

1. Dynamic Function Tests in Endocrinology

Compilation and editing of this volume:

Dr. Eresha Jasinge (Consultant Chemical Pathologist)

List of contributors

Consultant Chemical Pathologists

Dr. Saroja C.Siriwardene
Dr. Meliyanthi Gunatillaka
Dr. Deepani Siriwardhana
Dr. Chandrika Meegama

Consultant Physicians

Dr. Noel Somasundaram
Dr. Bandula Wijesiriwardane
Dr. Rushika Lanerolle

Consultant Paediatrician

Dr. Shyama De Silva

Coordinators

Consultant Histopathologists

Dr. Siromi Perera
Dr. Kamani Samarasinghe
Dr. Modini Jayawickrama

INDEX

CONTENT	PAGE NUMBER
1.1 Introduction	03
1.2 Investigation of Growth Hormone deficiency	03
1.3 Investigation of Growth Hormone excess	16
1.4 Investigation of Thyroid disorders	19
1.5 Investigation of disorders of Cortisol metabolism	52
1.6 Investigation of disorders of Aldosterone metabolism	68
1.7 Water Deprivation Test	74

1.1 Introduction

Endocrinology is the study of intra- and extracellular communication by messenger molecules as hormones (from the Greek hormon, meaning to excite, to arouse to activity).

Hormones vary widely with respect to their composition, transport, metabolism and mechanism of action.

Tests of endocrine functions are of two types.

- The basal secretion of the hormone is measured using a single blood or urine sample.
- Dynamic tests, on the other hand, require two or more samples and are used to test the integrity of the control mechanism of the hypothalamic-pituitary-end organ axis.

1.2 Tests for Growth Hormone Reserve (Deficiency)

These tests should be performed under the supervision of a paediatrician or a physician.

Blood samples should be (serum separated) sent to the relevant laboratory.

Basal growth hormone (GH) is usually $< 1\text{mU/l}$ in normal individuals except during pulses of secretion.

Therefore measurement of random growth hormone levels is unhelpful.

Available tests

Children (<18 yrs) (short stature)

- Glucagon stimulation test

Second line test Clonidine stimulation test or Exercise stimulation test

Investigations for anterior pituitary reserve

For adults only

Check both GH and ACTH reserve

- Insulin hypoglycaemic test or Glucagon stimulation test
- Second line test Clonidine stimulation test

or children and adults with contraindications for

ITT

- Glucagon stimulation test

Choice of appropriate specimens for analysis will be decided by the time and degree of achievement of hypoglycaemia.

Insulin Hypoglycemic Test (IHT)/ Insulin Tolerance Test (ITT)

Indication

Assessment of ACTH/cortisol and GH reserve

Rationale

The stress of insulin induced hypoglycaemia triggers the release of GH and ACTH from the pituitary gland in normal subjects.

GH response is measured directly; cortisol is measured as the indicator of ACTH secretion.

Contraindications

Epilepsy or unexplained blackouts

Ischemic heart disease or cardiovascular insufficiency

Severe long standing hypoadrenalism (liver glycogen stores are depleted → severe hypoglycaemia during ITT)

Glycogen storage disease

Precautions

ECG must be normal

Serum cortisol(09.00) must be > 100 nmol/L

Normal serum T₄ (replace first if low)

If above tests are abnormal, or in doubt, perform glucagon test

5% & 25% dextrose and i.v hydrocortisone 100 mg ampoules should be available

Procedure

□asting from midnight (review medication)

Weigh patient

Insert the IV cannula at 08.30

Draw the basal blood sample (0 min) for GH and glucose
Insulin (soluble) i.v bolus at 09.00

Usual dose: 0.15U/Kg

Cushing's and acromegaly: usually 0.3 U/Kg

Draw further blood samples at 30, 60, 90 and 120 min for venous plasma glucose (analyzed in the lab)and serum GH.

(If insulin dose is repeated 30, 60, 90, 120 and 150 min)

If cortisol deficiency is suspected draw samples for cortisol as well. However the analysis of cortisol in the lab will depend on achievement of hypoglycaemia and the response of growth hormone to it.

During the test patient should be observed for signs of hypoglycemia (tremors, sweating, tachycardia) to ensure that adequate stress has occurred.

If not clinically hypoglycaemic at 45 min then consider repeating insulin dose in full. The patient must be awake throughout and be able to answer simple questions.

With severe and prolonged hypoglycaemia (>20 min), or impending or actual loss of consciousness, or fits, it may rarely be necessary to terminate the test.

Give 25 ml of 25% dextrose i.v followed by an infusion of 5% dextrose but continue sampling if possible (the hypoglycaemic stimulus has been adequate).

Consider hydrocortisone 100 mg i.v at the end of test.

Give lunch and sweet drink at the end of the test.

Observe the patient for 2h after the test.

Time(min)	GH	Plasma glucose
0	√	√
30	√	√
60	√	√
90	√	√
120	√	√
150 (only if insulin is repeated)	√	√

Normal response

Venous plasma glucose concentration has fallen to < 2.2 mmol/l, this is a satisfactory evidence of sufficient stress.

Serum cortisol rises by > 200 nmol/L to at least 550 nmol/l
 Serum GH rises to > 20 mU/L

Interpretation

If adequate hypoglycaemia wasn't achieved cortisol or GH deficiency cannot be diagnosed.

Untreated hypothyroidism can also give subnormal results-treatment with thyroxine may be necessary for 3 months before ITT becomes normal.

Peak GH response < 10 mU/L suggests GH deficiency.

Responses 10-20 mU/L suggests partial deficiency.

➤ > 20 mU/L is regarded as normal

Recommendations

A. Should be carried out under the supervision and recommendation of a consultant. Samples should be sent to the relevant laboratory for analysis. Please discuss with the relevant laboratory before sending the specimens.

References

1. Tietz Text book of clinical Chemistry 3rd edition
2. Clinical chemistry in Diagnosis and Treatment, Philp D. Mayne, 6th edition
3. The Bart's Endocrine protocols. Peter J. trainer, Michael Besser

Glucagon Stimulation Test**Indication**

Assessment of ACTH/cortisol and GH reserve when ITT is contraindicated.

Rationale

Glucagon stimulates GH release.

A safer test than insulin hypoglycemic test in young children and infants as it doesn't usually cause the same degree of hypoglycaemia as is induced during an ITT.

Contraindications

- Recent or intercurrent illness
- Severe cortisol deficiency
- Glycogen storage disease
- Patients who haven't eaten for 48 h

In all these situations glycogen stores are low or cannot be mobilized and hypoglycemia may occur, especially in children

Side effects

Nausea, vomiting, abdominal cramps and a feeling of apprehension which may occur in the first 1-2 hours after injection.

Glucagon induces a rise in blood glucose (2-3 fold), maximal in the first hour, but this may be followed by symptomatic hypoglycaemia. .

Precautions

Serum cortisol > 100 nmol/L

Serum T₄ must be normal (replace first if low for several weeks)

Patient must be supervised for at all times

Preparation

□asting from midnight

(fasting should not be longer than 4-6 hrs in infants and young children)

Review medication as these may need to be withheld until after the test is completed.

Accurate weight

The patient must be on a bed for the duration of the test.

Water only is allowed until completion of the test.

Blood glucose should be monitored at the bedside throughout the test.

Insert i.v canula at 0830 h

Baseline blood (0 min) is collected for serum growth hormone and venous plasma glucose.

If the result shows GH deficiency same sample can be utilized to measure cortisol, if the clinician has requested.

Administer sc glucagon 0.01mg/Kg, up to a maximum of 1mg at 0900 (1.5 mg if > 90 Kg)

Draw blood samples at 60, 120, 150 and 180min

Child fed and must have normal blood sugar prior to discharge.

Observe minimum of 2 hrs after test.

Leave i.v cannula in situ until after child has eaten and blood sugar level returns to normal limits.

If bed side blood sugar < 4 mmol/L, give 0.5g/Kg dextrose as 25% dextrose i.v

Time(min)	glucose	GH
0	√	√
60	√	√
90	√	√
120	√	√
150	√	√
180	√	√

Normal response

plasma glucose: usually rises to peak around 90 min and then falls

Cortisol: rises by > 200 nmol/L to above 550 nmol/L

GH: rises to > 20 mU/L

Interpretation

As for the ITT

Peak GH is usually at around 120 min.

Peak GH response < 10 mU/l suggests GH deficiency.

Responses of 10-20 mU/l suggests partial deficiency.

Response > 20 is regarded as normal.

References

1. Endocrine test manual, department of endocrinology, Westmead Hospital for Children, Sydney, Australia 2002
2. The Bart's Endocrine Protocols: Peter J. Trainer & Michael Besser

Recommendations

A. Should be carried out under the supervision and recommendation of a consultant. Samples should be sent to the relevant laboratory for analysis. Please discuss with the relevant laboratory before sending the specimens.

Clonidine Stimulation Test

Indication

Assessment of GH reserve

Rationale

Clonidine is a potent stimulus to Growth hormone release via Growth hormone releasing hormone secretion.

In this test clonidine is administered orally and the growth hormone response in peripheral blood is measured.

Contraindications

- Sick sinus syndrome
- Compromised intravascular volume

Precautions

Systolic blood pressure (BP) falls by 20-25 mmHg in all subjects

In the event of more significant BP fall, elevation of the legs is recommended and record the BP every 15 min.

Patients should lie down during and 2h after test or until BP is satisfactory.

Adverse reactions

Drowsiness; all in blood pressure is expected, may last several hours. Effect will be prolonged in renal failure.

Preparation

asting from mid night

However a minimum fasting time of only 2 hours is required and short fasting times (< 4-6 hrs) should be applied in infants and young children.

Accurate height and weight and calculate body surface area (BSA).

Insert i.v cannula at 0830 h.

Draw the baseline blood (0min) for glucose and growth hormone at 0900 h

Give 0.15 mg/m² oral dose of Clonidine at 0900h

Draw samples of blood for growth hormone and glucose at 60, 90, 120 and 150 min.

Water is allowed during the test.

Time(min)	GH	Plasma glucose
0	√	√
60	√	√
90	√	√
120	√	√
150	√	√

Interpretation

Since the mechanism and locus of action is unclear, interpretation is of uncertain significance.

- Peak GH response < 10 mU/l suggests GH deficiency;
- Responses of 10-20 mU/l suggest partial deficiency.
- Response > 20 is regarded as normal.

References

1. Endocrine test manual, Department of endocrinology, Westmead hospital for children, Sydney, Australia 2001
2. The Bart's Endocrine Protocols: Peter J. trainer & Michael Besser

Recommendations

A. Should be carried out under the supervision and recommendation of a consultant. Samples should be sent to the relevant laboratory for analysis. Please discuss with the relevant laboratory before sending the specimens.

Exercise Stimulation test**Indication**

A screening test of GH secretion

Rationale

Exercise is a physiological stimulant of GH secretion.

Exercise to approximately 50% of maximal capacity is required and this is usually achieved on riding an exercise bike or by repeated stair climbing for about 15 min.

This test has a relatively high incidence of false positives for GH deficiency often due to inadequate exercise, yet is a safe and inexpensive test.

Contraindications

Limitation of exercise capacity by cardiovascular, respiratory or other systemic disease. Children under 8 often do not tolerate the enforced exercise well.

Preparation

fasting for at least 2 hours; in the morning.

IV sampling cannula

Patients with exercise induced asthma who normally take prophylactic medication before exercise should do so.

Method

Draw the pre exercise blood sample for growth hormone and glucose

Record baseline heart rate

Child is exercised vigorously for 20 min

Measure heart rate at approximately 5 min interval.

A heart rate of 140-160 is usually achieved.

The test should be stopped if the heart rate exceeds 180 or the child is markedly distressed or exhausted.

Offer cool water during test, but continue exercising.

After 20 min of exercise, child rests, collect the second sample at 40 min. (20 min post exercise) for growth hormone and glucose.

Interpretation

Peak GH response < 10 mU/l suggests GH deficiency.

Responses 10-20 mU/l suggest partial deficiency.

>20 mU/l is regarded as normal.

References

1. Duty Biochemist manual, Laboratory Medicine, Royal Perth Hospital, Australia,2001
2. Test Manual,Department of Endocrinology, Westmead Hospital for Children, Sydney, Australia,2002

Recommendations

A. Should be carried out under the supervision and recommendation of a consultant. Samples should be sent to the relevant laboratory for analysis. Please discuss with the relevant laboratory before sending the specimens.

1.3 Investigation of Growth Hormone Excess

Biochemical diagnosis of acromegaly/gigantism is made by assessing autonomous secretion of growth hormone. (GH)

This is done by measuring growth hormone levels during a 2-hour period after a standard 75-g oral glucose load. (glucose-tolerance test).

Random or basal GH level is not a specific or sensitive test for acromegaly given the pulsatile nature of GH secretion.

Elevation of random GH levels also occurs in stress, chronic renal failure, liver failure, diabetes mellitus and malnutrition.

Growth Hormone Assay

An optimal GH assay should have the following:

1. The sensitivity limit of the assay should be < 0.26 mU/L with interassay coefficient of variation of < 15%.
2. Assay should be validated with a normal range for suppressed GH after an oral glucose load (OGTT).
3. Have information on the antibody specificity to the GH isoform (the most abundant in circulation being the monomeric 22KDa isoform).

As there is lack of standardization of GH assays, this limits the comparisons of results between laboratories.

Specimen collection for Growth Hormone

Serum is the preferred specimen.

Serum specimens should be stored at 2 to 8^o C if they are not to be tested within 8h

If they must be stored for long periods, samples are frozen at – 200^oC.

Patient must be fasting and at complete rest for 30 min before collection.

Glucose Tolerance Test

Indication

Diagnosis of acromegaly

Contraindications

None

Precautions

Cases with known diabetes

A basal blood sugar must be checked

Procedure

The test should be performed after an overnight fast with the patient maintained at bed rest.

Insert an iv cannula

20-30 min after inserting the cannula draw baseline blood samples for glucose and growth hormone.

Note the time. (Time 0)

75g of oral glucose load at time 0

(Dissolve 75 g in 300 ml of water) This should be taken within 3-4 min

Paediatric dose 1.75g/Kg bodyweight

Draw samples of blood for growth hormone and plasma glucose at 30, 60, 90 and 120 min.

Interpretation

Serum GH suppresses to < 0.8mU/L (as measured by current two-site immunometric assays) after glucose in normal subjects.

□ Failure of GH to suppress after glucose is highly suggestive of GH excess.

□ Failure to suppress also occurs in

- chronic liver disease
- renal disease
- uncontrolled diabetes
- malnutrition
- anorexia nervosa
- pregnancy
- estrogen therapy
- puberty

References

1. Tietz text book of clinical chemistry 3rd edition
Carl A Burtis and E. R. Ashwood
2. The Bart's Endocrine protocols: P.J Trainer and Michael Besser
3. Lim E.M, Pullan P. Consensus statement review. Biochemical assessment and long term monitoring in patients with acromegaly: Statement from a Joint Consensus conference of the growth hormone research society and the pituitary society. The clinical Biochemist Reviews 2005; 26: 41-43

Recommendations

A. Should be carried out under the supervision and recommendation of a consultant. Samples should be sent to the relevant laboratory for analysis. Please discuss with the relevant laboratory before sending the specimens.

1.3 Thyroid Disorders

Thyroid function tests can be carried out in any hospital. Separated serum should be sent to relevant laboratories for the analysis.

Thyroid disorders are amongst the most prevalent of medical conditions, especially in women. Disorders of the thyroid include both overt and mild/subclinical hypo and hyperthyroidism, goiter and thyroid cancer.

Thyroid function tests (TFT)

Measurement of serum hormone levels are utilized to establish the diagnosis of diseases of the thyroid gland, or to monitor the response to therapy. The best tests are,

3rd generation Serum Thyroid stimulating hormone (TSH) and Serum Free Thyroxine (fT₄)

Alternately in the absence of the above, 2nd generation Serum Thyroid stimulating hormone (TSH) and Serum Total Thyroxine (TT₄) are acceptable.

Serum Total T₃ or, Serum Free T₃ measurements are only useful in specific situations and therefore are **not recommended for routine testing.**

Hypothyroidism

Primary Hypothyroidism

Screening

- Serum TSH (2nd or 3rd generation) as first line.

This **strategy** may be **cost-effective** for a wide range of clinical purposes but is not the best as it may be insufficient in situations where the pituitary-thyroid axis is disturbed such as, secondary hypothyroidism (hypopituitarism), during optimization of therapy in newly diagnosed primary hypothyroidism or hyperthyroidism.

The most appropriate initial screening would be a combination of serum (2nd or 3rd generation) TSH and fT₄.

Diagnosis

- The diagnosis of primary hypothyroidism requires the measurement of both TSH and fT₄.
- Subjects with a TSH of > 10 mU/L and fT₄ below the reference range have overt primary hypothyroidism and should be treated with thyroid hormone replacement.
- Subjects with subclinical hypothyroidism should have the pattern confirmed within 3-6 months to exclude transient causes of elevated TSH.

- The measurement of thyroid antibodies in subjects with subclinical hypothyroidism helps to define the risk of developing overt hypothyroidism.

Guiding treatment with thyroxine

- The measurement of both TSH and T_4 is required to optimize thyroxine replacement therapy.
- The primary target of thyroxine replacement therapy is to make the patient well and to achieve a serum TSH within the reference range.
- The corresponding T_4 will be **within or slightly above** its reference range.
- The minimum period to achieve stable concentrations of TSH **after a change in dose of thyroxine** is two months and **thyroid function tests should not normally be requested before this period** has elapsed.

Guiding treatment with tri-iodothyronine

- If tri-iodothyronine is used as a replacement hormone increasing doses should be used until serum TSH is within the reference range.
- The measurement of TSH is required to optimize tri-iodothyronine replacement therapy.

- The measurement of T_3 is of no value in patients on tri-iodothyronine replacement because of the variability after taking the replacement dose.

Assessing response to thyroxine therapy

- In determining the optimal dose of thyroxine the biochemical target is a TSH result that is detectable, not elevated and preferably within the reference range.

Long-term follow-up of patients on thyroxine

- Patients stabilized on long-term therapy should have serum TSH checked annually as a change in requirement for thyroid hormone can occur with ageing.

Subclinical (Mild) Hypothyroidism

Diagnosis

- Subclinical hypothyroidism is characterized by a TSH above the reference range with a T_4 measurement within the reference range.
- Should be confirmed by repeat thyroid function testing 3-6 months after the original test.

Guiding Treatment

- If the serum fT_4 concentration is normal but the serum TSH is > 10 mU/L, then treatment with thyroxine is recommended.
- If the serum fT_4 concentration is normal and the TSH is elevated but, <10 mU/L then thyroxine therapy is not recommended as a routine therapy.
- However, thyroxine may be indicated in non-pregnant patients having a goiter or in those who are seeking pregnancy.

Assessing response to therapy

- The aim should be to restore and maintain TSH within the reference range.
- TSH should be measured in 2-3 months, following a change in thyroxine dose.

Long- term Follow-up

In patients who receive thyroxine therapy

- TSH should be measured annually and the thyroxine dose altered to maintain TSH within the reference range.

In patients who do not receive thyroxine therapy

- Subjects with subclinical hypothyroidism who are thyroid peroxidase antibody positive should have an annual thyroid function test.

- Subjects with subclinical hypothyroidism who are thyroid peroxidase antibody negative should have repeat thyroid function testing approximately every 3 yrs.

Secondary hypothyroidism

Diagnosis

- First line TSH and fT_4 are required to identify secondary hypothyroidism.
- Secondary hypothyroidism should be distinguished from
 - non-thyroidal illness on the basis of clinical history, and free T_3 measurement,
 - from hyperthyroid patients on antithyroid therapy from the drug history.

Measurement of other anterior pituitary hormones are useful in the diagnosis of hypopituitarism as a cause of secondary hypothyroidism. Referral to an endocrinologist and selection of suitable dynamic function tests are recommended.

Guiding treatment

- Establish degree of hypopituitarism before commencing thyroxine replacement.
- Thyroid hormone replacement shouldn't be commenced in patients with cortisol deficiency as this could provoke an Addisonian crisis.

Assessing response to therapy

- Essential to monitor treatment by estimating $\square T_4$ and maintain the thyroid hormone concentration within the reference range.
- Measurement of TSH cannot be used to assess the response in patients with hypopituitarism.

Long term follow –Up

- An annual check of serum thyroxine concentration should be performed in all patients with secondary hypothyroidism, stabilized on thyroxine replacement therapy.

Congenital hypothyroidism

Diagnosis

- All newborn babies should be **screened** for congenital hypothyroidism by measurement of filter paper blood spot TSH using a sample collected **within 2-8 days of birth**, as part of a national screening programme. In the absence of such a national screening programme, clinically suspected babies should have a sample of venous blood collected for serum TSH assay. The result should be interpreted with the reference range for an age-matched (in days) healthy group.
- The measurement of TSH should be restricted to specialist laboratories and should have a turnaround time of < 5 days.

- **Confirmation of the diagnosis** of congenital hypothyroidism involves measurement of serum TSH and $\square T_4$ in both mother and neonate and TSH receptor antibody in the mother.
- All hypothyroid neonates should be treated as early as possible. Treatment must be started within the first 18 days of life.

Guiding treatment /follow-Up

- The measurement of both TSH and $\square T_4$ are needed to optimize thyroxine replacement in infants.
- Use age related reference ranges.

Hyperthyroidism

Primary Hyperthyroidism

Diagnosis

- The single most important biochemical test is serum TSH.(by a reputable second or third generation TSH assay with a functional sensitivity of < 0.02 mU/L)
- If the serum TSH concentration is within the reference range then a diagnosis of hyperthyroidism is effectively ruled out.
 - Exceptions are rare TSH dependant causes of hyperthyroidism such as,
 - TSH producing tumours of the pituitary
 - Thyroid hormone resistance

- In patients suspected of having hyperthyroidism all subnormal TSH results should trigger the measurement of $\square T_4$.
- If $\square T_4$ is not elevated in patients with subnormal TSH, $\square T_3$ should be measured to identify cases of T₃-thyrotoxicosis.
- The finding of a serum TSH concentration below the reference is not, however, specific for the diagnosis of hyperthyroidism.
 - Low serum TSH, especially if low but > 0.10 mU/L, often reflects “non-thyroidal illness” or therapy with a variety of commonly prescribed drugs.
 - The co-existence of hyperthyroidism and non-thyroidal illness may result in the finding of a normal $\square T_3$.

Once a biochemical diagnosis of hyperthyroidism has been made, following tests may be needed to indicate the cause and specialist referral should be sought.

Thyroid peroxides antibodies	(TPOAb)
TSH -receptor antibodies	(TSH-RAb)

The measurement of above antibodies is not routinely required to determine the cause of hyperthyroidism if the treatment plan is not altered.

Guiding Treatment

- The degree of elevation of serum $\square T_4$ or $\square T_3$ provides an indication of the severity of hyperthyroidism and should be interpreted in the context of clinical symptoms and signs to direct first-line therapy.

Assessing response to therapy

Thionamide therapy

- $\square T_4$ (or $\square T_3$ in cases of T₃-thyrotoxicosis) will be the marker of choice to guide thionamide therapy.
- Serum TSH alone is not adequate since TSH may remain suppressed for weeks-months after initiation of thionamides.
- Thyroid function tests should be performed every 4-6 weeks after commencing thionamides. The frequency of testing should be reduced to every 3 months once a maintenance dose is achieved.

Radioiodine therapy

- Measure serum $\square T_4$ and TSH in all patients. In most cases the $\square T_4$ will be the marker of choice to guide therapy.
- Thyroid function tests should be performed every 4-6 weeks for at least 6 months. Reduce the frequency of testing when the $\square T_4$ remains within the reference range, although an annual T $\square T$ is still needed.

Long –term Follow-Up

- Life –long thyroid function testing is needed for all patients who have received radioiodine therapy or surgery for hypothyroidism.
- Regular thyroid function testing is required for all patients being treated with long-term thionamides.

Subclinical (Mild) Hyperthyroidism**Diagnosis**

- Low serum TSH in the presence of normal concentrations of fT_4 and fT_3
- Exclude non thyroidal illness and drug therapies

Guiding treatment/follow- up

Patients with subclinical hypothyroidism that cannot be explained by non-thyroidal illness or drug therapy should have repeat serum TSH with fT_4 and fT_3

- The timing of repeat assessment should be based on the clinical picture
- More frequent testing may be appropriate if the subject is elderly or has underlying vascular disease, otherwise repeat biochemical testing after 3-6 months may be appropriate.
- Persistent subclinical hyperthyroidism should prompt specialist referral.

- Untreated subclinical hyperthyroidism should be followed into the long term by testing thyroid function every 6-12 months.

Inappropriate TSH

- Elevation of fT_4 and /or fT_3 is associated with an “inappropriately” detectable or elevated serum TSH concentration.
- This should stimulate the laboratory to consider errors or assay artifacts. Confirmation by repeat, including another assay is good practice.
- Once the laboratory has excluded such explanations then the cause of “true” inappropriate TSH should be considered.
- The measurement of serum SHBG and circulating free alpha subunit and other anterior pituitary hormones help to distinguish TSH-oma from thyroid hormone resistance.

Thyroid Function Tests in Pregnancy**Introduction**

- During pregnancy TT_4 and TT_3 are elevated. TSH is elevated in the first trimester.
- **Both TSH and fT_4** (and fT_3 also when TSH below the detection limit of a reputable assay) should be used to assess thyroid status and monitor thyroxine therapy in pregnancy.
- **Trimester- and method-specific reference intervals** should be used when reporting thyroid test values for pregnant patients.

Hypothyroidism

- The thyroid status of hypothyroid patients should be checked with TSH and fT_4 during each trimester. Measurement of T_3 is not appropriate.
- Normal TSH and fT_4 concentrations for the **gestational age** should be maintained.
- In hypothyroid patients the TSH should be checked and the thyroxine dose should be adjusted as soon as pregnancy is diagnosed.
- The dose of thyroxine will usually require a small increase, to ensure that the fT_4 level is in the upper reference range and the TSH in the low / normal range.
- An increase in the dose of T_4 is especially important for women who have been treated for thyroid cancer, to ensure that the TSH remains fully suppressed.
- After delivery the TSH should be checked, at 2-4 weeks post-partum. (at which time the dose of thyroxine can usually be reduced back to the pre-pregnancy level)
- Ideally, the following sequence of T fT should be performed in the hypothyroid woman during pregnancy

- * before conception if possible
 - * at time of diagnosis of pregnancy
 - * at antenatal booking
 - * at least once in second and third trimesters and again after delivery
- the newly diagnosed hypothyroid patient will need to be tested frequently(every 4-6 weeks) until stabilized

Hyperthyroidism

- If possible, thyroid function tests should be performed, prior to conception, in hyperthyroid women taking antithyroid drugs, and therapy modified if appropriate.
- Hyperthyroid women taking antithyroid drugs should have thyroid function tests checked at the time of diagnosis of pregnancy or at antenatal booking, when the therapy may need to be modified and the dose reduced.
- The newly diagnosed hyperthyroid patients should be monitored by measuring fT_4 (rather than TSH) monthly until stabilized.
- Monthly measurement of serum fT_4 is required in pregnant women receiving antithyroid drugs.

- Women who have been successfully treated previously for hyperthyroidism and are euthyroid at antenatal booking may be checked again in the second and third trimesters.
- All previously hyperthyroid females should be retested after delivery, as there is a higher chance of relapse at this time.
- Do not measure TSH-RAb again if it is low or negative at antenatal booking. A very high titer can predict the chance of intrauterine or neonatal thyrotoxicosis developing.

Post –Partum Thyroiditis

- Post-partum patients should have TSH and fT_4 measured at 6-8 weeks post-partum (or post-abortion) if they have any of the following.
- Goiter
- Non-specific symptoms that may suggest thyroiditis
- Previous history of post partum thyroiditis
- Previous history of autoimmune thyroid disease
- Positive TPO-Ab
- If the initial T_4 show a thyrotoxic pattern, do further tests to differentiate post-partum thyroiditis from Grave's disease.
- Start thyroxine therapy in a symptomatic patients if thyroid tests show hypothyroidism.

Screening for thyroid disease during pregnancy

- Pregnant women in the following categories should have thyroid function assessed either at diagnosis or at antenatal booking or even before conception, if feasible.
 - * type-1 diabetes
 - * previous history of thyroid disease
 - * current thyroid disease
 - * family history of thyroid disease
 - * goiter
 - * symptoms of hypothyroidism
- Thyroid function tests during pregnancy should comprise both TSH and fT_4

Neonatal thyroid assessment

Neonatal screening for congenital hypothyroidism

- All newborn babies should be screened for congenital hypothyroidism by measurement of blood spot TSH using a sample collected within 2-7 days after birth as part of a national screening programme.
- The peak TSH surge occurs within 30 min of birth and start to fall by 1-2 hours so that the concentrations reach adult levels by 2-3 days.
- The laboratories should be able to issue a result within 5 days.
- Confirmation of the diagnosis of congenital hypothyroidism involves measurement of serum TSH
-

and $\square T_4$ in both mother and neonate and TSH receptor antibody in the mother.

- All hypothyroid patients should be treated as early as possible. Treatment must be started within the first 18 days of life.

Neonatal hypothyroidism

- The measurement of both TSH and $\square T_4$ are required to optimize thyroxine replacement in infants. **Age related reference ranges should be used.**

Neonatal hyperthyroidism

- The diagnosis of neonatal hyperthyroidism requires measurement of both TSH and $\square T_4$. Both should be measured at regular intervals to guide treatment.

Thyroid Function Tests in Thyroid Cancer

Differentiated thyroid cancer

The role of TSH and thyroid hormones

Diagnosis

- Thyroid function tests do not directly aid the diagnosis of thyroid cancer, as patients are generally euthyroid.

Monitoring treatment and long term follow-up

- After thyroidectomy for thyroid cancer the TSH should be suppressed to and maintained at a level of <0.1 mU/L , in a reputable second or third generation TSH assay.

Thyroglobulin(Tg) and thyroglobulin antibodies(TgAb)

Diagnosis

- The measurement of serum thyroglobulin has no role in the diagnosis of thyroid cancer.

Monitoring therapy and long term follow-up

- Samples for thyroglobulin assay should not be collected for at least 4-6 weeks after thyroidectomy or iodine therapy.
- In a sensitive assay (RIA or IRMA) detectable thyroglobulin usually indicates the need for further investigation to identify the source. The laboratory should advise of the cut-off level for a particular assay method.
- Perform $T\text{□}T$ whenever thyroglobulin and thyroglobulin antibodies are measured. .
- The requesting clinician should indicate on the request form whether the patient is on thyroxine therapy.

- The sensitivity of serum Tg measurement for detecting recurrence is enhanced by an elevated TSH level. Hence, **Tg should be preferably measured when the serum TSH is > 30 mU/L**(after thyroxine withdrawal or the use of recombinant human TSH). There is no need for TSH stimulation if the serum Tg is already detectable.
- If TgAb are detectable, measurement should be repeated at regular intervals.(6monthly) If undetectable they should be measured at follow-up when thyroglobulin is measured. The development of increasing TgAb may indicate recurrence of tumor.
- For routine follow-up of patients in remission, serum Tg can be measured while the patient is taking TSH suppressive treatment.
- The frequency of Tg measurement during follow-up of thyroid cancer depends on the clinical condition of the patient.
- Tg results are method dependent. Clinicians should use the same laboratory and Tg assay on a long-term basis, to ensure the continuity in monitoring.
- Laboratories should not change methods without prior consultations with clinical users of the service.

Medullary thyroid cancer

Calcitonin

Diagnosis

- A pre-operative value for serum calcitonin (preferably fasting) should be measured in patients with medullary thyroid cancer (MTC) to establish a baseline for the long-term follow up.

Monitoring therapy

- The response to primary surgery can be assessed both clinically and by the measurement of serum calcitonin.
- Post-operative samples should be collected at least 10 days after thyroidectomy and should be fasting if possible.
- Life long calcitonin measurement is recommended for patients with medullary cancer. The frequency of measurement will depend on both clinical status and the previous calcitonin result.

Thyroid function tests in medullary cancer

- T₄T₃ have no place in the diagnosis of MTC.
- During follow-up T₄T₃ should be monitored.

- TSH suppression is not appropriate for the treatment of MTC. So thyroid hormone replacement should follow the guidelines for treating hypothyroidism.

Laboratory Investigations for Thyroid Dysfunction

Provision of Laboratory Tests

- The laboratory should be able to issue T₄T₃ results within 48 hours from receipt of the specimen though in the majority of cases there is no urgency for receipt of routine T₄T₃.
- More rapid response is desirable in thyrotoxic crisis and myxedema coma as they are medical emergencies.

Grouping of Thyroid Function Tests

- Blood tests which establish if there is thyroid dysfunction(TSH, fT₄, fT₃, TT₄, TT₃)
- Tests to elucidate the cause of thyroid dysfunction(thyroid auto antibodies)

Measurement of TSH and thyroid hormones should be performed to determine the patient's thyroid status before ordering the more demanding tests that seek to determine the cause of the thyroid dysfunction.

Tests to establish if there is thyroid dysfunction

Thyrotrophin(TSH)

- The measurement of TSH by a **sensitive immunometric** assay provides the single most sensitive, specific and reliable test of thyroid status in both overt and subclinical primary thyroid disorders.
- TSH alone is not a reliable test for detecting thyroid dysfunction arising from hypothalamic-pituitary dysfunction.
- It is essential that laboratories use a reliable and sensitive method for TSH that meets the following conditions.
 1. The functional sensitivity should be used to define the lowest concentration of TSH that can be determined in routine use.
 2. Functional sensitivity is defined from the 20% between-run coefficient of variation (CV) determine by a recommended protocol.
 3. Laboratories should use a TSH method with a functional sensitivity of < 0.02 mU/L
 4. Prior to the introduction of a TSH method, the laboratory should validate the functional sensitivity quoted by the manufacturer. Quality assurance procedures should be in place to ensure that the functional sensitivity of the assay is regularly monitored.

Free T₄ (FT₄) and Free T₃ (FT₃)

- Laboratories should be aware of how their assay performs in a variety of clinical situations including thyroid disorders, pregnancy, non-thyroidal illness, certain medications (heparin, phenytoin, furosemide, carbamazepin, salicylate) and familial binding protein abnormalities.
- Clinicians should be made aware, by the laboratory, of the expected assay performance in the clinical settings listed above.
- Laboratories should obtain from kit manufacturers details of how their assay compares with equilibrium dialysis in the clinical situations listed above.
- **Free hormones can increase in samples on storage** but because of assay design (eg inclusion of albumin in reagents) not all free hormone methods detect such changes. Laboratories should be aware of how storage affects free hormone concentrations when measured by their own method. Appropriate action should be taken to minimize such sample deterioration.
- Freeze samples that cannot be assayed within 48 hours of collection.
- Interference from anti-thyroid antibodies is method dependent. Laboratories should know how the presence of such antibodies would affect their assay.

Total T₄ (TT₄) and total T₃ (TT₃)

- It is common to find abnormal TT₄ and TT₃ in some euthyroid patients.
Eg Changes in serum thyroid binding proteins (pregnancy)
Changes in affinity for hormones (non-thyroidal illness drugs such as salicylates)

Reference ranges

Adults

- TSH 0.4- 4.5 mU/L
- □T₄ 9.0-25 pmol/L
- □T₃ 3.5-7.8 pmol/L
- TT₄ 60-160 nmol/L
- TT₃ 1.2-2.6 nmol/L

TSH (3rd generation immunochemiluminometric assay)

- | | | | |
|---|------------|-----------------|-----------------|
| * | Premature | (28-36 wk) | 0.7-27.0(miu/L) |
| * | Cord blood | (>37 wk) | 2.3-13.2 |
| * | Children | birth to 4 days | 1.0-39.0 |
| | | 2-20 wk | 1.7-9.1 |
| | | 21wk-20y | 0.7-6.4 |

*	Adults	21-54 y	0.4-4.2
		55-87 y	0.5-8.9
*	Pregnancy	□first trimester	0.3-4.5
		Second trimester	0.5-4.6
		Third trimester	0.8-5.2

TSH surges immediately after birth, peaking at 30 min at 25-160 mIU/L and values decline back to adult values in the first week of life.

FT₄ (by direct equilibrium assay)

			pmol/L
*	Newborn	(1-4 days)	28.4-68.4
	Children	(2wk-20yr)	10.3-25.8
	Adults	(21-87y)	10.3-34.7
	Pregnancy	(□first trimester)	9.0-25.7
		(2 nd & 3 rd trimester)	6.4-20.6

Total T₄

		nmol/L
	Cord	95- 168
	Children	
	1-3 days	152-292

	1-2 wk	126-214
	1-4 mo	93-186
	4-12 mo	101-213
	1-5 yr	94-194
	5-10 yr	83-172
	10-15 yr	72-151
	Adults (15-60 yr)	
	Males	59-135
	□emales	65-138
	Adults> 60 yr	65-138
	New born screen (whole blood)	
	1-5 days	>97
	6 days	> 84

- Where possible manufactures reference ranges should be confirmed locally using an adequate population size of at least 120 ambulatory subjects.
- Since TSH, free and total thyroid hormones change during pregnancy. trimester related reference ranges should be available.

Quality control and quality assurance

Internal quality control (IQC)

- All laboratories must run IQC that comprise human serum pools
- The analyte concentrations for the pools should be chosen to ensure that assay performance is monitored within the euthyroid, hyperthyroid and hypothyroid ranges. Laboratories should define allowable limits of error for each of these results.

External quality control (EQC)

- All laboratories must participate in an accredited EQA scheme.
- Laboratories should ensure that assay performance meets the minimum performance specified by the EQA scheme.

Follow-up of unusual test results

Unusual combinations of TSH and thyroid hormone results may have a pathological source but more commonly result from poor compliance or assay interference in one or more assays.

- Laboratories should have protocols available to determine if results are analytically valid or due to assay interference.

Such protocols may include:

dilution tests

Removal of heterophilic antibody and HAMA using commercial tubes (for hormone assay)

Remove of anti-thyroid antibodies using polyethylene glycol precipitation

Confirmation by an alternative assay which if possible should have been validated against a reference method

Laboratory tests used to determine the cause of thyroid dysfunction

1. Thyroid peroxidase antibodies (TPOAb)

- The measurement of TPOAb is of clinical use in diagnosis of autoimmune thyroid disorders
 - As a risk factor for autoimmune thyroid disorders
 - As a risk factor for hypothyroidism during treatment with interferon alpha, interleukin-2 or lithium
 - As a risk factor for thyroid dysfunction during lithium or amiodarone therapy.
- **TPO results are method dependent** and this should be recognized.
- Functional sensitivity should be determined for the TPOAb method using the same protocol as for TSH
- A sensitive and specific **immunoassay should be used** to measure TPOAb, **not an agglutination test.**

2. Thyroglobulin antibodies(TgAb)

- In iodine sufficient areas it is of no value to measure both TgAb and TPOAb in non-neoplastic conditions.
- The only reasons to measure Tg antibodies are
 - a. in differentiated thyroid cancer to determine possible interference from these antibodies in immunoassays for thyroglobulin.
 - b. Serial measurements may prove to be useful as a prognostic indicator
- Assays should be performed using a **sensitive immunoassay not an agglutination method**
- Determine functional sensitivity as for TSH
- Thyroglobulin and TgAb be measured in the same specimen.
- If Tg Ab is being used for monitoring purposes the method should not be changed without consultation with the users.

3. TSH receptor antibodies(TSH-RAb)

- The measurement of TSHR-Ab is particularly helpful in pregnancy to investigate hyperthyroidism of uncertain origin to investigate patients with suspected “ euthyroid Graves” opthalmopahty.
□or pregnant women with a past or present history of Graves disease

To identify neonates with transient hypothyroidism due to TSH blocking antibodies

4. Thyroglobulin (Tg)

- In patients with differentiated thyroid cancer measurement of serum Tg is used in monitoring, but is not of value in the initial diagnosis.
- Samples for Tg measurement should be preferably be taken when the TSH is elevated either after withdrawal of T4 therapy or following administration of recombinant human TSH. □or patients in remission and under routine follow-up it is acceptable in the first instance to sample during T4 administration.
- Laboratories and manufactures should inform clinicians of the possibility of interference due to endogenous TgAb and indicate the most likely nature of the interference(false elevation/false reduction in measured Tg)

5. Calcitonin

Specimen collection

- Ideally a fasting morning specimen should be obtained to enable optimal comparison with reference values.(If this is not possible specimens can be collected at any time of day)
Calcitonin in serum is unstable. **Specimens should be kept on ice. Red cells then should be separated**

within 30 min of collection and serum frozen immediately.

- Post-operative samples should be collected at last 10 days after thyroidectomy and should also be fasting samples if possible.
- ☐or provocative testing samples are usually collected 5 min prior to administration of calcium/pentagastrin and then at intervals of 2.5 and 7 min after.

Methodology

- Laboratories must decide whether to use a method that recognizes primarily monomeric calcitonin (IMA) or a method with broader specificity (RIA).

Specimen collection and storage

TSH

Serum that is free of haemolysis and signs of lipaemia is preferred.

Specimens are stable for 5 days at 2 to 80 C and for at least 1 month when stored frozen.

☐or newborn screening, whole blood can be collected by heel prick 48 to 72 hours after birth.

Thyroxine

Serum is the preferred specimen

Serum is best stored at 2 to 80C if they will not be tested within 24 hours.

If longer periods of storage are necessary, freeze the specimen.

☐rozen specimens are stable for at least 30 days.

Avoid repeat freezing and thawing.

Do not analyse grossly haemolysed specimens.

Triiodothyronine

Serum is the preferred specimen.

Serum is best stored at 2 to 80C if they will not be tested within 24 hours.

If longer periods of storage are necessary, freeze the specimen.

☐rozen specimens are stable for at least 30 days.

Avoid repeat freezing and thawing.

Thyroid binding globulin

Serum is the preferred specimen.

Serum is best stored at 2 to 80C if they will not be tested within 24 hours.

If longer periods of storage are necessary, freeze the specimen.

☐rozen specimens are stable for at least 30 days.

Avoid repeat freezing and thawing.

Patients should discontinue thyroid hormone replacement therapy long enough (6 weeks) to have an elevated TSH level prior to specimen collection.

Anti-thyroid antibodies

Serum is the preferred specimen.

Serum is best stored at 2 to 80C if they will not be tested within 24 hours.

If longer periods of storage are necessary, freeze the specimen.

Calcitonin

Serum is the preferred specimen.

Ideally a fasting morning specimen should be obtained.

Calcitonin results may be affected by visible haemolysis or lipaemia and assay of such specimens should be avoided if possible.

Calcitonin in serum is unstable. **Specimens should be kept on ice.**

Red cells then should be separated within 30 mins of collection and serum frozen immediately.

Post operative samples should be collected at least 10 days after thyroidectomy.

Selective use of thyroid function tests

- The use of first-line TSH will fail to identify some patients with thyroid disorders.
- Situations in which TSH usually provides the correct estimate of thyroid status
 - I. In overt primary hyperthyroidism TSH is nearly always below 0.10 mU/L
 - II. In overt primary hypothyroidism serum TSH is always increased.
 - III. In subclinical disorders, TSH will be the most sensitive indicator of failing thyroid function, and serum T_4 and T_3 are often normal. Before the diagnosis of subclinical thyroid disorders can be made, causes of abnormal TSH other than thyroid disorders such as pregnancy, non-thyroidal illnesses, drug treatment and assay interference must be excluded.

- The measurement of TSH with T_4 should allow the detection of almost all cases of thyroid dysfunction as long as the results of both tests are correctly interpreted.
- Additional tests such as T_3 may be needed in some circumstances.

References

1. Tietz text book of Clinical chemistry. Carl A. Burtis, Edward R. Ashwood, 3rd edition

Recommendations:

B. Thyroid function tests can be carried out in any hospital lab. Samples should be sent to the relevant laboratory for analysis. Please discuss with the relevant laboratory before sending the specimens.

1.5 Investigation of Disorders of Cortisol Metabolism

- Cortisol excess
- Cortisol deficiency

A random cortisol estimation is difficult to interpret due to the variability of cortisol excretion during the day and should be avoided if possible.

As with cortisol, 17 hydroxyprogesterone (17OHP) has a marked circadian rhythm.

Diurnal rhythm and adult values are reached by 3 months.

Cortisol Excess

Patient assessment

Laboratory tests shouldn't be used in isolation, results should be interpreted together with a full clinical assessment of the patient.

Choice of test

First-line screening tests must have high sensitivity to minimize the incidence of false negative results and reference (cut-off) values chosen to achieve these aims.

Screening tests

Overnight Dexamethasone Suppression
Test is particularly recommended
Or
24-hour urinary free cortisol

Definitive Tests

Are indicated if a positive result is found on screening.

Overnight Dexamethasone Suppression Test

This test can be performed in any hospital. Separated serum should be sent to relevant laboratory for analysis.

Principle

Dexamethasone is a potent glucocorticoid which, when given as a 1 mg oral dose at night, will normally suppress ACTH secretion and hence suppress the cortisol level the following day morning.

It is a useful initial screening test for Cushing's syndrome.

Patient preparation Not required

Sample Serum

Procedure

The patient is given 1 mg Dexamethasone (small children may require a relatively smaller dose) to be taken at 11.00 pm (± 1 hour)

Blood is taken at 9.00 am (± 1 hour) the following morning for cortisol measurement.

Interpretation

Normal subjects will have a **9 am cortisol of < 50 nmol/L** after 1 mg of Dexamethasone.

Following are the causes for serum cortisol level to be > 50 nmol/L (failure to suppress) following overnight dexamethasone test.

1. Cushing's syndrome
2. Stress
3. Obesity
4. Oral contraceptive use
5. Pregnancy
6. Estrogen therapy
7. Alcoholism
8. Acute or chronic illness

9. Failure to take dexamethasone
10. Treatment with phenobarbitone, phenytoin, carbamazepin
11. Glucocorticoid therapy with prednisolone or hydrocortisone
12. Endogenous depression

Recommendations

- A. Should be carried out under the supervision and recommendation of a consultant. Samples should be sent to the relevant laboratory for analysis. Please discuss with the relevant laboratory before sending the specimens.**

24-hour Urine Free Cortisol (UFC)

1. This should be determined using an assay which incorporates an extraction step.
 2. Creatinine should be measured in the 24-hour sample to verify the adequacy of collection.
 3. Results should be interpreted with caution in young children and in patients with significant renal dysfunction
 4. The reference range should be quoted on reports; the upper limit should not exceed 300 nmol/24 hours.
- * **UFC may not identify patients with mild hypercortisolism and therefore UFC cannot be considered a universal single screening test for the detection of Cushing's syndrome.**

This test is useful in

- pregnancy
- Patients on oral contraceptive treatment or estrogens

Recommendations

- A. Should be carried out under the supervision and recommendation of a consultant. Samples should be sent to the relevant laboratory for analysis. Please discuss with the relevant laboratory before sending the specimens.**

Low Dose Dexamethasone Suppression Test

This should be performed under the supervision of a Consultant. Separated serum should be sent to relevant laboratory for the analysis.

Indications

Establishment of diagnosis of Cushing's syndrome.

Contraindications None

Precautions

Care in diabetes mellitus and active peptic ulceration.

For inpatients each dose of dexamethasone should be written up as an individual dose.

Procedure

Draw a sample of blood at 9.00 am on day 0 for serum cortisol.

(Basal sample) Give dexamethasone 0.5 mg orally strictly 6-hourly at 9.00, 15.00, 21.00 and 3.00 (9 am, 3 pm, 9 pm and 3 am) for 48 hours, commencing immediately after the basal sample.

(Children < 10 years 5µg/Kg)

Blood sample for cortisol at 9.00 hours (9.00 am) after 48 hours on day 2 (6 hrs after last dose)

Normal response

Basal serum cortisol is in the normal resting am range at 9.00(170-700 nmol/L)

After 48 hours serum cortisol is suppressed to < 50 nmol/L

Interpretation

A serum cortisol < 50 nmol/L after 48 hours excludes Cushing's syndrome unless clinical suspicion is very high

Very rarely patient's with Cushing's syndrome may show normal suppression.

- A number of conditions may cause non-suppression with low -dose dexamethasone in the absence of Cushing's syndrome.

Severe endogenous depression Patients may have an abnormal circadian rhythm but show normal cortisol response to an ITT.

Alcoholism(Alcoholic pseudo- Cushing's) Rapidly reverses on stopping drinking

Severe stressful illness /infection Do not perform the test

Failure to take dexamethasone correctly Check with the patient

Oestrogen therapy Pregnancy, Oral contraceptives due to high globulin binding proteins

Corticosteroid therapy Prednisolone & hydrocortisone cross react in the cortisol assay.

Recommendations

- A. Should be carried out under the supervision and recommendation of a consultant. Samples should be sent to the relevant laboratory for analysis. Please discuss with the relevant laboratory before sending the specimens.**

Dexamethasone suppression of cortisol

(extended)

Test for differential diagnosis of Cushing's syndrome.

This should be performed under the supervision of a Consultant. Separated serum should be sent to relevant laboratory for the analysis.

Principle

The multiple Dose Dexamethasone suppression test (DST) consists of administration of Dexamethasone

0.5 mg every 6hrs for 48 hours (low does Dexamethasone suppression test) followed by 2mg Dexamethasone every 6hrs for 48 hrs

Clinical use

The test aids in the differential diagnosis of hypercortisolism in patients who do not suppress after an overnight Dexamethasone suppression test.

Sample Serum for cortisol

Side effects some subject report sleep disturbances

Procedure

The test is performed as an in-patient procedure.

No preparation is required but the ward staff must be aware of the importance of correctly timed collection of specimens.

Insert an iv cannula

Blood is collected daily at 9.00a.m. for cortisol measurements.

At 9.00a.m on day 1, commence Dexamethasone 0.5 mg orally 6hrs for 48hours after drawing the basal blood sample at 9.00.m.

At 9.00 a.m. on day3, increase the dose to 2mg every 6hrs for 48 hours.

Day	Time	Dosage	Cortisol	
Day 1	0900	0.5mg	✓	
	1500	0.5mg		
	2100	0.5mg		
Day 2	0300	0.5mg		
	0900	0.5mg	✓	
	1500	0.5mg		
	2100	0.5mg		
	Day 3	0300	0.5mg	
		0900	2.0 mg	✓
1500		2.0 mg		
	2100	2.0 mg		
	Day 4	0300	2.0 mg	
		0900	2.0 mg	
1500		2.0 mg		
	2100	2.0 mg		
	Day 5	0300	2.0 mg	
		0900	2.0 mg	✓

The test is carried out according to the table above. It is important to collect the 0900 hour blood sample before giving oral Dexamethasone.

Guide to interpretation

Patients with pituitary dependent Cushing's syndrome(Cushing's disease) usually do not suppress (<50% of basal) with low dose but the majority of patients will suppress with high dose.

(A significant minority of patient's (15%) with Cushing's disease do not suppress)

Suppression is defined as **50% reduction** in basal cortisol.

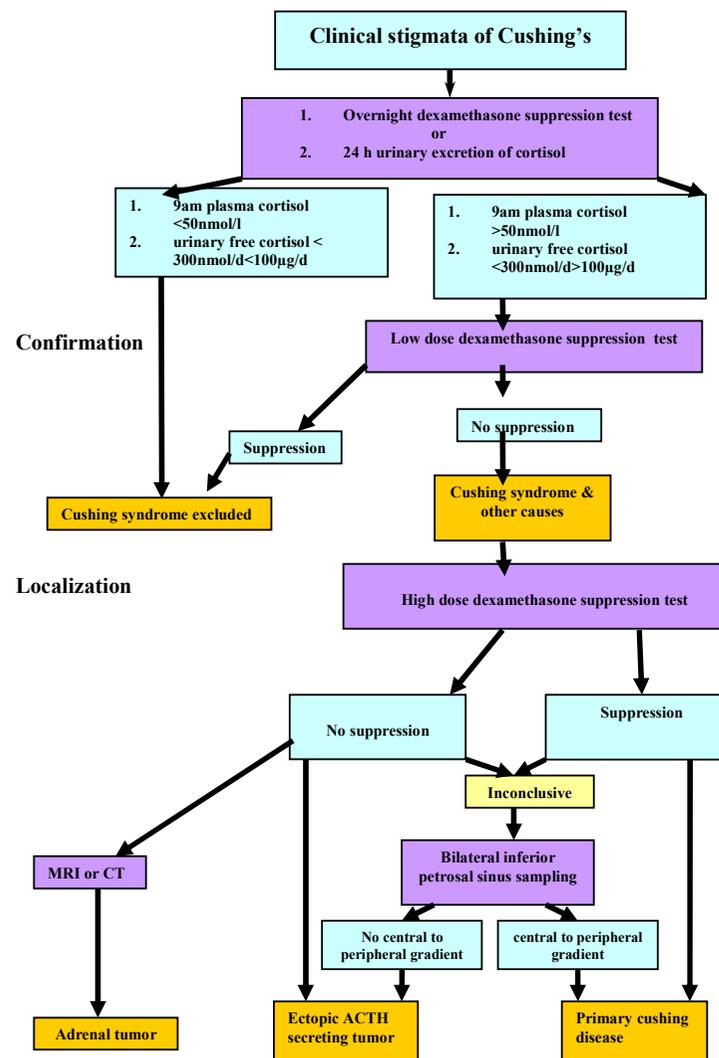
Patients with false positive results in the overnight Dexamethasone suppression test should suppress in the low dose period.

Patients with adrenal tumors or ectopic ACTH production tumors fail to suppress with rare exceptions.

Patients with adrenal adenomas have ACTH levels below the reference range, while patients with ectopic ACTH producing tumors have ACTH levels within or above the reference range.

Recommendations

A. Should be carried out under the supervision and recommendation of a consultant. Samples should be sent to the relevant laboratory for analysis. Please discuss with the relevant laboratory before sending the specimens.



Sample collection and storage

Serum cortisol

Serum is the preferred specimen. Collect blood(2ml) into a bottle without a preservative. No special handling procedures are necessary. Specimens must be stored refrigerated overnight at 20C to 80 C. reezing is preferred for long-term stability.

24-hour free cortisol

A complete 24-hour urine specimen is collected with or without 10g boric acid to maintain pH < 7.5.

If collected without preservative urine should be refrigerated during the collection period.

After measuring the total volume, a thoroughly mixed aliquot (~ 10 ml) is stored frozen at -200C .

Care should be taken to ensure an appropriately timed, complete 24-hour collection because an incorrectly timed sample is the largest source of error with this method.

ree cortisol determination on randomly collected urine are discouraged because of the variation and pulsatile characteristic of cortisol secretion.

Adrenocorticotrophic Hormone(ACTH)

ACTH is easily oxidized, strongly adsorbs to glass surfaces, and can be rapidly degraded by plasma proteases into immunoreactive fragments during freezing and thawing of the specimen.

actors that influence plasma ACTH, such as prior administration of corticosteroids, time of day at

which the specimen is taken and stress from a poorly performed venipuncture, must be taken into account.

Blood specimen should be collected into prechilled polystyrene(plastic) tubes containing EDTA. Immediately placed on ice, and centrifuged at 40C .

Supernatant is then transferred to another plastic tube and stored at -200C or colder.

Immediately prior to setting up the ACTH assay ,frozen specimen should be thawed and centrifuged to remove any fibrin clots, which may interfere with the assay.

Precision and Bias

Each laboratory should ensure that appropriate internal quality control and external quality control assessment procedures are in place.

Any laboratory consistently unable to meet the following criteria and which cannot change to a superior assay should refer samples elsewhere.

- a) or serum cortisol, the precision should be less than 15% and bias < 15%.
- b) or urine free cortisol, the precision should be < 25%.

References

1. Queensland health pathology services: Royal Brisbane Hospital , Tests list, collection details 2001
2. All Wales Clinical biochemistry audit group standards for screening for Cushing's syndrome, 2001

3. C.Bhagat. Consensus Statement Review: Diagnosis and Complication of Cushing's Syndrome: a consensus statement. *The Clinical Biochemist Reviews* 2004; 25; 149-151
4. Tietz Text Book of Clinical Chemistry. Carl A. Burtis, Edward R. Ashwood. 3rd edition
5. Bonstein S.R, Stratakis C.A., Chrousos G.P. Adrenocortical tumours: Recent Advances in Basic Concepts and Clinical Management. *Annals of Internal Medicine* 1999; 130:759-771

Cortisol Deficiency

Synacthen Stimulation of Cortisol-(short) Test for Adrenal Insufficiency

Rationale

A low plasma/serum cortisol that does not rise after ACTH administration (Short Synacthen test) confirms impaired adreno-cortical reserve.

If the impaired response persists after prolonged ACTH administration (Long Synacthen Test), primary adreno-cortical insufficiency is indicated rather than secondary adrenal atrophy due to impaired ACTH secretion; the latter results either from prolonged corticosteroid therapy or hypothalamic-pituitary disease.

Tetracosactrin (Synacthen or Cortrosyn) is the synthetic analogue of ACTH that is now most commonly used for the ACTH stimulation test.

Contraindications

- Known sensitivity to ACTH
- Pregnancy

Patient preparation

The following drugs should be withheld 24 hours prior to the test:

- Glucocorticoids eg prednisolone or cortisone
 - Metapyrone
- If steroids are required within this 24 hour period, preferred drug is Dexamethasone.
Patient doesn't require to fast, nor be at rest.

Procedure

Cannulate the patient and take baseline sample (0 min) for cortisol.

Administer the synacthen dose

250 µg Synacthen im or as a slow iv bolus

0-6 months 36 µg/Kg

6 months-2 yrs 125 µg

≥2 years 250 µg

Draw further two blood samples at 30 and 60 min for cortisol.

17OHP may also be requested (indications, congenital adrenal hyperplasia CAH, hirsutism, infertility)

Interpretation

A normal response is an increase in the serum cortisol level of . 200 nmol/L over the baseline and or the final value > 550 nmol/l.

A failure to respond suggests adrenal failure primary or secondary. A long tetracosactrin test is required to confirm primary adrenal failure

A peak 17OHP response of > 30 nmol/l is indicative of CAH. In addition, a 30 min 17OHP; cortisol ratio >0.1 suggests CAH, < 0.023 is normal.

Values between 0.023 and 1.0 suggest heterozygosity for 21 hydroxylase deficiency.

References

1. Tietz text book of Clinical biochemistry 3rd edition Duty Biochemists Manual, Department of clinical biochemistry, Royal Perth hospital, Sydney, Australia, 2001

Recommendations

A. Should be carried out under the supervision and recommendation of a consultant. Samples should be sent to the relevant laboratory for analysis. Please discuss with the relevant laboratory before sending the specimens.

Long Synacthen Test

There are two protocols. Please contact the following laboratories. (NHSL, MRI, Faculty of Medicine Galle, Faculty of Medicine Peradeniya)

1.6 Investigation of Disorders of Aldosterone Metabolism

Aldosterone-producing tumours are usually associated with hypertension with or without hypokalaemia.

Screening tests for an aldosterone producing tumour include evaluation of hypertension and measurement of serum potassium.

Screening tests

- Serum potassium
- Urinary potassium

To demonstrate renal losses of potassium in the presence of hypokalaemia the patient should be on at least 120 mmol/l of sodium for 3 days before investigation, since a low sodium intake may normalize mild hypokalaemia.

Samples	Spot Urine (needs simultaneous blood and urine specimens) 24-hour urine
---------	---

- Plasma aldosterone : rennin ratio in an upright position
- >20 suggests primary hyperaldosteronism

Confirmatory tests

These should be performed under the supervision of a Consultant. Separated serum should be sent to relevant laboratory for the analysis.

- Saline suppression test
- Oral salt loading test

Recommendations

A. Should be carried out under the supervision and recommendation of a consultant. Samples should be sent to the relevant laboratory for analysis. Please discuss with the relevant laboratory before sending the specimens.

Saline Suppression Test

Rationale

Aldosterone is the major mineralocorticoid synthesized in the adrenal cortex.

Rapid volume expansion with intravenous saline should suppress plasma aldosterone in normal subjects but not in patients with primary aldosteronism.

Procedure

The test is carried out in the ward under supervision.

Patients may have fluids but otherwise should be fasted on the day of the test.

Withhold the following drugs

- β -adrenoceptor blocking drugs - 2 weeks before testing
- dihydropyridine calcium channel blocker - 2 weeks before testing
- Spironolactone - 6 weeks before testing
- Loop diuretics - 6 weeks before testing

Diltiazem can be used to control blood pressure during testing for aldosterone excess.

Serum potassium should be maintained within the reference range (>3.5 mmol/L) prior to commencement and during the test.

The subject is awakened at 0600 h and kept in an upright posture for 2h.

Record Blood Pressure.

BP must be $< 190/110$ mm/Hg before proceeding

Insert IV line and send blood for urgent potassium

Blood is drawn for determination of plasma aldosterone and electrolytes at 0800h. The subject then assumes a supine position, and 2L of isotonic saline, 0.9 g%, is infused over a 4-h period.

Record BP, pulse every 15 min during the infusion for first hour, every half an hour thereafter.

Blood is drawn from the **non-IV infusion arm** for plasma aldosterone and electrolytes determination at 1200h

Potassium should be ≥ 3.5 mmol/L before patient leaves.

Observe patient one hour prior to discharge. Patient may have coffee/tea and food at this time.

Cancel the test if

- $K^+ < 3.5$ mmol/L
- $BP > 190/110$ mm/Hg

Contraindications

- Heart failure
- Uncontrolled hypertension

Interpretation

Normal individuals show a plasma aldosterone level of ≤ 140 $\mu\text{mol/L}$ after saline infusion.

Levels ≥ 277.4 $\mu\text{mol/L}$ are usually seen in patients with autonomously functioning aldosterone – secreting tumours.

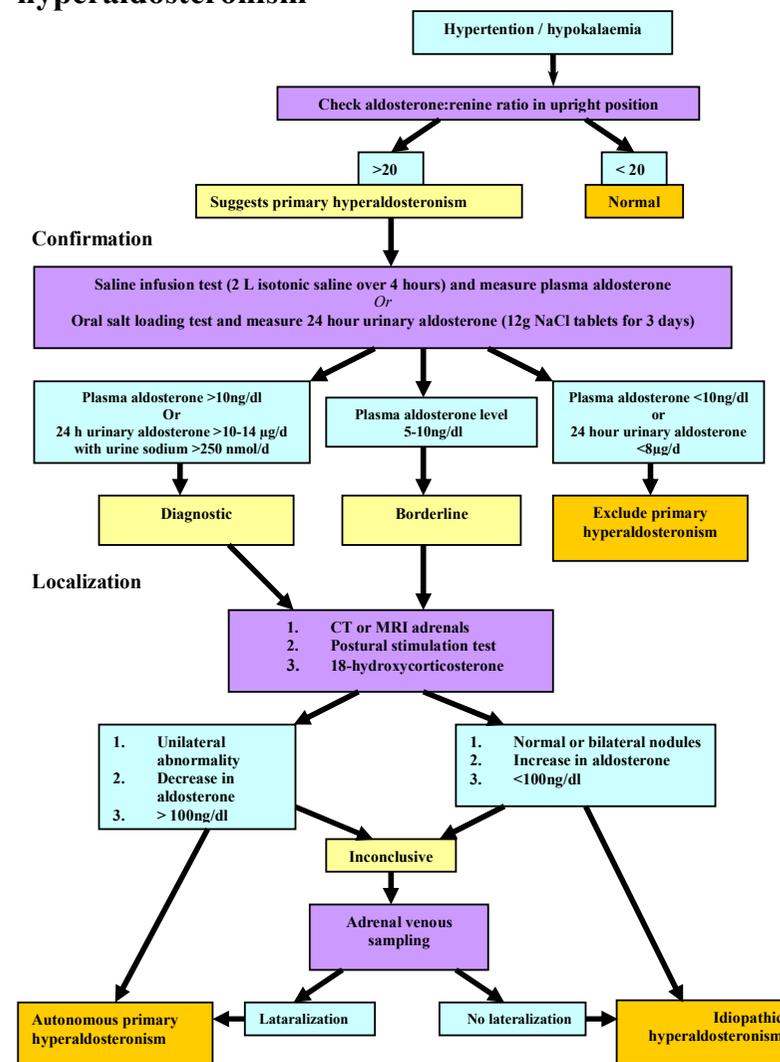
Recommendations

A. Should be carried out under the supervision and recommendation of a consultant. Samples should be sent to the relevant laboratory for analysis. Please discuss with the relevant laboratory before sending the specimens.

References

1. Tietz textbook of Clinical chemistry 3rd edition. Duty Biochemist Manual, Royal Perth Hospital, Australia, 2001
2. Bonstein S.R, Stratakis C.A., . Chrousos G.P. Adrenocortical tumours: Recent Advances in Basic Concepts and Clinical Management. Annals of Internal Medicine 1999; 130:759-771

Diagnostic algorithm for primary hyperaldosteronism



Specimen collection and storage

Aldosterone

Plasma (preserved with heparin, EDTA) or serum can be used. If an upright blood specimen is to be collected, the subject should be in an upright position (standing or seated) for at least 2 h before collection. Specimen should be stored frozen in an airtight container and are stable for up to 2y at -200C.

□or urine assays, a complete 24-hour urine specimen is collected with boric acid as a preservative. 50% acetic acid should also be added to the urine collection to achieve a pH between 2.0 and 4.0. Specimens should not be acidified with strong mineral acids, such as hydrochloric acid.

Aliquots are stored frozen at -200C. It is recommended that a urine sodium also be performed to facilitate the interpretation of the result.

Renin

Blood should be collected in to an EDTA containing bottle.

After centrifugation at **room temperature**, the plasma is removed and frozen at -20⁰C. or lower.

□reeze thaw cycles should be avoided because of the possible activation of prorenin.

Plasma should be transported frozen to the laboratory.

At the time of collection, blood should not be chilled or placed on ice because irreversible cryoactivation of pronin can occur, leading to falsely high estimates of plasma rennin activity.

1.7 Water Deprivation Test

These should be performed under the supervision of a Paediatrician or a Physician. Separated serum should be sent to relevant laboratory for the analysis.

Osmolality should be analyzed only by an osmometre.

Transport the samples to the laboratory as soon as they are collected.

Laboratory should analyze them promptly.

Rationale

This test is to establish the diagnosis of diabetes insipidus in a patient with polyuria, polydipsia, and possibly hypernatremia.

With dehydration and increased plasma osmolality, arginine vasopressin (AVP) is released from the hupothalamus/posterior pituitary gland. Under the influence of AVP, water is reabsorbed from the collecting duct lumen into the hyper osmotic renal medulla along the osmotic gradient. This results in the production of concentrated urine.

Contraindications

If the urine osmolality is > 800 mOsmol/Kg water without exogenous 1-desamino-8-D arginine vasopressin (dDAVP), the test is unnecessary and the patient is considered normal.

Before commencing the test hypothyroidism, cortisol deficiency and osmotic diuresis must be excluded.

DDAVP should not be used in patients with vascular disease especially coronary atherosclerosis and renal failure.

It is important to monitor vital signs during the dehydration procedure.

If weight loss exceeds 2Kg (or 5% in children) or the clinical condition deteriorates, the test **must** be stopped. If the test is performed as above, adverse effects are rare.

To ensure adequacy of dehydration, plasma osmolality before administration of dDAVP should be > 288 mOsmol/Kg water.

Side effects

dDAVP may produce water intoxication only if there has been an increase in urine osmolality. Because of this, patients must not be allowed to drink freely after completion of the test if they have responded to the drug. The early sign of water intoxication are drowsiness, listlessness and headache.

Economization of resources

The following patients can be exempted from following the complete test protocol after assessment of Posterior Pituitary Reserve in the following manner.

Basal investigations

Serum osmolality

Urine osmolality

(Obtained simultaneously on rising or as soon as possible thereafter in the OPD)

Interpretation

If

Serum osmolality is 275-295 mosmol/Kg

Urine osmolality > 600 mosmol/Kg

Urine/serum osmolality ratio >2

The patient will be considered as having normal posterior pituitary reserve and further testing is not indicated.

Precautions

Before commencing the test hypothyroidism, cortisol deficiency and osmotic diuresis must be excluded.

dDAVP (1-desamino=8-D-arginine vasopressin) should not be used in patients with vascular disease especially coronary atherosclerosis and renal failure.

It is important to monitor vital signs during the dehydration procedure.

If weight loss exceeds 2Kg (or 5% in children) or the clinical condition deteriorates, the test must be stopped. If the given protocol is followed, adverse effects could be minimised.

Water deprivation test is not indicated if serum sodium is > 145 mmol/L. Check the serum osmolality.

If the serum osmolality is > 300 mosm/Kg water in a patient suspected of having diabetes insipidus, or in very small children, testing can be done without subjecting the patient to a period of dehydration.

Check basal serum and urine osmolality
Give dDAVP (see below for dose)
Test serum and urine osmolality 2 hours later.

Procedure

The laboratory must be given notice in advance of commencement of test/specimen collection

Ensure availability of a ward environment in which the patient can be observed and in which the patient does not have access to any water, including hand washing basins, toilets etc.

If mild polyuria exists, the subject should be fasted from 2200 hours (10.00 pm) the night before the test, if moderate to severe polyuria exists, the subject should be fasted from 0700 hours on the day of the test. In children the test should be carried out in the day time under supervision.

Prior to fasting (before 0700 hours- 7.00 am) , weigh patient, take random urine(1-2 ml) for osmolality (do not use any preservative) and collect blood (2 ml) for osmolality and serum sodium.

Collect urine hourly from 0800 hours (8.00 am) on day of the test into a clean dry container and send immediately to the laboratory for measured osmolality.

Weigh patient hourly from 0800 hours until completion of the test.

Continue until urine osmolality shows < 30 mOsmol/Kg water difference between three successive collections.

Draw a sample of blood (1ml) for measured osmolality and sodium when the plateau is reached. The laboratory will inform this to the ward.

After blood has been collected, inject 5 units of aqueous vasopressin subcutaneously or administer intranasal desmopressin acetate in the following doses.

Adults	0.4 ml (40 micrograms)
Children	0.2 ml (20 micrograms)
Infants	0.1 ml (10 micrograms)

One hour later collect urine for measured osmolality and blood sample for measured osmolality and sodium.

If there is no definite change in urine osmolality repeat measurement of osmolality in urine one hour later.

Guide to Interpretation

	Urine osmolality (mosmol/Kg water) before dDAVP	Increase in urine osmolality after dDAVP	Serum osmolality (mosmol/ Kg water) before dDAVP
Normal	≥ 800 (> 400 usually)	< 9% (No rise in urine osmolality)	< 300 Serum Na is normal
Diabetes Insipidus -Central hypothalamic -Nephrogenic	< 800 (< than that of serum) usually < 300 usually < 300	> 50% to reach ≥ 800 < 10%	May be > 300
Partial central	Urine osmol > plasma	Increase to < 800 (peak of 300-600)	
Partial nephrogenic	300 < urine < 800	No response	

In complete nephrogenic diabetes insipidus, AVP levels exceed 3 ng/L when plateau osmolality is reached, whereas in complete central diabetes insipidus, AVP levels do not exceed 1.5 ng/l.

Psychogenic polydipsia can be excluded when

1. Serum Na > 145 mmol/L or serum osmolality > 300 mosmol/Kg water
2. Urine/ plasma osmolality < 1.5

Osmolality should be measured by an osmometer.

Recommendations

A. Should be carried out under the supervision and recommendation of a consultant. Samples should be sent to the relevant laboratory for analysis. Please discuss with the relevant laboratory before sending the specimens.

Sample Collection**Osmolality**

Collect fresh urine (1 ml) and serum (1 ml) samples into a plain bottle without a preservative.

Transport to the laboratory as soon as they are collected.

Laboratory should analyze them promptly.

Antidiuretic hormone (ADH) or (AVP)

Blood specimens for ADH are collected into chilled tubes containing EDTA as an anticoagulant.

Specimens should be transported to the laboratory on ice and centrifuged at 4⁰C within 30 min of collection.

Plasma is then removed and stored at frozen at -20⁰C until analysis is performed.

Significant deterioration occurs with prolonged storage.

Reference ranges

Serum

neonate may be as low as	266	mOsmol/kg H ₂ O
child and adult	275-295	mOsmol/kg H ₂ O
> 60 yr	280-301	mOsmol/kg H ₂ O

Urine random 50-1200 depending on the fluid intake

References

1. Tietz text book of Clinical chemistry Burtis CA, AshwoodER (Eds) 3rd edition
2. Biochemistry manual, Department of Clinical Biochemistry, Westmead Hospital for children, Australia 2002