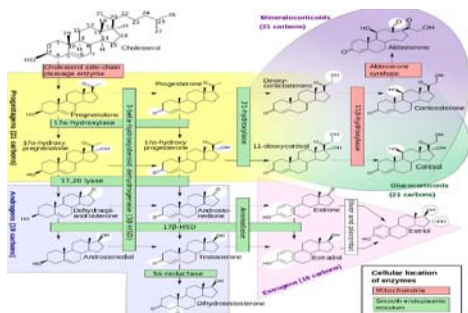


Investigation of adrenal disease

Adrenal disease

- Cortisol synthesis may fail for a number of reasons:
 - Defective enzyme in pathway or destruction of the gland.
 - Large amounts of ACTH in the blood lead to adrenal hyperplasia when cortisol synthesis is defective.

Cortisol synthesis



Congenital Adrenal Hyperplasia (CAH)

- Commonest form defect 21 hydroxylase
- 1 in 12000 newborns
- Defects of other steroidogenic enzymes 10 times less common.
- In the absence of 21 hydroxylase, cortisol and sometimes aldosterone cannot be produced effectively
- Consequently 17 hydroxypregnenolone and 17-OHP accumulate and are available for the action of 17,20 lyase to produce excess androgens (male hormones)
- Females with 21 hydroxylase deficiency are born with ambiguous genitalia due to androgen exposure in foetal life.

Congenital Adrenal Hyperplasia (CAH)

- The affected boys may become sick at home after early discharge from hospital.
- Affected girls with ambiguous genitalia are more likely to remain in hospital and can be monitored for electrolyte changes. The plasma potassium concentrations tend to rise before the plasma sodium falls.

Mild CAH (non salt losing or simple virilising)

- This type may present later with precocious puberty due to excess androgen production.
- In adults non classic form of CAH can occur. Basal 17-OHP concs are raised above normal and this can be further increased in a synacthen test.
- Synthesis of adrenaline is decreased in patients with defects in cortisol synthesis because normal medullary organogenesis is dependent on glucocorticoid secretion from the adrenal cortex.
- Patients with CAH can become hypoglycaemic during and after moderate exercise.

Investigation of CAH

- Low cortisol high concs of substrate of defective enzyme
- 21 hydroxylase most common 17-hydroxyprogesterone conc needs to be determined in blood along with cortisol, ACTH,renin, aldosterone
- 1st three days, 17-OHP can be detected in blood samples when measured specifically eg by mass spectrometry or an immunoassay performed on a solvent extraction of the plasma to isolate free steroid from sulphated steroids that interfere in a direct immunoassay.
- Plasma concs of cortisol will be low and renin high due to renal salt loss.
- Urine steroid profile should be requested because urine analysis by gas chromatography and mass spectrometry is a definitive test that displays the nature of the excess steroids (gas chromatography retention time and mass spectrum of each of the abnormal steroids).

Investigation of CAH

- Pregnanetriol is the main urinary metabolite of 17-OHP in children and adults and the excretion rate is high in CAH due to 21-hydroxylase deficiency.
- Using capillary column gas chromatography and mass spectrometry the metabolites 17-hydroxyprogesterone and 21-deoxycortisol are markers for the disease. In the first 3 days of life urine contains maternal and placental steroids so diagnosis of CAH on the basis of urine steroids should not be attempted in that period.
- If 17-OHP not elevated concs of 11 deoxycortisol for 11 hydroxylase and DHEAS for HSD3B2
- A 17 alpha hydroxylase defect should be considered when investigations of a patient with primary infertility show persistently raised progesterone.

Genetics

- Gene deletions and large gene conversions can be detected by genomic Southern blot analysis
 - Restriction enzyme digestion, allele specific oligonucleotide hybridisation, single-stranded conformation polymorphisms, and allele specific polymerase chain reaction (PCR) have been used for molecular diagnosis of point mutations.
 - Care must be taken with genetic tests where more than one copy of a gene is present; this is quite common with CYP21A2 genes.
 - In some cases the relevant gene will have to be sequenced. This testing is useful when families wish to have further children. Amniotic fluid can be taken at 11-12 weeks gestation for genetic analysis.
 - If the foetus is an affected girl, then treatment with dexamethasone throughout pregnancy can reduce the virilization that might have been experienced with living girl siblings. The non classic form of CAH is due to specific mutations amongst those seen in CYP21A2.

Addisons Disease

- Addisons disease presents with symptoms including fatigue, muscle weakness, weight loss, vomiting, diarrhoea and sweating. Historically caused by tuberculosis infection.
- Adrenal enzyme antibodies are now known to block the activity of 21-hydroxylase.
- Addisons disease is caused by cortisol deficiency. In the absence of cortisol production many patients have some signs of hyperpigmentation that can be in scar tissue or skin creases inside the cheek
- This is due to increased concentrations of melanocyte stimulating hormone as a consequence of increased pituitary processing of the ACTH precursor molecule pro-opiomelanocortin (POMC) following adrenal failure.
- The long term use of corticosteroids and sudden withdrawal can also be responsible.
- In newborn infants there may be a failure of adrenal development or a defect in cortisol synthesis.

Addisons Disease

- Rare forms of adrenal failure are due to lack of ACTH secretion or receptor action.
- The adrenal gland can also be destroyed by the high levels of very long chain fatty acids (VLCFA) that accumulate in the adrenal, brain, and myelin in adrenoleukodystrophy.
- Blood tests in adrenal failure will show hypoglycaemia, hyponatraemia, hyperkalaemia and metabolic acidosis. Low plasma concentrations of cortisol will not be increased with synacthen.
- A low cortisol (<100 nmol/L) at 8am suggests adrenal insufficiency and a synacthen test with measurements of cortisol should be used to confirm this.
- If a biosynthetic defect is thought to be the cause then the basal and stimulated samples should be analysed to check the concentrations of the intermediates before and after stimulation.
- Recent use of glucocorticoids may also lead to a state of adrenal hypoplasia lasting for some time. Herbal preparations may contain illegally added medicines.

Cushings

- Pituitary tumour producing excess cortisol resulting in the presence of central obesity, a round full-moon shaped face, reddened skin stretch marks (striae) and pigmentation.
- **Cushings syndrome** refers to the clinical picture from excess cortisol secretion. Administration of exogenous corticosteroids can also produce the picture of Cushings (iatrogenic). Rheumatoid arthritis patients are often prescribed very large doses of glucocorticoids.
- When the cortisol excess is due to autonomous or exogenous sources the conc of ACTH in the blood will be reduced.
- Similar syndrome seen in the case of an ectopic (usually lung) ACTH secreting tumour or a tumour of the adrenal cortex secreting cortisol.

Cushings lab tests

- Cortisol excess will be reflected in an increase of free cortisol in the blood and since this is filtered at the kidney a 24 hour urine free cortisol measurement is a good first line test.
- Blood samples taken at 8am and midnight will show high cortisol concs at midnight and a loss of diurnal rhythm of cortisol in affected patients. Salivary cortisol is sometimes used – these require sensitive and specific assays.
- Ectopic tumours – v high ACTH concs
- High cortisol – obesity, stress, depression, alcoholism

Cushings provocative tests

- Low dose dexamethasone suppression test (0.5 mg four times a day for two days) will suppress cortisol in normal subjects and eliminate those where previous high cortisol concentrations were due to stress.
- High dose dexamethasone (2 mg four times a day) will not suppress cortisol in Cushings disease.

Conns Syndrome

- Resulting from an adrenal tumour secreting aldosterone (primary aldosteronism)
- Patients with this condition have high blood pressure and hypokalaemia and it can lead to muscle weakness, headache and alkalosis.
- Tumours vary in size from 0.5 cm and not all are visualised on imaging.
- Previously hypokalaemia was thought to be an important marker of the condition. Incidence was low (less than 1% hypertensive patients). Recent studies of patients with normokalaemia have revealed 10-20% of patients with familial and drug resistant hypertension have hyperaldosteronism.
- Aldosterone : Renin ratio is test of choice for primary aldosteronism when bp is controlled with drugs that do not affect the renin-angiotensin-aldosterone system.
- Since potassium ion concentrations in the adrenal cortex are important for the enzymes in the last step of aldosterone synthesis, patients with hypokalaemia should be given potassium supplements prior to blood sampling for aldosterone and renin measurements

Confirmation of primary aldosteronism

- High aldosterone to renin ratio
 - Be careful when offering interpretative advice as many units are used for both analytes.
- Aldosteronism can be due to a unilateral adenoma, bilateral adrenal hyperplasia or dexamethasone suppressible types
- Catheterization studies will help localise a unilateral tumour but catheterization of adrenal veins requires a skilled radiologist.
 - A catheter is inserted into a vein in the groin and moved up into the vena cava.

Confirmation of primary aldosteronism

- For each blood sample taken as the catheter is repositioned a peripheral blood sample should be taken for aldosterone concentrations
- Left adrenal vein easier to locate than right.
- Blood samples from tumour are assayed for aldosterone and cortisol
- Plasma aldosterone concs can be considerably higher than peripheral levels and will need to be assayed in dilution.
- Cortisol is measured to confirm catheter in vein.

Clinical significance of other abnormal steroid results

- Low aldosterone can be found if liquorice is consumed.
- Liquorice suppresses the inactivation of cortisol to cortisone so cortisol remains available at high concentrations in the kidney where it acts with the mineralocorticoid receptor.
- Sodium retention leads to hypertension and suppression of renin. Similar picture in 11beta-hydroxysteroid dehydrogenase type 2.
- Plasma aldosterone concs low due to suppression of renin through action of deoxycorticosterone as a mineralocorticoid.
- Hyporeninaemic hypoaldosteronism is seen in kidney damage in particular in DM patients.

Clinical significance of other abnormal steroid results

- Aldosterone results can be abnormal in renal disorders affecting electrolyte transport; and can mimic defects of aldosterone production through high renin activity:
- Bartter's or Gitelman's
- Or low renin activity (Liddle's and Gordon's)
- High renin activity and aldosterone concs are seen in a number of situations (congestive heart failure, cirrhosis, and nephritic syndrome) and only rarely with defects of aldosterone action (defects of epithelial sodium transport channel or defects of the aldosterone receptor called pseudohypoaldosteronism)

Clinical significance of other abnormal steroid results

- Children with premature adrenarche have increased plasma concs of DHEA and DHEAS for age.
- Dehydroepiandrosterone sulphate concs are elevated in children and adults with adrenal tumours and somewhat raised in CAH due to HSD3B2 deficiency. During childhood testosterone and androstenedione production and their plasma concs are lower than in the first year and at puberty.

Disorders of the adrenal medulla

- Excess catecholamine secretion – increased heart rate, high blood pressure with postural hypotension, palpitations, headaches, weight loss, skin pallor.
- Plasma metanephrines screening test in samples from patients with drug resistant hypertension especially when an adrenal mass has been seen.
- Tests should be conducted without stress and after review of medications likely to affect results eg TCA and phenoxybenzamine.
- Negative screen for metanephrines virtually rules out a catecholamine secreting tumour.

Catecholamine methods

- In the methods for urine metanephrines the conjugated compounds are hydrolysed before analysis
- Tandem mass spectrometry will probably replace methods based on HPLC with other detectors.
- Some genetic tests are being introduced
- Plasma concs of chromagranin A may become an additional marker for phaeochromocytoma.
- Abdo imaging with CT or magnetic resonance are useful when localizing a tumour.

Questions

- What are the reasons for failure of cortisol synthesis?
- What is the most common defect in Congenital Adrenal Hyperplasia?
- Describe Congenital Adrenal Hyperplasia.
- How can gene deletions be detected?
- What are the symptoms of Addisons Disease?
- What is the concentration of cortisol suggestive of adrenal insufficiency?
- Describe Cushings Syndrome?
- What are the lab tests for Cushings?
- Describe the methods for catecholamine measurement?
