing's syndrome

Dexamethasone supression test

- Screening test
- Plasma cortisol is measured after 1 mg of oral
- dexamethasone administration ,the previous night before test.
- The next morning 8.00 am cortisol level of less than 5ug /dl is considred normal.
- Higher level is indicative of hyperfunction.

Laboratory investigations

3. Low dose Dexamethasone suppression test :

- 0.5 mg Dexametason (oral) given 6 hourly for 2 days
- blood for plasma cortisol collected 6 hour after last dose
- urine for UFC is collected before & on the 2nd day of Dexa

- Result:
  - UFC suppress by 50% (< 70nmol/24h) normal
  - plasma cortisol suppress < 140 nmol/L pseudo-Cushing
  - no suppression of UFC & Pl. cortisol Cushing's synd

Ufc – urinary free cortisol

2. Elucidation of the cause

- Plasma ACTH
  - Normal < 50 ng/L
  - Low – adrenal causes
  - Elevated
    - Slight – pituitary dependent Cushing's
    - Gross – ectopic secretion of ACTH

Elucidation of the cause

- CRH Test
  - Differentiate ectopic ACTH secretion and Cushing's disease.
  - Cushing's disease – plasma ACTH increases 50% over baseline and cortisol increase by 20%
  - Ectopic ACTH or adrenal tumour – no response

Elucidation of the cause

- Imaging
  - CT scan of adrenal gland: TRO adrenal tumor
  - MRI of pituitary gland: majority microadenoma (< 10mm). MRI reveal lesion in 50 - 60% of cases
  - CT scan/MRI of thorax & abdomen: ectopic ACTH producing tumor

Treatment

- Depend of Cushing's syndrome depends on the etiology:
  - Adrenal adenoma
  - Adrenal Carcinoma – resection
  - Cushing’s disease - transphenoidal hyposectomy
  - Drug (block cortisol synthesis) - metyrapone

Thank u