Clinical Practice Guidelines for Endocrinology Investigations

2015

Pakistan Society of Chemical Pathologists

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Disclaimer: All efforts have been made to include most recent and reliable information in this booklet but knowledge and best practice in this field keeps on changing very rapidly. So it is the responsibility of the health practitioners to make decision regarding patient care based on their patient experience and any further update on the subject.

Authors of the booklet declare no conflict of interest of any sort or any nature.

Dedicated to the teacher of the teachers, father of Chemical Pathology in Pakistan and the first FRCPath (Chemical Pathology) in the sub-continent:

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Foreword

One of the most important tasks of any professional organization is to provide evidence-based guidance for safe and efficient patient care. **Pakistan Society of Chemical Pathologists (PSCP)**, since its inception,is playing this role in the field of Chemical Pathology and Endocrinology. Chemical Pathologist is the right specialist to develop and implement scientific practices based on latest update, which are not only patient friendly but also feasible in the available resources. Present set of Clinical Practice Guidelines have been compiled to facilitate medical officers and specialists in Laboratory Medicine, Adult and Paediatric Medicine, Obstetrics/Gynecology, Critical Care and many other fields.

These guidelines are based on best available research evidence and practice experience. Only Grade 1A and Grade 1B evidence have been used as per GRADE system, which is in fact a tradeoff between benefits on the one hand, and risks, burden, and costs on the other. Most of the evidence has been obtained from sources like 'UpToDate', 'MEDSCAPE', standard text books and landmark studies and articles from authentic medical journals.

Each guideline has been primarily prepared by one author but reviewed by other authors, making it an authentic multi author publication. One or more closely related Endocrine diseases have been discussed in one guideline and consist of a brief introduction and diagnostic and monitoring strategies most suitable in our set up. Useful algorithms have also been added for quick reference. Where necessary detailed protocols of the dynamic function tests have also been given

Present guidelines will be first in the series. We will formulate and publish guidelines for other diseases coming in the previews of

Chemical Pathology under the auspices of PSCP. A continuous revision system of these clinical practice guidelines will also be adopted to produce subsequent edition to keep pace with the developments in the medical field.

We do not claim that the contents of guidelines are free of errors and omissions. We expect comments and feedback not only from Chemical Pathologists but from our clinical colleagues, too. Here we acknowledge the efforts of trainees Chemical Pathology AFIP Rawalpindi, particularly Dr. Qurat-Ul-Ain, for carrying out meticulous proof reading.

On behalf of all the authors I will request you to contact us with your valuable inputs to be included in the next editions.

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Contents

Note: Beautiful title of this booklet has been designed by Lt Col Zujaja Hina Haroon, Consultant Chemical Pathologist, AFIP Rawalpindi.

PSCP Guidelines 1/2015

Hyperglycaemia and Diabetes Mellitus (DM)

Background: DM is the most common Endocrinal / Metabolic Disorder. Any Clinical Chemistry Laboratory has a substantial workload from patients undergoing diagnosis or monitoring of the disease. It is defined as a state of *"Chronic Hyperglycaemia"* with disturbance in carbohydrate, lipid and protein metabolism. Deficiency of insulin is NOT an essential feature of DM. In fact many type 2 DM patients are Hyperinsulinaemic. So serum insulin estimation has no role in the diagnosis of DM.

Classification[1]:

1. Type 1 diabetes (β-cell destruction)
2. Type 2 diabetes (Progressive insulin secretary defect
3. Other specific types of diabetes
    a. Genetic defects in β-cell function and insulin action
    b. Disease of the exocrine pancreas
    c. Drug or chemical-induced
    d. Secondary diabetes due to endocrine disorders
4. Gestational diabetes mellitus

Clinical Stages of DM[1]

1. Normoglycaemia
2. Impaired Glucose Regulation (Now called ***Pre-Diabetes***)
    a. Impaired Fasting Glycaemia (IFG)
    b. Impaired Glucose Tolerance (IGT)
3. Diabetes Mellitus (DM)
    a. DM not requiring insulin
    b. DM requiring insulin for Control
    c. DM requiring insulin for Survival

Criteria of Diagnosis of DM[1]:

Based on Plasma Glucose (Sample collected in NaF-EDTA tube)

1. Fasting plasma glucose (FPG) > 7.0 mmol/l (>126 mg/dl). Repeat at interval of at least one week or when stress-free ***or***
2. Two-hour plasma glucose > 11.1 mmol/l on Oral Glucose Tolerance Test (OGTT). The OGTT should be performed by a glucose load of 75 g dissolved in water
3. In a patient with classic symptoms of hyperglycemia, a random plasma glucose ≥11.1 mmol/L (>200mg/dl)

4. Random Blood Glucose: There is no role of random blood glucose in the diagnosis of DM except in certain categories e.g. infants and patients presenting in altered conscious states.
5. Two hours post-prandial glucose has no role in the diagnosis and advising 2 hours after breakfast glucose to rule out DM should be stopped.
6. Urine glucose is an obsolete test and should not be used.

Based on Glycosylated Haemoglobin (A1C):

Pre-requisites: A1C carried out on an NGSP Certified method e.g. HPLC e.g. (D-10 by Biorad) or Turbidimetric Inhibition Immunoassay (using instruments like Advia 1800® and Modular P800® etc.)

Important: ***A1C can be used for the diagnosis*** (Cut off > 6.5%)

Units of A1C:

a. Traditional Units is % of total haemoglobin (Hb)
b. SI Unit: mmol / mol of Hb
c. Conversion Equation: A1c % = [0.09148 x mmol] + 2.152

Criteria for the diagnosis of Pre-diabetes[2]

1 Impaired Fasting Glucose (IFG) : FPG 5.6-6.9 mmol/l (100-126 mg/dl)
2 Impaired Glucose Tolerance (IGT) : 2-h plasma glucose load in the OGTT (see below) is between 7.8-11.0 mmol/l (140 – 200 mg/dl)
3 Pre-diabetes: A1c : 5.7-6.4 %

Oral Glucose Tolerance Test (OGTT)

Indication

1 Plasma Fasting glucose between 5.6 mmol/L (100 mg/dl) and 7.0 mmol/L (126 mg/dl)
2 Patients with family history of diabetes mellitus
3 Patients with previous history of large babies

Contraindication:

Plasma Fasting glucose >7.0 mmol/L (126 mg/dl)

Preparation:

1 Carbohydrate intake greater than 150 g per day
2 Patient should not be taking anti-diabetic agent and should be free of physical and mental stress.

Precautions:

1 Patient should be sitting comfortably for throughout the test
2 No smoking, diet or physical or mental stress during the test

Procedure:

1 Sample is taken for Fasting Plasma Glucose
2 Sample is analysed for glucose or glucose is estimated by POCT device

3 If glucose is > 7.0 mmol/L or 126 mg/dl, patient should NOT be given oral glucose but advised a repeat of FPG after three days.
4 If FPG is < 7.0 mmol/L or 126 mg/dl then give 75 g glucose in 250 ml of water to be taken in five minutes. A flavoured solution may also be used.
5 A plasma sample should be taken exactly TWO hours after glucose intake.
6 Plasma sample one hour glucose intake is NOT required for the differential diagnosis of hyperglycaemia and should NOT be drawn and analysed.

Interpretation: (Based on 2 h Post Glucose Load):

1 Normal: < 7.8 mmol/L (140 mg/dl)
2 IGT: 7.8 – 11.1 mmol/L (140-200 mg/dl)
3 DM: > 11.1 mmol/L (200 mg/dl)

Monitoring of DM

A1C: Cut-off value for monitoring is 7.0 %

Estimated Average Glucose (eAG)[3]

1. eAG is a new way to talk to patients about diabetes management.
2. The measurement of A1C—expressed as a percentage—is usually not very clear to patients who use glucometer or lab values.
3. This may make A1C targets difficult for patients to translate into action.
4. Doctors can now report A1C results to patients using the same units (mg/dl or mmol/l) that patients see routinely in blood glucose measurements.
5. It can be calculated by following formula:

eAG (mmol/L) = A1c (%)x1.59-2.59

Tests for diagnosis and monitoring of Diabetic Nephropathy

Albumin: Creatinine Ratio (ACR)

1 The effect of variations in urine volume on the urine albumin concentration can be avoided by calculation of the urine albumin-to-creatinine ratio in a spot urine specimen.
2 Albumin should be estimated in urine by turbidimetric inhibition immunoassay (TINIA) and creatinine can be measured by Jeff's Kinetic method after dilution of urine samples.
3 A value 3.4 to 34 mg/mmol of creatinine (or 30 to 300 mg/g of creatinine) suggests that albumin excretion is between 30 and 300 mg/day and, therefore, that microalbuminuria is present.
4 Values >34 mg/mmol (or 300 mg/g) are indicative of frank albuminuria.
5 This classification system requires that at least two of three specimens fall within the microalbuminuric or frank albuminuric range over a three- to six-month period.

6 Clinical Chemistry laboratories of public sectors may select samples for ACR by carrying out urine protein test by strips. If proteins are clearly present, then perform urine protein creatinine ratio as per following method.

Protein: Creatinine Ratio (PCR)

7 Protein in the urine can be quantitatively measured in samples which are clearly positive on urine strips for protein.
8 A dye-binding method (e.g. pyrogallol) or turbidimetric method may be used, kit commonly used for protein estimation in CSF.
9 The PCR value >50 mg /mmol of creatinine is indicative of significant proteinuria or severely increased albuminuria (NKF-KDIGO guideline 2012).

Metabolic Syndrome

Three of these five criteria should be fulfilled:

1 Waist Circumference: > 87.5 cm (35 inches) in women and > 100 cm (40 inches) in men (abdominal obesity)
2 Triglyceride: > 1.70 mmol/L (150 mg/dl) or higher)
3 Blood Pressure: >130/85 mmHg or higher
4 Fasting blood glucose:> 5.6 mmol/L (100 mg/dl) or higher
5 HDL-C:< 1.29 mmol/L (50 mg/dl) in women and <1.1 mmol/L (40 mg/dl) for men

Demonstration of Insulin Resistance

HOMA-IR:It is a convenient and non-invasive method for the demonstration of IR. Its formula is:

Fasting Plasma Glucose (mmol/L) x Serum Insulin (mIU/L) / 22.5.

Normal Sensitivity is upto 2.24.

Target Glycaemic Levels DM in Ambulatory Patients

1. Fasting glucose of 3.9 to 7.2 mmol/L (70 to 130 mg/dl) or
2. Postprandial glucose (90 to 120 minutes after a meal) < 10 mmol/L (180 mg/dl) or
3. A1C value of ≤7.0 percent for most patients.

Target Glycaemic Levels in Critically Ill Patients:

8.0-10 mmol/L or 144-180 mg/dl (based on NICE-SUGAR Study; NEJM 2009)[4]

References:

1. Standard of Medical Care 2015 (IADPSG) (www.iadpsg.org) and American Diabetes Association (ADA) (www.diabetes.org)
2. www.UpToDate.com 2015
3. ADA. Classification and Diagnosis. Diabetes Care 2011;34(suppl 1):S12)
4. NICE-SUGAR Study; NEJM 2009

PSCP Guidelines 2/2015

Hyperglycaemia in Pregnancy

Background: Diabetes mellitus (DM) has dreadful complications in pregnancy. It can present with more than one ugly faces:

a. Pre-gestational DM:This term is sometimes used for women who are known cases of DM and become pregnant. Diagnosis of these cases is similar to the DM discussed in previous guidelines.

b. Overt DM: Relatively newer class describes women who had pre-existing diabetes but were unaware of it. Full blown DM ***diagnosed at any stage of pregnancyis called Overt DM***. The incidence of this category is increasing with worsening life styles and increased prevalence of type 2 DM in women. WHO has endorsed this category in 2013 but with the term '***Diabetes is Pregnancy'*** (without the word 'Overt')[1].

c. Gestational DM (GDM): Women with onset or first recognition of abnormal glucose tolerance ***during any stage of pregnancy*** (other than those in overt category).

There is a need to diagnose pre-existing DM or overt DM as early as possible because hyperglycaemia at this stage can lead to foetal anomalies. If left undiagnosed and untreated the patient may not only have abnormal foetus but also complications related to increased diabetogenic hormones and increased insulin resistance after 24th weeks. These guidelines have been formulated to minimize the risk ofhyperglycaemia by advocating early and efficient diagnosis and monitoring.

Identification of High Risk Patients

1. Ethnicity (e.g. Sub-continent countries)
2. Strong family history of diabetes
3. Prior history of GDM
4. Previous delivery of a baby > 4.1 kg
5. Manifestations of Insulin Resistance e.g. Metabolic Syndrome or PCOS
6. Other manifestations of glucose intolerance

Recommended Screening Protocol

Screening at first ante-natal visit: If TWO or more ***risk factors*** are present then screening should be done at first visit (before 24 weeks) by protocols used for non-pregnant adults (see below). If overt DM or GDM is diagnosed at this stage patient should be managed not only for her glycemic control but also for diabetic complications.

Screening at 24-28 weeks: ***All*** women who have not been diagnosed as GDM or overt DM earlier should undergo a 75 g Oral Glucose Tolerance Test (OGTT) as one step at 24-28 weeks of gestation.

Diagnostic Criteria for Overt DM[2]: Overt DM is diagnosed at any stage of pregnancy if one of the following is present:

a. Glycosylated Haemoglobin (A1c)*: ≥6.5%
b. Fasting Plasma Glucose: ≥7.0 mmol/L (126 mg/dl)
c. Random blood glucose**: ≥11.1 mmol/l (200 mg/dl)
d. 2-h plasma glucose (following a 75g oral glucose load): ≥11.1 mmol/l

** Should be carried out only in first trimester by an NGSP certified method.*
*** Should NOT be advised except in situations where suspicion of DM is strong*

Diagnostic Criteria for GDM[3]: The diagnosis of GDM at any stage of pregnancy is diagnosed if any one of the following is present following a 75g oral glucose load:

a. Fasting : Between 5.1-6.9 mmol/l (92 -125 mg/dl)
b. 1 hour: >10.0 mmol/l (180 mg/dl)
c. 2-hour: Between 8.5 – 11.0 mmol/l (153-199 mg/dl)

Monitoring of Hyperglycaemia in Pregnancy[4]: Following glycaemic targets are recommended for control of hyperglycaemia which may be achieved by metformin or insulin

a. Pre-prandial ≤ 5.3 mmol/L (95mg/dl)
b. 1 hour post meal ≤ 7.8 mmol/L (140mg/dl) or
c. 2 hour post meal ≤ 6.7 mmol /L (120mg/dl)

Follow-up after pregnancy[4,5]: At 6-12 weeks test after delivery, FPG or A1c or 75g OGTTshould be done. Further monitoring depends on the results:

a. Normal : Reassess after every 3 years
b. Pre Diabetes (7.8 to 11mmol/L): Reassess every year
c. Diabetes (>11.1mmol/L): Reassess quarterly

References:

1. Standard of Medical Care 2015 (IADPSG) (www.iadpsg.org) and American Diabetes Association (ADA) (www.diabetes.org)
2. The American Congress of Obstetricians and Gynecologists (ACOG) (www.acog.org)
3. Diagnostic Criteria and Classification of Hyperglycaemia First Detected in Pregnancy. WHO Report 2013
4. Diabetes in pregnancy. www.UpToDate.com
5. "The Hyperglycemia and Adverse Pregnancy Outcomes (HAPO)" (N Eng J Med 2008;358:19 at www.NEJM.com).

Hypoglycaemia

Categories: Hypoglycaemia can be broadly divided into following categories:

1. Hypoglycaemia in Adults
    a. Hypoglycaemia in Non-Diabetics
        i. Ill or medicated individuals
        ii. Seemingly well individuals
    b. Hypoglycaemia in Diabetics
2. Hypoglycaemia in Neonates

Hypoglycaemia in Non-Diabetics

Whipple's Triad: Helps to establish the existence of a hypoglycemic disorder. Only those patients in whom Whipple's triad is documented require evaluation and management of hypoglycemia:

1. Symptoms consistent with hypoglycemia
2. A low plasma glucose concentration measured with a precise method (not a home glucose monitor) when symptoms are present
3. Relief of those symptoms after the plasma glucose level is raised

Causes of Hypoglycaemia in Adults

A. Ill or medicated individual

1. Drugs : Insulin or insulin secretagogue, Sulphonylureas, Alcohol/Ethanol
2. Others : Beta blockers, , Salicylates, quinine, indomethacin, Lithium, getifloxacin
3. Critical illnesses : Hepatic, renal, or cardiac failure, Sepsis (including malaria), predisposing illness, hospitalized patients
4. Hormone deficiency : Cortisol, Glucagon and epinephrine, hypopituitarism/ Addison's disease
5. Non-islet cell tumor hypoglycemia (NICTH)

B. Seemingly well individual

Endogenous hyperinsulinism:

1. Insulinoma
2. Islet hyperplasia
3. Nesidioblastosis
4. Post - gastric bypass hypoglycemia,
5. Autoimmune hypoglycemia
6. Severe exercise

Accidental, surreptitious, or malicious hypoglycemia

Investigations of Endogenous hyperinsulinism:

1. Fasting Hypoglycaemia: Observe during fasting upto 72 h.
2. Post-prandial Hypoglycemia: Observe for 5 h after mixed meal test

Causes of Hypoglycemia in Childhood

1. Transient neonatal hypoglycemia
2. Hyperinsulinaemia
   a. Islet cell hyperplasia
   b. Insulinoma
3. Inherited metabolic disorders including
   a. Glycogen storage diseases
   b. Galactosaemia
   c. Hereditary fructose intolerance
   d. Fatty acid B-oxidation defects
   e. Prematurity
   f. Small-for-dates
   g. Endocrine disorders
   h. Starvation
   i. Drugs
   j. Ketotic hypoglycaemia

Definition of Hypoglycaemia in DM: *All episodes of an abnormally low plasma glucose concentration (with or without symptoms) that expose the individual to harm.*

Blood glucose (SMBG) level ≤3.9 mmol/L (70 mg/dL)

At this level patient should take defensive options including:

1. Repeating the measurement
2. Avoiding critical tasks such as driving
3. Adjusting the subsequent treatment regimen
4. Ingesting carbohydrates.

Clinical classification ofHypoglycaemia in DM (ADA)

1. **Severe hypoglycemia:** An event requiring the assistance of another person to actively administer carbohydrate, or other resuscitative actions. Plasma glucose measurements may not be available during such an event, but neurological recovery attributable to restoration of plasma glucose to normal is considered sufficient evidence that the event was induced by a low plasma glucose concentration.
2. **Documented symptomatic hypoglycaemia** An event during which typical symptoms of hypoglycaemia are accompanied by a measured plasma glucose concentration 3.9 mmol/L (≤70 mg/dl) is classified as a documented symptomatic hypoglycaemic event.

3. **Asymptomatic hypoglycaemia** Asymptomatichypoglycaemia is classified as an event not accompanied by typical symptoms of hypoglycaemia but with a measured plasma glucose concentration of ≤70 mg/dl (3.9 mmol/L).
4. **Probable symptomatic hypoglycemia** An event during which typical symptoms of hypoglycemia are not accompanied by a plasma glucose determination (but that was presumably caused by a plasma glucose concentration ≤70 mg/dL [3.9 mmol/L].
5. **Pseudohypoglycaemia**An event during which the person with diabetes reports typical symptoms of hypoglycemia, and interprets those as indicative of hypoglycemia, but with a measured plasma glucose concentration >70 mg/dL (3.9 mmol/L). This category reflects the fact that patients with chronically poor glycemic control can experience symptoms of hypoglycemia at plasma glucose levels >70 mg/dL (3.9 mmol/L) as glucose levels decline into the physiological range.

Extended OGTT for Reactive hypoglycaemia

Introduction: Hypoglycaemia after a carbohydrate meal may occur in three situations:

1. In otherwise normal subjects (functional)
2. In some tumours of pancreatic and extra-pancreatic tissues
3. In early adult-onset diabetes mellitus.
4. In patients who have had gastric surgery (alimentary).

It is Important to point out that 10-20% of normal subjects will develop hypoglycaemia (plasma glucose less than 2.8 mmol/l) within 2-3 hours after a carbohydrate load; however, they do not develop hypoglycaemic symptoms. The most useful investigation is a 5 hours glucose tolerance test with estimation of plasma glucose levels at 30 minutes intervals.

This is the commonest cause of reactive hypoglycaemia and occurs 2-4 hours after meals when patients develop adrenergic symptoms associated with a low plasma glucose level. The cause is unclear but it tends to occur in patients with emotional problems. The frequency of attacks is greater during periods of emotional stress. 72 hours fast is usually well tolerated. Some authorities do not agree that this condition exist; for example, it could represent normal reactive hypoglycaemia in a subject having adrenergic symptoms related to some other disorder. The fact that biochemical hypoglycaemia is difficult to demonstrate in functional reactive hypoglycaemic subjects after a meal of complex carbohydrates would seem to support this approach.

In the reactive type a 5 hours glucose tolerance test, taking blood for glucose and insulin values at 30 minutes intervals, is the most useful test and will generally provide the diagnosis. If the history is suggestive of fasting hypoglycaemia determine the blood glucose and insulin levels on several occasions after an overnight fast. If this fails to demonstrate hypoglycaemia then proceed with a 72 hours fast taking samples for glucose and insulin at 6-12 hours intervals or if symptoms appear. Exercising the patient during or at the end of the fast is also a useful procedure to attempt the precipitation of hypoglycaemia. If hypoglycaemia occurs during fasting plasma insulin values which are inappropriately high for the glucose are suggestive of insulinoma.In hypoglycaemia due to non-pancreatic tumours the insulin levels will be appropriately low.

Indication for extended OGTT: Evaluation of patients suspected of having reactive hypoglycemia (5 hours OGTT).

Preparation of Patient:-

1. The patient must be ambulatory and free from pyrexia, acute illness or trauma for at least two weeks.
2. He/she should have diet containing at least 150g carbohydrates per day for three days prior to test. Carbohydrate restriction results in a decreased tolerance for glucose and a diabetic curve may be observed in a normal individual.
3. Any drug that alters blood glucose level should also be discontinued for 3 days prior to testing (e.g. salicylates, steroids, thiazide diuretics, anticonvulsants etc.). If patient is already under treatment with hypoglycemic drugs e.g. insulin, or the sulphonylurea group, these should be discontinued at least on the day of the test.
4. To avoid circadian variation and to obtain a greater reproducibility the test should be done in the morning between 0700-0900 hours.
5. The patient must have 9-16 hours fast. An average 12 hours fast is recommended. After the evening meal the patient should be permitted only water.
6. Heavy tea and coffee drinkers should reduce their consumption during the days preceding the test.
7. No smoking is recommended during fast or at least in the morning before OGTT and during the OGTT.
8. No physical exercise is allowed during the test.
9. Patient should be seated quietly and relaxed for 30 minutes before the test. It will allow patient to relieve himself from undue anxiety and tension.
10. If not already done, it is advisable to determine the patient's fasting plasma glucose level prior to the OGTT. In case a definite hyperglycemia exists, glucose load is contra-indicated.

Test Procedure for OGTT:-

Glucose load: Considerable variation exists with respect to the loading dose of glucose employed. In the past 50 g of glucose was used for adults, but it has proved to be an inadequate stimulus for some patients. The criteria of 1 g/kg body weight in adults are also in use, but at times weight of person is not known.

National Diabetes Data Group of USA has made following recommendations which have also been accepted by WHO expert Committee.

a. For adults 75 g glucose.
b. For children: 1.75 g/kg body weight with a maximum of 75 g.
c. Glucose load is given in water (25 g/100 ml) and patient should drink it within 5 minutes. It is noted as zero time. The blood samples are taken at half hourly intervals upto 5 hours after obtaining fasting sample.

A 5 hours test is generally adequate for the diagnosis of hypoglycemia. If during the test nausea, fainting, sweating or other hypoglycemic symptoms occur, a blood specimen for glucose should be drawn immediately and the procedure is discontinued.

Note: glucose levels on venous blood taken from the anti cubital fossa may be spuriously low because of glucose extraction by the forearm muscles. Thus, in the evaluation of these patient arterialized blood (from vein on back of warmed hand) or capillary blood (finger prick, ear lobe) should be used for glucose estimations.

Interpretation:-

A variety of criteria have been recommended from time to time for the interpretation of the oral glucose tolerance test.

1. If the history is suggestive of fasting hypoglycaemia determine the blood glucose and insulin levels on several occasions after an overnight fast.
2. Exercising the patient during or at the end of the fast is also a useful procedure to attempt the precipitation of hypoglycaemia.
3. If hypoglycaemia occurs during fasting plasma insulin values which are inappropriately high for the glucose are suggestive of insulinoma. In hypoglycaemia due to non-pancreatic tumours the insulin levels will be appropriately low.
4. *The value of **Fasting Plasma Glucose** for hypoglycaemia is 2.8 mmol/l in presence of clinical symptoms.*

Factors Affecting Fasting Plasma Glucose Tolerance:-

There are so many factors which can affect and disturb the glucose tolerance of an individual which explain poor reproducibility of OGTT, and pose severe difficulties in interpretation. It must be, therefore, emphasized that the above mentioned diagnostic criteria are valid only after the exclusion of factors known to disturb glucose tolerance.

Dietary Factors:- Low carbohydrate and low caloric diet reduce glucose tolerance in normal subjects. Impaired glucose tolerance has been observed

in persons who have restricted their carbohydrate intake in an anticipation of the test. Diet should contain at least 150g carbohydrate daily for 3 days preceding the test. Excess tea and coffee should also be avoided during the days preceding the test.

Physical Activity:- Glucose tolerance is impaired by physical inactivity.

Intercurrent Disease and Injury: Physical stress and acute illness reduce glucose tolerance. OGTT should not be performed at least 1-2 months after recovery e.g. from acute myocardial infarction, trauma, burns, operations etc.

Psychological Stress: Glucose tolerance should not be tested during or shortly after a major emotional disturbance.

Endocrine Diseases: Most of the endocrine ***hypersecretory*** states impair glucose tolerance. (vi) Hyperthyroidism and pheochromocytoma are the most frequent. The anti-insulin action of the hormones concerned makes it impossible to interpret OGTT until the associated endocrinopathy has been adequately treated.

Pregnancy: Placental hormone production particularly after the first trimester decreases insulin sensitivity. About 60% of women with gestational diabetes mellitus (GDM) become overtly diabetic within 15 years.

Drugs:

Decreased glucose tolerance:Most important are glucocorticoids and thiazide diuretics.

Improved glucose tolerance: Oral hypoglycemic agents, salicylates.
All medications must be withdrawn at least 3 days before the test.

Effect of Age on Blood Sugar:

With advancing age after 3rd decade there is decrease in glucose tolerance. It might be due to decreasing physical activity, muscle mass or obesity.

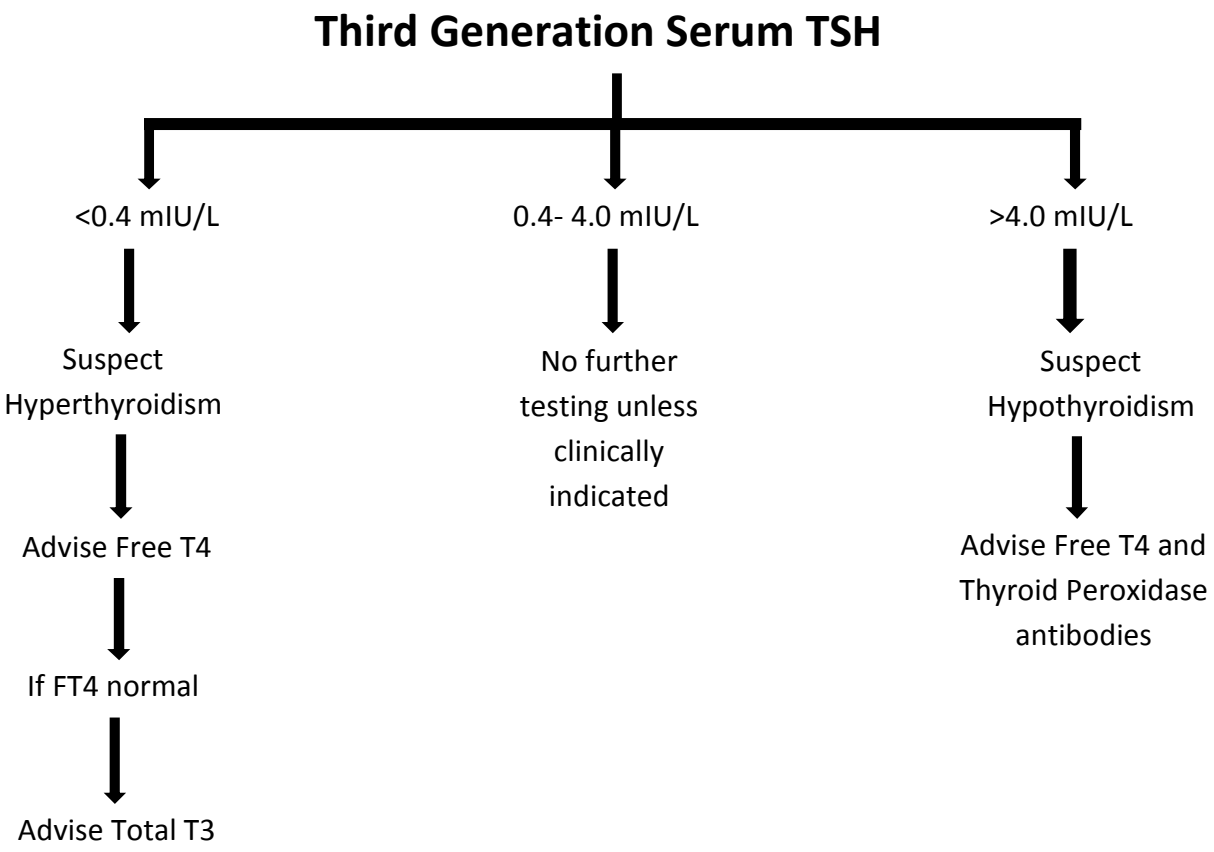
Reference

1. Robert F. Dons and Frank H. wians,Jr. Endocrine and Metabolic disorder Clinical lab testing manual 4th ed2009.
2. UpToDate www.UpToDate.com

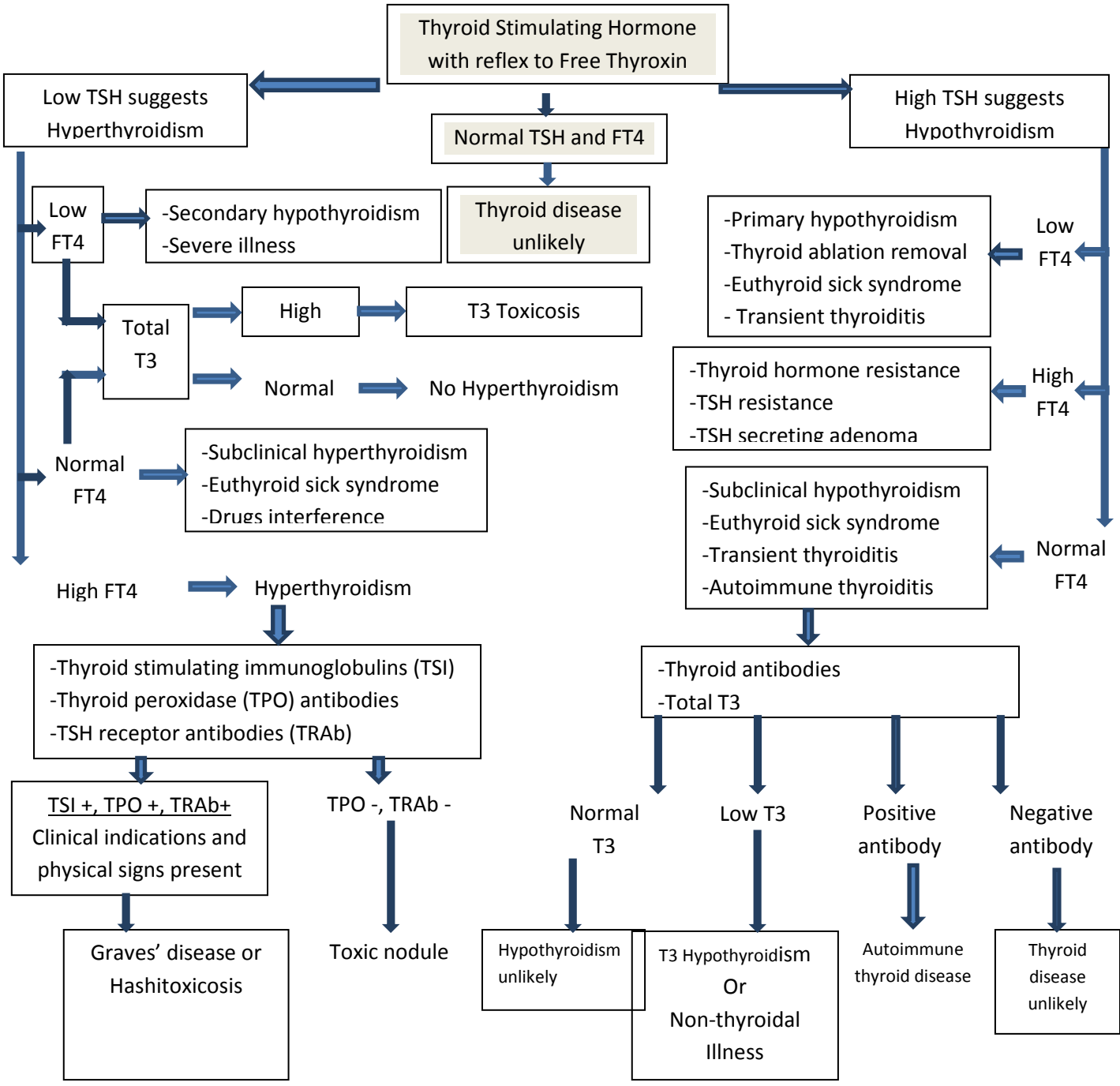
Thyroid Disorders

Thyroid Function tests

1. Should be prescribed only if thyroid disease is clinically indicated
   a. Easy fatigue, cognitive impairment or decline in quality of life
   b. Increased or decreased metabolism (Heat or cold intolerance, weight loss or gain, depression or anxiety etc.)
   c. Goiter on physical examination
   d. Family history of autoimmune thyroiditis
2. Should be preferred in non-hospitalized patients without known or suspected pituitary disease
3. Screening for Thyroid Dysfunction should be avoided in severely ill patients
4. Sampling does not require specific timings or relation with meals
5. Begin evaluation by prescribing enhanced third generation assay of serum Thyroid Stimulating Hormone (TSH). Followed by serum free Thyroxin (FT4) and then serum total Triiodothyronine (T3) levels as indicated below


Third Generation Serum TSH
<0.4 mIU/L
0.4- 4.0 mIU/L
>4.0 mIU/L
Suspect Hyperthyroidism
No further testing unless clinically indicated
Suspect Hypothyroidism
Advise Free T4
Advise Free T4 and Thyroid Peroxidase antibodies
If FT4 normal
Advise Total T3

Algorithm for Suspected Thyroid Disorder



Parathyroid Disorders

Background

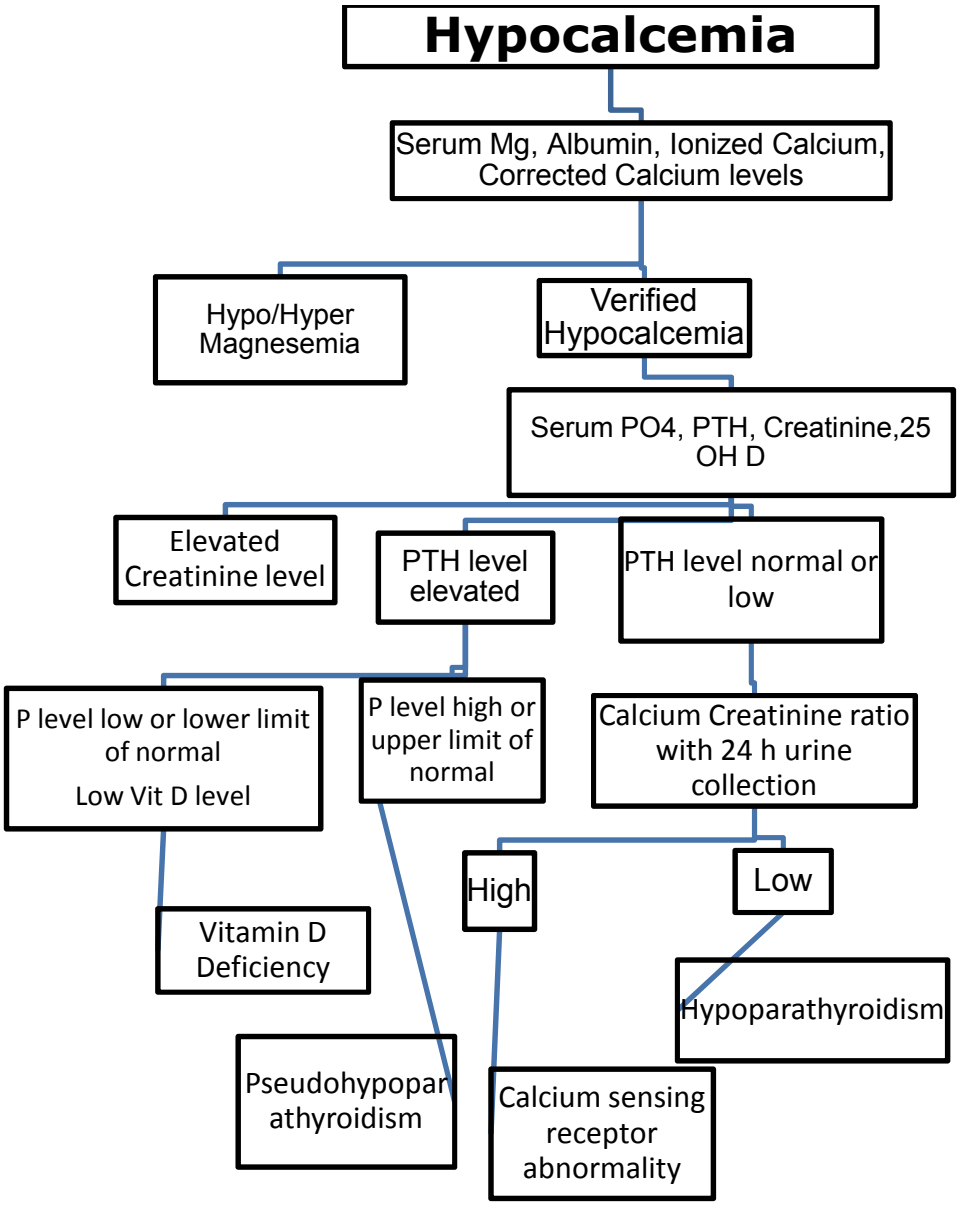
In healthy individuals, any decrease in serum Calcium below the normal range triggers a pronounced increase in PTH, whereas high calcium concentration provides a negative feedback to the release of PTH by the parathyroid gland. Two most common causes of hypercalcaemia are primary hyperparathyroidism (HPT) and hypercalcaemia of malignancy (HOM). In HPT hypercalcaemia is present with elevated PTH while in HOM, PTH is low or within normal reference range. Hypocalcaemia with a low PTH level, on the other hand, is to be expected in hypoparathyroidism, either postsurgical or idiopathic.

Specimen Requirements and Sample Processing

1. ***For the intact hormone, the in vivo half-life is 2 to 5 minutes.*** Specimen requirements may depend on the specific method. Serum, EDTA and heparinized plasma are the recommended samples.
2. After separation serum or plasma should be frozen if analysis is delayed.
3. Samples can be stored at -20^{o}C for 10 days.

Test Protocol: While collecting sample for PTH following protocol be adopted:

1. Take proper history of the patient
2. Take sample for serum calcium, serum phosphate, serum magnesium and serum alkaline phosphatase for correct interpretation of results
3. Urinary calcium detection is helpful in detection of cases of lithiasis, hyperparathyroidism and familial hypocalciureic hypercalcaemia (FHH).


Hypocalcemia
Serum Mg, Albumin, Ionized Calcium, Corrected Calcium levels
Hypo/Hyper Magnesemia
Verified Hypocalcemia
Serum PO4, PTH, Creatinine,25 OH D
Elevated Creatinine level
PTH level elevated
PTH level normal or low
P level low or lower limit of normal
Low Vit D level
P level high or upper limit of normal
Calcium Creatinine ratio with 24 h urine collection
High
Low
Vitamin D Deficiency
Hypoparathyroidism
Pseudohypoparathyroidism
Calcium sensing receptor abnormality

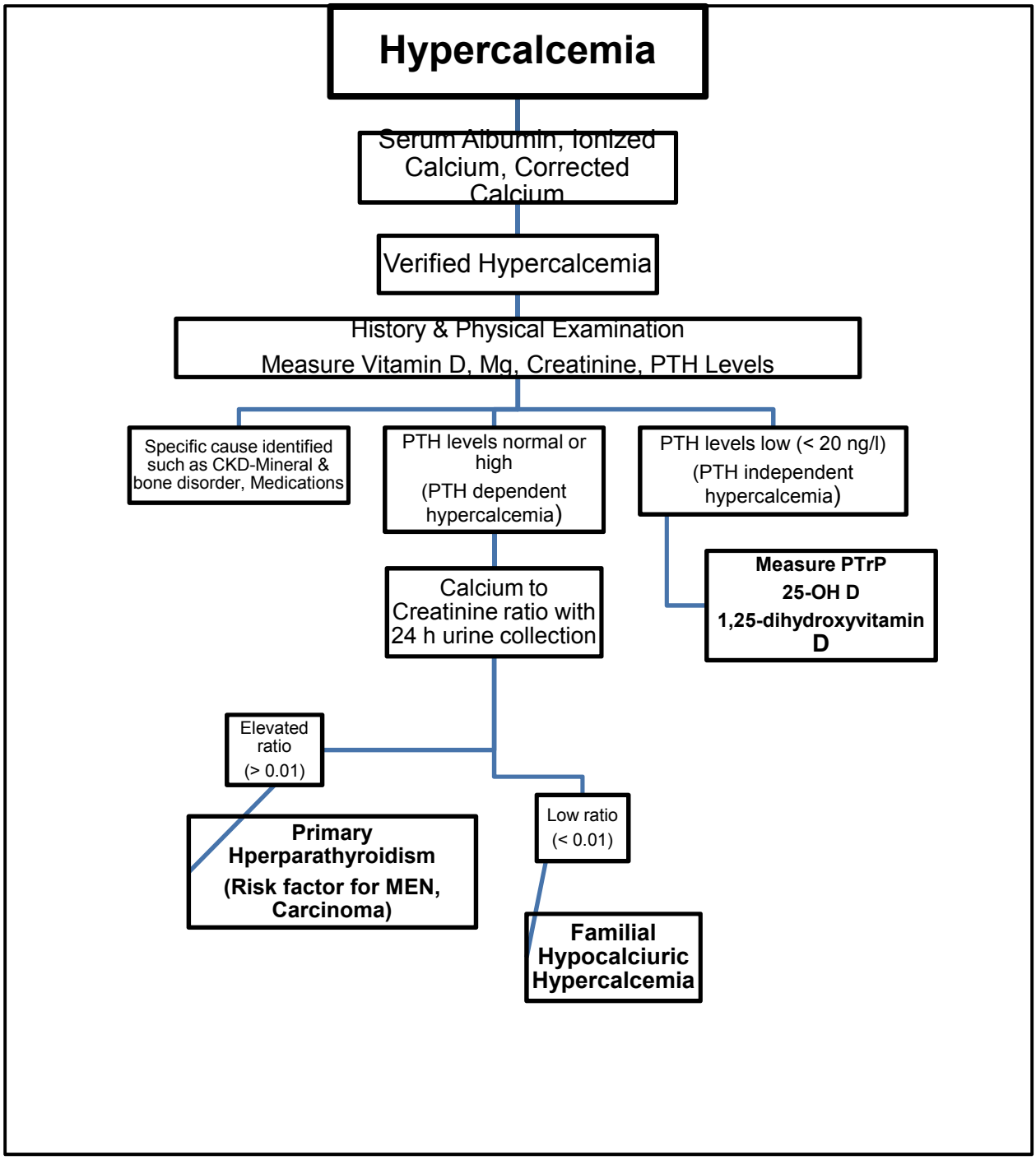

Hypercalcemia
Serum Albumin, Ionized Calcium, Corrected Calcium
Verified Hypercalcemia
History & Physical Examination
Measure Vitamin D, Mg, Creatinine, PTH Levels
Specific cause identified such as CKD-Mineral & bone disorder, Medications
PTH levels normal or high (PTH dependent hypercalcemia)
PTH levels low (< 20 ng/l) (PTH independent hypercalcemia)
Measure PTrP
25-OH D
1,25-dihydroxyvitamin D
Calcium to Creatinine ratio with 24 h urine collection
Elevated ratio (> 0.01)
Low ratio (< 0.01)
Primary Hperparathyroidism (Risk factor for MEN, Carcinoma)
Familial Hypocalciuric Hypercalcemia

Table: Serum Calcium, Serum Phosphorous and PTH Levels in Parathyroid and Vitamin D Disorders

Disorders	Serum Ca	Serum P	Plasma iPTH
Primary Hyperparathyroidism	Increased	Decreased	Increased
Secondary Hyperparathyroidism	Decreased to normal	Decreased	Increased
Tertiary Hyperparathyroidism	Increased	Decreased (Increased in CKD)	Increased
Vitamin D Deficiency	Decreased	Decreased	Increased
Vitamin D Excess	Increased	Increased	Decreased
Familial Hypocalciureic Hypercalcaemia	Mildly increased Ca Urine calcium to creatinine clearance ratio <0.01	Decreased	Normal
Neonatal Severe Primary Hyperparathyroidism	Marked increased	Decreased	Increased
Hypoparathyroidism	Decreased	Increased	Decreased
Pseudohypoparathyroidism	Decreased	Increased	Normal
Pseudopseudohypoparathyroidism	Normal	Normal	Normal

Vitamin D Disorders

Background: Vitamin D deficiency (VDD) is a world-wide epidemic with recent estimates indicating more than 50% of the population at risk. This pandemic of inadequate vitamin D (VDD, vitamin D insufficiency VDI) has been found in all age group even those who are otherwise healthy and are not prone to deficiency. In Pakistan prevalence of VDD has been reported upto 92% (70% - 97% in AKU Karachi and 81%-92% in Lahore) ambulatory patients in various situations.

Vitamin D occurs in the nature in two forms:

1. Cholecalciferol (D3) is of animal origin derived from parent molecule, 7-dehydrocholesterol.
2. Ergocalciferol (D2) is derived from plant origin precursor ergosterol.

We recommend following cut-off values for the estimation of Vitamin D status (Institute of Medicine Cut-offs)

1. Vitamin D Deficiency: Serum 25 (OH) D <25 nmol/l
2. Vitamin D Insufficiency: Serum 25 (OH) D 25-50 nmol/l
3. Desirable level : Serum 25 (OH) D 50-100 nmol/l
4. Vitamin D intoxication: Serum 25(OH) D >250 nmol/l

References

1. UpToDate. (online) Cited on 22 Mar 2013. Available at: https://www.uptodate.com
2. Holick MF. High Prevalence of Vitamin D Inadequacy and Implications for Health. Mayo Clin Proc. 2006;81(3):353-373.
3. Enders D, Rude RK. Mineral and Bone Metabolism. In Burtis CA, Ashwoods ER and Bruns DE, (edi) Teitz Textbook of Clinical Chemistry. 4th ed. W. B. Saunders Company; 2006;pp 1891-1963.
4. "The 10 Parathyroid Rules of Norman". [online]. Cited on 30 Jan 2013. Available from: www.parathyroid.com/10_parathyroid_rules.htm.
5. Prentice A. Vitamin D deficiency: a global perspective: Nutrition Reviews 2008; 66:(2):S153-154.
6. Zuberi LM, Habib A, Haque N, Jabbar A. Vitamin D Deficiency in Ambulatory patients. J Pak Med Assoc 2008;58(9):482-484.

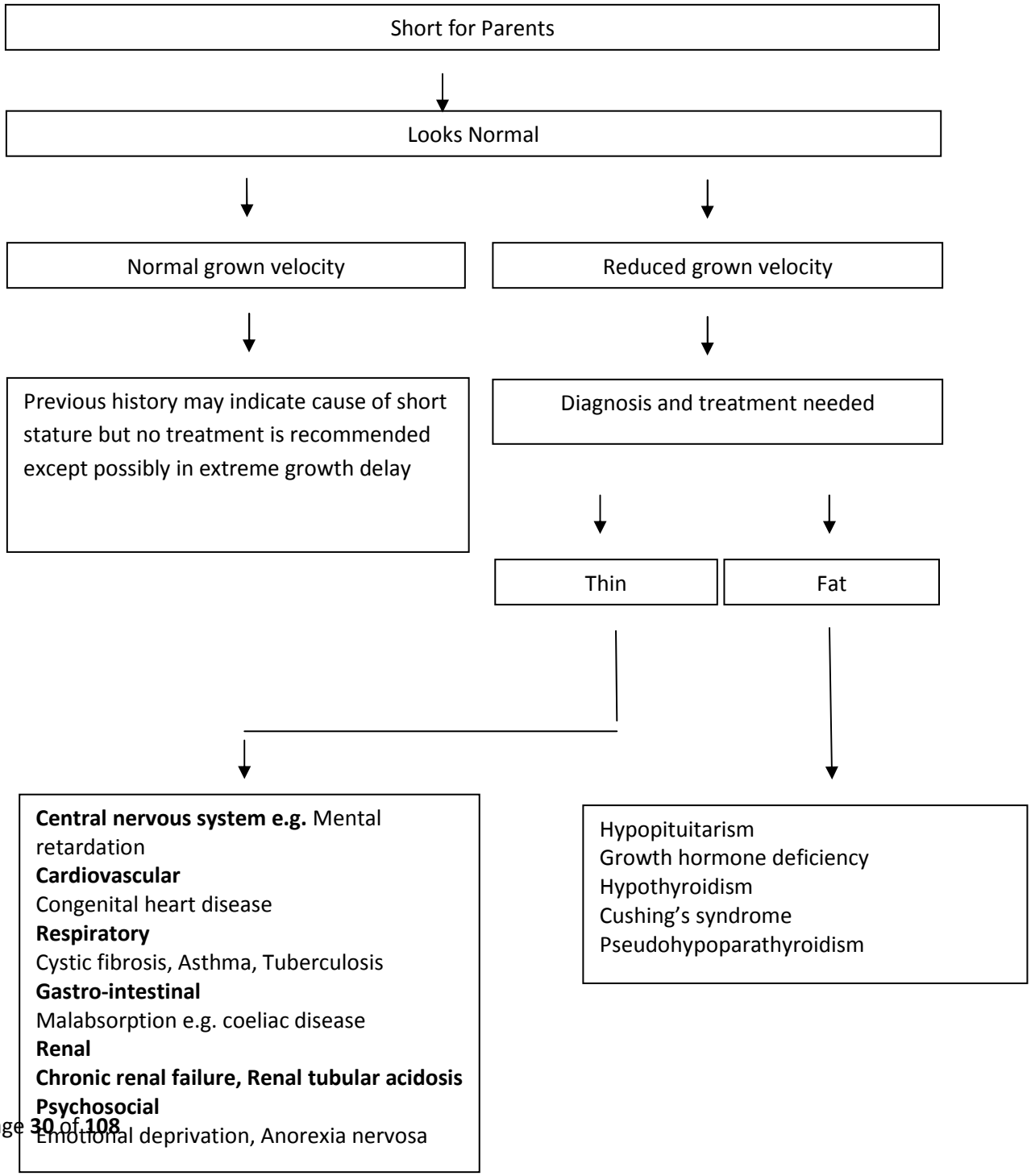
Short Stature

Short stature is a common cause of concern amongst children, adolescents and their parents. Three per cent of the population have a height below their third percentile and will be noticeably short; however, probably fewer than one out of a hundred of these will have a primary endocrine defect. In order to detect these children, careful clinical assessment and measurements of the height velocity are needed to separate those who are small, but growing normally, from those who are failing to grow. For the latter, a diagnosis is needed in order to institute appropriate treatment. The state of nutrition may help to point the diagnostic pathway but any child who is growing slowly should have diagnostic investigation to determine the cause. In assessing all short patients a detailed history, physical examination and urine analysis are required, together with a radiograph of the non-dominant hand and wrist. The bone age can be assessed and therefore the growth potential. Short stature which is out of keeping with the family background is likely to be significant. The mid-parental height (the mean of the parental centile heights) can be calculated in order to estimate height expectation. Information should also be obtained about the growth pattern in the parents, especially concerning the time of onset of the pubertal growth spurt. Physical examination may reveal an abnormal looking child with either dysmorphic features or disproportionate short stature (short limbs or short back and limbs). *Depending upon the history and clinical examination following biochemical tests may be carried out before embarking on growth hormone dynamic function tests:*

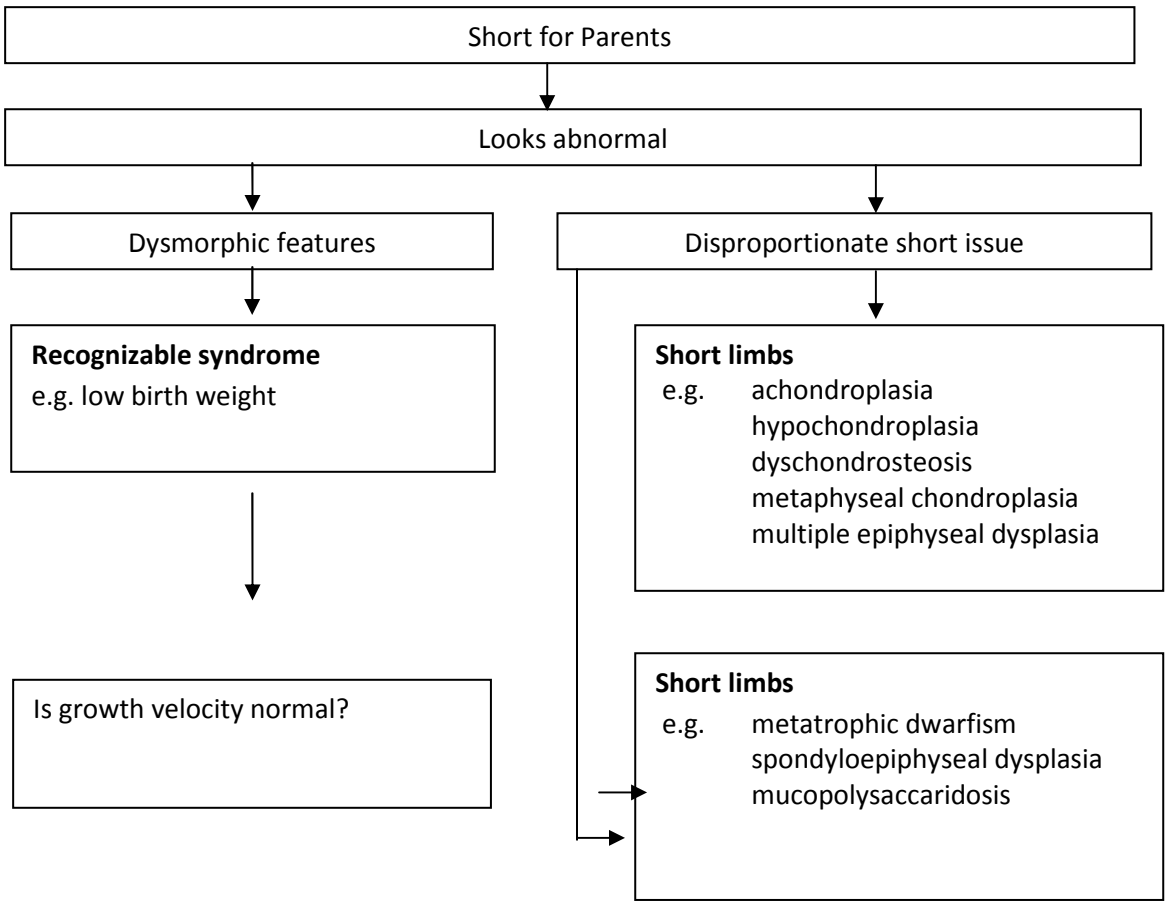
1. Serum TSH
2. Serum anti gliadin antibodies
3. Serum anti reticulin antibodies
4. Serum anti Tissues transglutaminase antibody
5. Serum urea & creatinine
6. Serum electrolyte
7. Serum PTH
8. Serum calcium & phosphate
9. Serum total vitamin D
10. Serum IGF-1
11. Serum IGFBP3

The major causes of short stature in children with a normal appearance and those with disproportionate short stature are illustrated in figures I and II followed by test protocols of dynamic function tests for Growth Hormone

Evaluation of short stature patients with normal appearance



Evaluation of Short Stature Patients with Abnormal Appearance



Dynamic Function Tests for Growth Hormone Evaluation

Background:

GH stimulation tests include:

1. Exercise stimulation test
2. L-Dopa stimulation test
3. Glucagon stimulation test
4. Insulin tolerance test
5. Arginine stimulation test

Diagnosis of GH related Growth Failure

For establishing GH deficiency as a cause of GH related growth failure (short stature), at least two provocative tests should be suggestive of GH deficiency

1. Exercise Stimulation Test

Principle:

Strenuous physical exercise causes GH secretion in normal subjects.

Preparation:

1. The patient should be fasting overnight (no calorie intake for at least 8 hours).
2. The test should be performed early in the morning (0800 hours).

Procedure:

1. Basal venous blood sample is obtained for GH
2. The patient is subjected to vigorous exercise on a tread mill for 20 minutes.
3. Pulse is monitored during the test.
4. A venous blood specimen is drawn immediately after the termination of exercise.
5. Both the samples are centrifuged and GH is estimated in serum portion of both the samples.

Interpretation:

In children-

GH level > 20 mIU/l, GH deficiency is unlikely.

GH levels < 20 mIU/l is suggestive of GH deficiency and needs confirmation by a second provocation test.

In adults: Normal response > 15 mIU/l

2. L- DOPA Stimulation Test

This test is used in patients suspected of having GH deficiency.

Principle

L- Dopa stimulates growth hormone (GH) secretion from the anterior pituitary gland measurements of which (GH) serve as a test of anterior pituitary function.

Preparation

The patient should be fasting overnight (no calorie intake for at least 8 hours).

The test should preferably be carried out in the morning at 0800 hours.

Procedure

1. Basal venous blood sample is obtained for GH.
2. L- Dopa is administered orally preferably with food and milk according to the following dosage schedule:
   a. Patient > 30 Kg: 500 mg
   b. Patient between 15 - 30 Kg: 250 mg
   c. Patient < 15 Kg: 125 mg
3. Venous blood sample is collected 60 minutes after L- Dopa administration.

Side effects

Transient nausea and occasional vomiting

Interpretation

In children-

GH level > 20 mIU/l, GH deficiency is unlikely.

GH levels < 20 mIU/l is suggestive of GH deficiency and needs confirmation by a second provocation test.

In adults-

Normal response > 15 mIU/l

Arginine Stimulation Test

Principle

IV administration of arginine hydrochloride stimulates GH release in normal subjects.

Preparation

The patient should be fasting overnight (no calorie intake for at least 8 hours).

The test should preferably be carried out in the morning at 0800 hours.

Patient should be in recumbent position.

Procedure

1. Basal venous blood sample is obtained for GH.
2. Weigh the patient
3. Place an indwelling cannula in the ante cubital fossa with good access for administration of arginine.
4. A 10% solution of arginine hydrochloride, 0.5 g/kg (maximum dose= 30g) is infused intravenously over 30 minutes.
5. Venous blood sample is collected at 30, 60 and 90 minutes after the infusion is begun.

Interpretation

In children-

GH level > 20 mIU/l, GH deficiency is unlikely.

GH levels < 20 mIU/l is suggestive of GH deficiency and needs confirmation by a second provocation test.

In adults-

Normal response > 15 mIU/l

4. Glucagon Stimulation Test

Introduction

This test is used in the assessment of growth hormone (GH) and ACTH / cortisol reserve, especially when insulin-induced hypoglycaemia is contraindicated e.g. history of convulsions, hypoglycaemia or diabetes mellitus.

Principle

Glucagon works by stimulating the release of GH and ACTH by a hypothalamic mechanism, indirectly stimulating cortisol secretion. GH response to glucagon is slow so the late samples are important.

Contraindication

1. Starvation of >48 hours or glycogen storage disease (may result in hypoglycaemia)
2. Severe hypoadrenalism (09.00h cortisol <55 nmol/l)
3. Hypothyroidism (may reduce GH and cortisol response)
4. Pheochromocytoma or insulinoma (may provoke an attack)

Preparation

1. The patient should be fasting overnight (no calorie intake for at least 8 hours).
2. The test should preferably be carried out in the morning at 0800 hours.
3. Patient should be in recumbent position.

Prerequisites

Thyroid function (TSH and free t4) and cortisol levels must have been checked to rule-out panhypopituitarism.

Preparation

1. The patient should fast overnight (no calorie intake for at least 8 hours) and be recumbent during the test.
2. Weigh the patient

Procedure:

1. Place an indwelling cannula in the ante cubital fossa with good access for administration of glucose, if required.
2. Check fasting plasma glucose by glucometer.
3. Administer glucagon i.m. According to dose of 0.03-0.1mg/kg body weight (maximum dose 1.0mg).
4. Observe for signs of hypoglycemia i.e. tachycardia, palpitation, sweating, headache, confusion, drowsiness, loss of consciousness & abdominal pain and check plasma glucose level with help of glucometer.
5. Adequate hypoglycaemia is plasma glucose < 2.5 mmol/l or 50% of the basal glucose value which is symptomatic.
6. Take 3ml venous blood in plain tubes for GH/ cortisol and 3 ml in fluoride tube for glucose and give a sweet drink to child.
7. Repeat sampling at 60, 90, 120, 150 and 180 minutes. Maximum response will be at 2-3 hours.
8. Once the test is completed, the subject should be given a supervised meal.
9. The adults should not drive for at least two hours after the test.

Emergency management of hypoglycaemia

1. Hypoglycaemia should be reversed if there are severe symptoms i.e. loss of consciousness, cardiac symptoms, extreme anxiety or fits.
2. If possible blood samples may be collected because adequate stimulation has been achieved.
3. If necessary, i.v. 50% dextrose 0.4ml/kg (i.e. 28ml for a 70 kg adult) should be administered and blood sampling continued as per protocol.
4. Do not give glucagon unless venous access is lost.

Side effects

Nausea is a common side-effect (30%) and, rarely, patients may vomit.

Interpretation

1. An adequate cortisol response is defined as a rise in plasma cortisol concentration to greater than 550 nmol/L or > 200 nmol/l over basal level.
2. GH response (In children):An increase in GH concentration to > 20mIU/l indicates adequate pituitary GH reserve.
3. GH response (In adults) : Normal response > 15 mIU/l

5. Insulin Tolerance Test (ITT)

Indications:

Assessment of GH and ACTH/ Cortisol reserve

Contraindications:

1. Age > 60 years
2. Ischemic heart disease with abnormal ECG
3. Epilepsy
4. Grossly overweight patients
5. Severe panhypopituitarism, hypoadrenalism (0900 hours Cortisol < 100 nmol/l)

Principle:

ACTH and GH are both released as a part of stress mechanism triggered by hypoglycaemia. GH response is measured directly. Cortisol is measured as an indicator of ACTH response.

Prerequisites:

1. Patient's detailed evaluation which includes clinical history, physical examination and measurement of height and weight (percentile).
2. Base line investigations to be done which include:
    a. Blood CP and ESR
    b. Renal function tests
    c. Liver function tests
    d. Thyroid function tests
    e. Morning serum Cortisol level
    f. X ray wrist for bone age
    g. Exercise or levodopa stimulation test or serum IGF and/or IGFBP3 level
3. This test should not be performed on children outside a specialist paediatric endocrine unit. Detailed explanation of the procedure and side effects associated with test should be communicated to the parents.Written consent by the parents should be taken before the procedure.
4. Physician should be in attendance throughout the test.
5. Equipment / medicine required before test procedure are

1. 50 mL 50% dextrose solution
2. Glucagon inj for IM administration in case IV access is lost
3. Glucose test strips and lancets with glucometer
4. Indwelling cannula gauge 19 to 22
5. Fluoride and plain test tubes

6. Resuscitation equipment

Preparation

1. The patient should fast overnight (no calorie intake for at least 8 hours) and be recumbent during the test.
2. Weigh the patient

Procedure:

1. Place an indwelling cannula in the ante cubital fossa with good access for administration of glucose, if required.
2. Check fasting plasma glucose by glucometer.
3. Calculate Inj insulin dosage as follows and administer.
   a. 0.15 U/ kg for subjects with normal pituitary function
   b. 0.10 U/ kg for subjects with hypopituitarism
   c. 0.2- 0.3 U/ kg for subjects with acromegaly, Diabetes or Cushing's syndrome
4. Observe for signs of hypoglycemia i.e. tachycardia, palpitation, sweating, headache, confusion, drowsiness, loss of consciousness & abdominal pain and check plasma glucose level with help of glucometer.
5. Adequate hypoglycaemia is plasma glucose < 2.5 mmol/l) or 50% of the basal glucose value which is symptomatic.
6. If there have been no clinical signs of hypoglycaemia by 30 min, the dose of insulin should be repeated and the test should be continued with blood samples time again from 0 min.
7. Take 3ml venous blood in plain tubes for GH/ cortisol and 3 ml in fluoride tube for glucose and give a sweet drink to child.
8. Repeat sampling at 30, 60, 90 and 120 minutes. At least two blood specimens should be taken following adequate hypoglycaemia.
9. Once the test is completed, the subject should be given a supervised meal.
10. The adults should not drive for at least two hours after the test.
11. Protocol for the test is tabulated below.

At 0 min	Take 3ml blood in plain tubes for GH/ Cortisol and 3 ml in fluoride tube for glucose & inject insulin intravenously as pre calculated dose
At induction	Take 3ml blood in plain tubes for GH/ Cortisol and 3 ml in fluoride tube for glucose
At 30, 60, 90 and 120 min	Take 3ml blood in plain tubes for GH/ cortisol and 3 ml in fluoride tube for glucose

Emergency management of hypoglycaemia

1. Hypoglycaemia should be reversed if there are severe symptoms i.e. loss of consciousness, cardiac symptoms, extreme anxiety or fits.
2. If possible blood samples may be collected because adequate stimulation has been achieved.
3. If necessary, i.v. 50% dextrose 0.4ml/kg (i.e. 28ml for a 70 kg adult) should be administered and blood sampling continued as per protocol.
4. Do not give glucagon unless venous access is lost.
5. Once the test is completed, the subject should be given a supervised meal.
6. The adults should not drive for at least two hours after the test.
7. Hypothyroidism impairs the GH and Cortisol response. If hypothyroidism is confirmed, the need for a repeat ITT may be reconsidered after 3 months of thyroxin therapy.
8. Patients with dual deficiency (hypothyroidism and hypoadrenalism) should have corticosteroid replacement commenced prior to thyroxin as the later has been reported to precipitate an Addisonian crisis.

Interpretation:

1. An adequate Cortisol response is defined as a rise in plasma Cortisol concentration to greater than 550 nmol/l or > 200 nmol/l over basal level.
2. GH response (In children): An increase in GH concentration to > 20mU/l indicates adequate pituitary GH reserve.
3. GH response (In adults): Normal response > 15 mIU/l

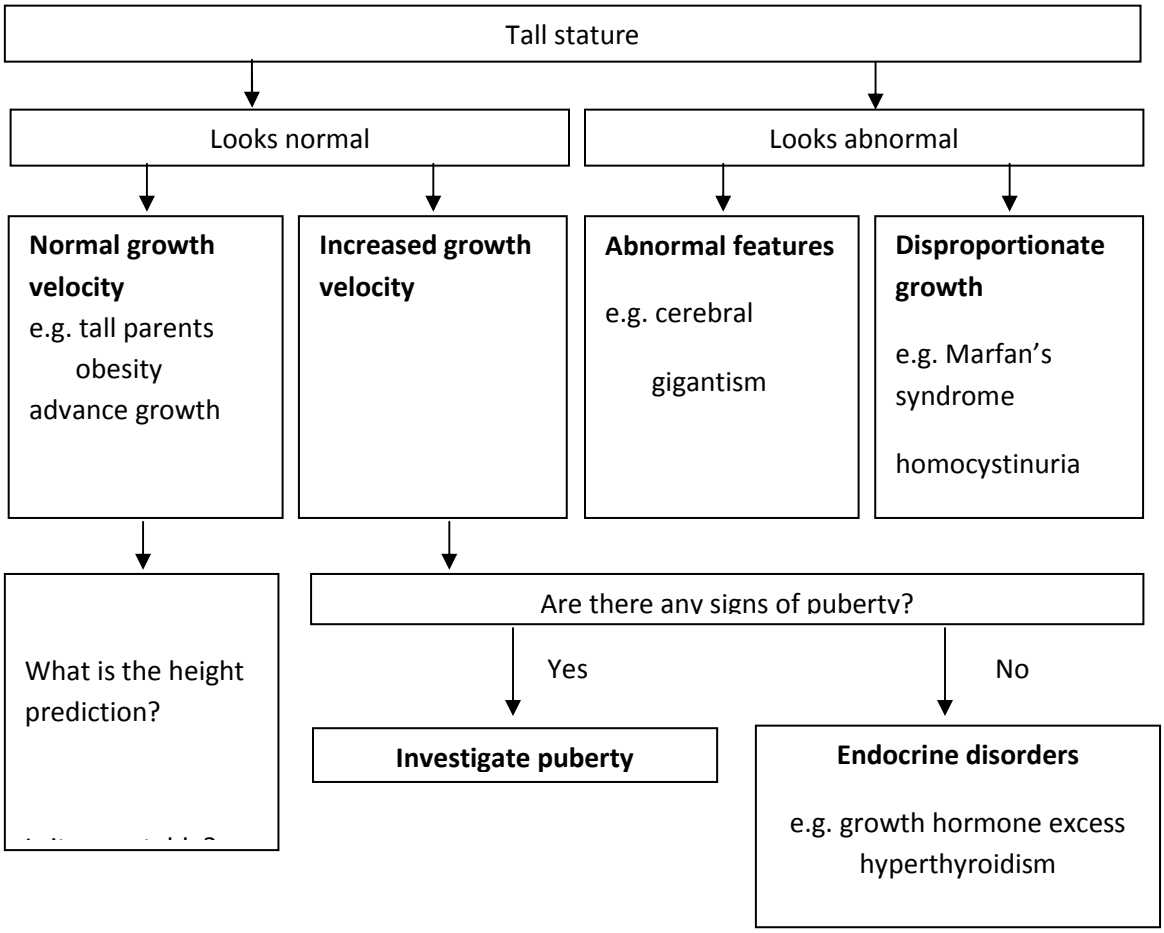
Reference

Burtis CA, Ashwood ER, Bruns DE. Teitz Textbook of clinical chemistry and molecular diagnostics; 5th edition: 2012.

Acromegaly

There are relatively few pathological causes of tall stature, most children representing the upper end of normal distribution of height. Most often there is a family history of tallness in or both parents. Ultimate height can be predicted by assessment of bone age. The major causes of tall stature are illustrated in fig III followed by protocol for growth hormone suppression test.

Flow Diagram to Evaluate Tall Stature



1. Growth Hormone Suppression Test

Introduction:

The test is of value in confirming the presence of active acromegaly or gigantism, particularly in the early stages.

Principle:

In the presence of either active acromegaly or gigantism, the normal suppression of growth hormone (GH) by food or glucose does not occur.

Patient preparation:

The patient should be fasting overnight and the test should be performed early in the morning (0800 hour). The patient should not be receiving GH-stimulating drugs.

Procedure:

This is as for the oral "OGTT". The patient reports in the morning in fasting state. Basal sample for GH and fasting blood glucose is taken. He is given 75 grams glucose in 250-300 ml water to drink. Blood samples for GH and glucose are collected at 30, 60, 90 and 120 min later. Serum is separated and used for GH and glucose assays.

Quality control:

GH assay is assessed by using 2 levels of controls. The results of these controls are entered on the Levy Jenning charts. The results are assessed in the light of Westgard Rules.

Normal response:

The normal response is for serum GH to be suppressed to <3 mIU/l (1 ng/ml) at some point during the period of the test.

Interpretation:

In the patient with active disease, there is failure of a high resting serum GH to suppress and indeed there may be a paradoxical rise. Often there is also evidence of decreased glucose tolerance. A paradoxical rise may also occur in renal failure and diabetes mellitus.

Failure of suppression is sometimes seen in advanced liver disease, heroin addiction and anorexia nervosa.

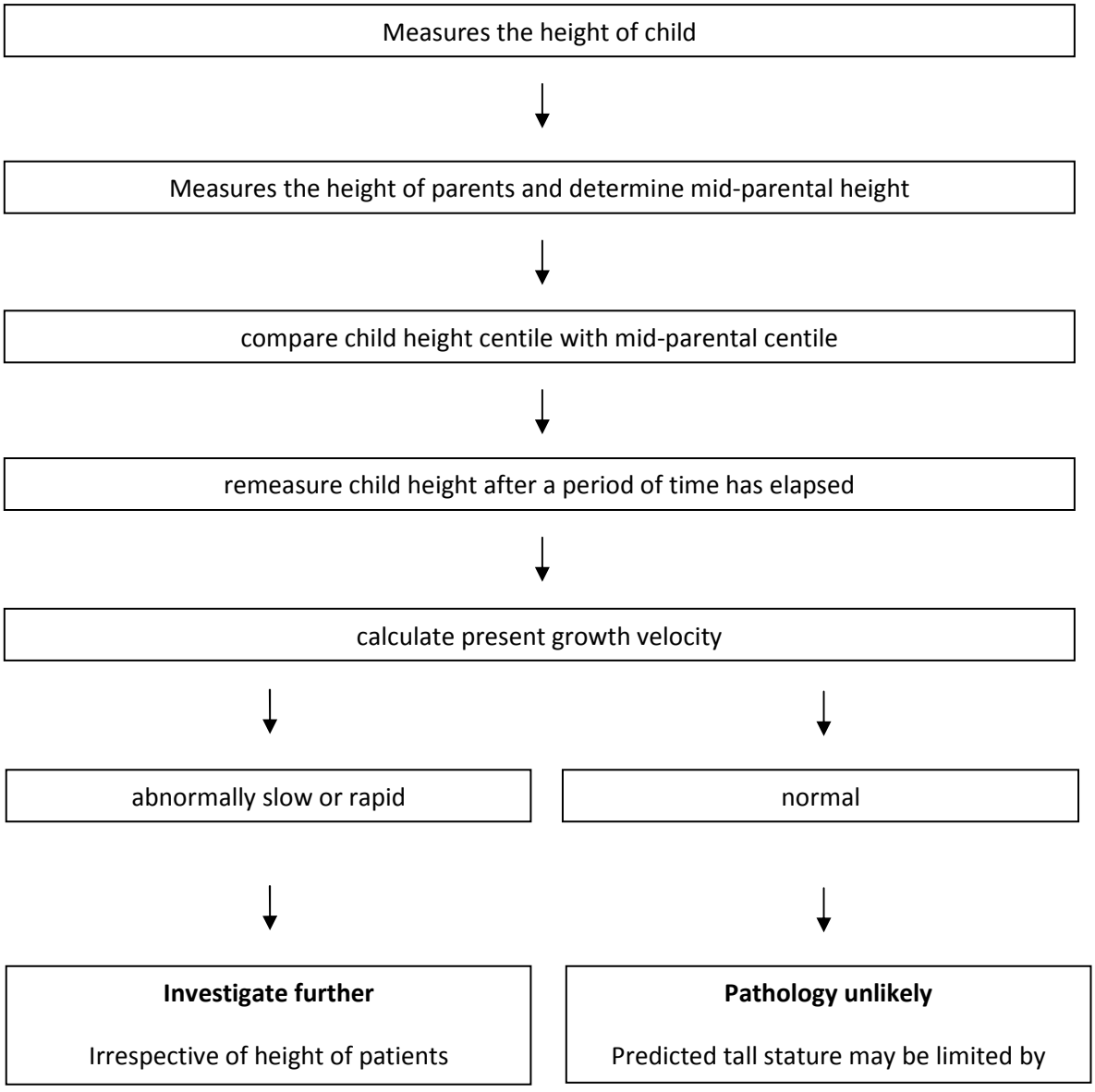
Comments:

This is a useful test for confirming suspected early acromegaly, or for establishing whether or not obvious acromegaly is still active. In burnt out acromegaly, the basal serum GH level returns gradually towards normal, although impaired glucose tolerance may persist.

Reference:

Diagnosis of Acromegaly. www.UpToDate.com (2015)

Summary of Essential Steps in the Assessment of Growth Disorders



Addison`s Disease

Introduction:

The clinical presentation of adrenal insufficiency is variable, depending on whether the onset is acute, leading to adrenal crisis, or chronic, with symptoms that are more insidious and vague. Therefore, the diagnosis of adrenal insufficiency depends upon having an appropriate level of clinical suspicion. Adrenal crisis should be considered in any patient who presents with peripheral vascular collapse, whether or not the patient is known to have adrenal insufficiency. Likewise, isolated corticotrophin (ACTH) deficiency, although rare, should be considered in any patient who has unexplained severe hypoglycaemia or hypernatremia. Prolonged administration of pharmacologic doses of synthetic glucocorticoids is by far the most common cause of ACTH deficiency and consequent adrenal insufficiency.

However, patients treated with glucocorticoid therapy rarely present with adrenal crisis, although sudden withdrawal of glucocorticoids can result in exacerbation of the disorder for which they were being given (e.g. asthma, inflammatory disease, or organ transplantation), symptoms of glucocorticoid deficiency, or hypotension.

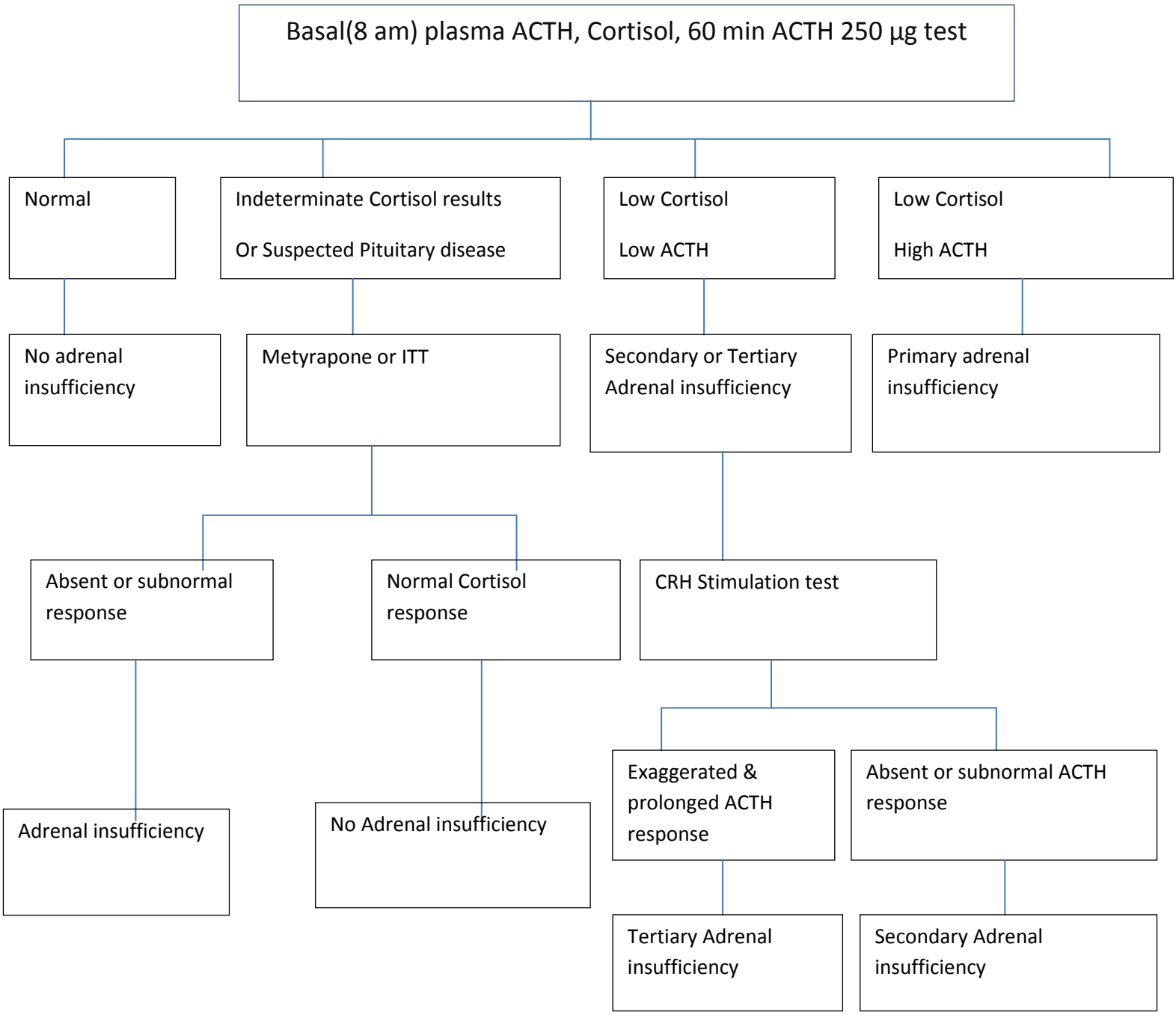
Evaluation of the Patient with Possible Adrenal Insufficiency:

1. **Measure 8 am Serum Cortisol and Plasma ACTH:** These two tests should be performed in the first sample as soon as patient is suspected of Addison Disease. A delay in diagnosis can be detrimental as patient may go into adrenal crisis if not diagnosed and treated early.The diagnosis of Addison Disease is ruled out if basal serum cortisol value is ≥ 18µg/dl or 500nmol/L.
2. **Short ACTH Stimulation Test.** In all other patients a short ACTH stimulation test should be performed.
3. Caution should be taken in interpreting the results in patients with abnormalities of cortisol binding globulin (CBG) or albumin, such as

patients with cirrhosis of nephrotic syndrome, or those taking oral estrogens. In these settings, decreased or increased levels may lead to an incorrect diagnosis. Salivary (≥ 5.8ng/ml or 16nmol/L excludes adrenal insufficiency) or serum free cortisol have been suggested as alternatives.

4. If new or recent onset ACTH deficiency is suspected (e.g., pituitary surgery within the past two weeks), the low dose ACTH stimulation test is suggested.
5. The underlying etiology of the adrenal insufficiency should then be determined.

Diagnostic Approach to Addison Disease



Short Synacthen Test (High Dose)

Introduction:

The N-terminal 24 amino acid residues of ACTH comprise the bioactive portion of the 39 residue molecule, and is commercially available in synthetic form (tetracosactrin) suitable for either *intramuscula*r or *intravenous* injection in precise dosage (e.g. Synacthen, Ciba-Geigy). Injection of an adequate dose is followed by a marked cortisol secretion by the normal adrenal cortex.

Patient Preparation:

1. Test is performed in morning (8am). Fasting is not required.
2. If possible glucocorticoids medication should be ceased 24 hours prior to test to minimize possible suppression.
3. Patient taking glucocorticoids other than Dexamethasone should be shifted to dexamethasone after medical consultation because other glucocorticoids interfere with assay.

Contraindications:

1. Suspected Cushing's syndrome
2. Pregnancy
3. Viral infections,
4. Heart failure,
5. History of drug allergy.

The test should be conducted under medical supervision.

Possible side effects: -

Rare allergic reactions have been reported.

Protocol for Adults:-

Time (min)

0	Basal blood sample for cortisol.
	(0.7ml) 250μg Synacthen IM. or IV.
30 & 60	Specimens for serum Cortisol.

Protocol for Paediatric Patients:-

Time (min)

0 Basal venous blood sample for cortisol.

Synacthen 36μg/ Kg in neonates or 125μg 6 months -2 y, 250μg over 2y of age given by IM or IV route

30 and 60 venous blood samples for serum Cortisol

Test Procedure:-

This test should be done under supervision of a doctor. History of the patient is taken and necessary physical examination is carried out and blood samples for serum cortisol basal and after stimulation are taken. The samples are immediately transported to processing room where serum is separated and test is carried ***out.***

Interpretation:-

A normal adrenocortical response is defined as:

1. A post stimulation ***Cortisol*** value exceeding the basal value by more than 200 nmol/l. Or
2. A post stimulation ***Cortisol*** value exceeding 550 nmol/l.

The normal increment is inversely related to the basal level, and the 30 min cortisol level exceeds that in the 60 min sample.

3. A failure to respond is consistent with adrenal failure, while a sluggish response (60-min values > 30 min) is suggestive of adrenal dysfunction secondary to hypothalamic/pituitary disease or steroid therapy.
4. Depending on the clinical question, e.g. possible adrenal cortex atrophy due to lack of stimulation, a prolonged stimulation test may be needed to further investigate a sluggish response.

Short Synacthen Test (Low Dose)

Introduction:

A test involving more physiological plasma concentrations of ACTH theoretically provides a more sensitive index of adrenocortical responsiveness.The N-terminal 24 amino acid residues of ACTH comprise the bioactive portion of the 39 residue molecule, and is commercially available in synthetic form (tetracosactrin) suitable for either *intramuscula*r or *intravenous* injection in precise dosage (e.g. Synacthen, Ciba-Geigy). Injection of an adequate dose is followed by a marked cortisol secretion by the normal adrenal cortex.

Patient Preparation:

1. Test is performed in morning (8am). Fasting is not required.
2. If possible glucocorticoids medication should be ceased 24 hours prior to test to minimize possible suppression.
3. Patient taking glucocorticoids other than Dexamethasone should be shifted to dexamethasone after medical consultation because other glucocorticoids interfere with assay.

Contraindications:

1. Suspected Cushing's syndrome
2. Pregnancy
3. Viral infections,
4. Heart failure,
5. History of drug allergy.

The test should be conducted under medical supervision.

Possible Side Effects: -

Rare allergic reactions have been reported.

Protocol for Adults:-

Time (min)

0	Basal venous blood sample for cortisol.
	1μg* Synacthen IV.
30 & 60	Specimens for venous serum ***Cortisol***.

*There is no commercially available preparation of "low dose" cosyntropin. One prepares the low-dose solution of cosyntropin locally; the vials of cosyntropin currently available contain 250 mcg and come with sterile normal saline solution to be used as diluent. Instructions are as follows:

Inject 1 mL of the diluent into the vial of cosyntropin to produce a 250 mcg/mL solution and shake thoroughly.

Using a 1 mL tuberculin syringe, withdraw 0.2 mL (i.e., 50 mcg cosyntropin) and inject it into a vial containing 24.8 mL of sterile normal saline solution to produce a 2 mcg/mL solution.

After shaking thoroughly, again using a 1 mL syringe, withdraw 0.5 mL (1 mcg cosyntropin) or the appropriate volume for the patient's surface area, and inject the entire volume immediately intravenously.

Test Procedure:

The test should be done under supervision of a doctor. History of the patient is taken and necessary physical examination is carried out and blood samples for serum cortisol basal and after stimulation are taken. The samples are immediately transported to processing room where serum is separated and test is carried ***out.***

Interpretation:-

1. A normal adrenocortical response is defined as:
2. A post stimulation ***Cortisol*** value exceeding the basal value by more than 200 nmol/l. Or
3. A post stimulation ***Cortisol*** value exceeding 550 nmol/l.
4. The normal increment is inversely related to the basal level, and the 30 min cortisol level exceeds that in the 60 min sample.
5. A failure to respond is consistent with adrenal failure, while a sluggish response (60-min values > 30 min) is suggestive of adrenal dysfunction secondary to hypothalamic/pituitary disease or steroid therapy.
6. Depending on the clinical question, e.g. possible adrenal cortex atrophy due to lack of stimulation, a prolonged stimulation test may be needed to further investigate a sluggish response.

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Cushing Syndrome / Disease

Introduction:The possible presence of Cushing's syndrome is suggested by certain symptoms and signs. Unfortunately, none of these are pathognomonic, and many are nonspecific (e.g., obesity, hypertension, menstrual irregularity, and glucose intolerance). As a result, the diagnosis must be confirmed by biochemical tests. The diagnosis of Cushing's syndrome is further complicated by following:

Exogenous Glucocorticoids: Before evaluation for possible Cushing's syndrome, it is essential that a careful history has excluded exogenous glucocorticoid intake.

Physiologic Hypercortisolism: Hypercortisolism can occur in several disorders other than Cushing's syndrome. Examples include:

1. Patients who are physically stressed, such as by a severe bacterial infection
2. Patients with severe obesity, especially those with visceral obesity or polycystic ovary syndrome
3. Patients with psychological stress, especially patients with a severe major depressive disorder and melancholic symptoms
4. Rarely, patients with chronic alcoholism

When such patients present with clinical features consistent with Cushing's syndrome, they may be referred to as having pseudo-Cushing's syndrome. However, the distinction is not always simple.

First Line Tests for Patient with Possible Hypercortisolism:

The initial diagnostic tests for hypercortisolism should be highly sensitive, even though the diagnosis may be excluded later by more specific tests. We agree with the approach outlined by the evidence-based 2008 Endocrine Society Clinical Guidelines. *The First Line Tests are:*

1. Late night salivary cortisol
2. Twenty four urinary cortisol

3. 1-mg overnight Dexamethasone suppression test or longer low dose DST (2 mg/day for 48 h)

The important points to be kept in mind are:

1. Urinary and salivary cortisol measurements should be obtained at least twice.
2. At least two first-line tests should be abnormal to establish the diagnosis of Cushing's syndrome.
3. The urinary cortisol excretion should be unequivocally increased (threefold above the upper limit of normal for the assay), or the diagnosis of Cushing's syndrome is uncertain and other tests should be performed.
4. The diagnosis of Cushing's syndrome is confirmed when two tests are unequivocally abnormal.
5. The patient should undergo additional evaluation if the test results are discordant or only slightly abnormal.
6. If test results are normal, the patient does not have Cushing's syndrome unless it is extremely mild or cyclic. Additional evaluation is only suggested if symptoms progress or cyclic Cushing's syndrome is suspected.

Diagnostic Tests:

Late evening Serum Cortisol: Measurement of serum or salivary cortisol in the late evening is based upon the fact that the normal evening nadir in serum cortisol is preserved in obese and depressed patients but not in those with Cushing's syndrome. Evening Cortisol<2 mcg/dL (50 nmol/L) excludes Cushing’s syndrome.Most patients with Cushing's syndrome have early morning serum cortisol concentrations within or slightly above the normal range. In contrast, serum cortisol concentrations one hour after sleep are almost always high (greater than 7.5 µg/dL [207 nmol]) and are often equal to the early morning values (i.e., they have an abnormal or absent circadian rhythm).

Daily urinary cortisol excretion: Twenty-four hour urinary cortisol excretion provides a direct and reliable practical index of cortisol secretion.Twenty-four hour urinary cortisol excretion is an integrated measure of the serum free cortisol concentration (i.e., cortisol that is not

bound to cortisol-binding globulin [CBG, transcortin] or other serum proteins). The two most important factors in obtaining a valid result are collection of a complete 24-hour specimen and a reliable reference laboratory.It may be easier for the patient to collect an overnight urine sample from 10 PM to 8 AM, rather than a 24-hour sample, as the initial test. When cortisol excretion is expressed as a function of urinary creatinine excretion, this test appears to give results as reliable as the 24-hour collection.The patient can be assumed to have Cushing's syndrome if basal urinary cortisol excretion is more than three times the upper limit of normal (which may vary somewhat in different assays) and one other test is abnormal. The patient should then be evaluated for the cause of the hypercortisolism. On the other hand, patients with equivocally raised values (above normal but less than three times the upper reference value) may have physiologic hypercortisolism and should either be reevaluated after several weeks or be subjected to one or more of the other tests.

Low-Dose Dexamethasone Suppression Tests:

Introduction: Exogenous dexamethasone substitutes for endogenous cortisol in suppressing ACTH release. The dexamethasone dosages used should reliably suppress ACTH secretion by the normal pituitary gland, leading to suppression of cortisol secretion and subsequent reductions in serum cortisol concentrations and urinary excretion of cortisol and cortisol metabolites. There are two forms of the low-dose dexamethasone suppression test: the 1mg "overnight" and the two-day 2 mg test.

Clinical significance: This test is indicated as first line test in patients with clinical suspicion of Cushing syndrome.

Principle: In normal subject the administration of a supraphysiological dose of glucocorticoid results in suppression of ACTH and cortisol secretion. In endogenous Cushing's syndrome, there is a failure of this suppression.

Patient preparation: There should have been no treatment with glucocorticoid drugs (including topical preparations) for several weeks. Mineralocorticoids do not interfere with this test. The test may be performed on inpatients or outpatients.

Procedure: At 2300h dexamethasone (1 mg) is given orally. A venous blood sample is taken at 9.00 am on the following day for serum Cortisol.

Sample handling: This is as for serum ***Cortisol*** estimation. Samples are transported immediately to processing room, where serum is separated and used for assay.

Normal response:

There is marked suppression of serum Cortisol level to less than 50 nmol/L after the dexamethasone dose.

Limitations:

1. No specific data for interpretation in paedriatric population.
2. Not suitable for patients with abnormal CBG e.g. marked hepatic microsomal p 450 enzyme induction by drugs leads to dexamethasone being eliminated rapidly, thereby causing inadequate suppression of serum cortisol.
3. About 5% of normal subject also show a positive response. (95% sensitivity)

Interpretation:

A significant proportion of patients with depression (in whom there is loss of the normal diurnal variation in serum cortisol levels) show early escape from the suppression of serum cortisol normally seen on the second day as evidenced by a concentration of >***140*** nmol/l or > 50 % of the ***basal*** value. However, many patients with depression fail to show this escape by exhibiting low serum cortisol concentrations may also be found in individuals with organic hypofunction of the adrenal cortex but these patients would show low levels in base line sample too. Marked hepatic microsomal p 450 enzyme induction by drugs leads to dexamethasone being eliminated rapidly, thereby causing inadequate suppression of serum cortisol. Some patients with other disorders, including anorexia nervosa without obvious depression, weight loss from other causes and patients with dementia associated with enlarged cerebral ventricles, show false positive responses, i.e., they, too display escape from suppression. About 20% of normal subject also show a positive response. The test is negative in patients with pure anxiety states and schizophrenia, but it must be remembered that these disorders may be associated with an element of depression in which case the test could be positive. A repeat test following treatment for the depression, which remains positive, suggests a poor prognosis.

Comments:

If the 9.00 am blood sample taken on the second day shows a low serum cortisol concentration as compared with a normal base line value on the first

day this confirms compliance with the taking of an adequate dose of dexamethasone. Some authorities recommend measurement of serum dexamethasone concentrations as a further check on compliance in addition to assaying serum cortisol.

High-Dose Dexamethasone Suppression Tests:

Clinical significance:

This test is useful in determining the cause of established Cushing's syndrome and particularly in the differentiation of pituitary dependent Cushing's disease from the other causes of Cushing's syndrome including ectopic ACTH production by tumors, adrenocortical adenomas and adrenocortical carcinomas. The latter 3 disorders do not usually show suppression.

Principle

A high dose of dexamethasone administered over a short period of time differentiates pituitary-dependent Cushing's disease from Cushing's syndrome of other etiology by causing suppression of plasma ACTH /serum cortisol in the former.

Patient preparation

The patient ***can*** be admitted to hospital. There should ***be*** no treatment with Glucocorticoids drugs (including topical preparations) for several weeks; mineralocorticoids do not interfere with this test. The test should not be performed in the presence of cardiac failure.

Procedure

Morning (0800-0900 hours) basal samples ***is taken*** for serum ***Cortisol***. Dexamethasone (8 mg) is administered orally ***at*** 2300 ***hours*** on same night. ***On next morning***, venous blood (5 ml) is collected into a plain bottle for serum Cortisol.

Sample handling

This is same as for estimation of serum ***Cortisol***. Samples are transported to processing room, where serum is separated and used for assay.

Normal response

There is marked suppression of serum ***Cortisol*** to **>** 50% of the base line level after ***high*** dose of dexamethasone.

Interpretation:

Suppression of serum ***Cortisol*** level in patients with Cushing's syndrome points to a pituitary dependent etiology. Failure of suppression is a feature of both adrenocortical tumors (adenoma and carcinoma) and ectopic ACTH producing tumors. Failure of suppression may ***also*** occur particularly in some patients with pituitary disease. ***However,*** an extremely high serum ***Cortisol*** level favors the diagnosis of adrenocortical carcinoma or ectopic ACTH producing tumours. A paradoxical response (i.e. a rise of serum ***Cortisol***) to dexamethasone administration should alert one to the possibility of cyclical Cushing's syndrome.

Comments

This test is time consuming and not without adverse clinical effects, particularly in patients with incipient cardiac failure, hypertension and peptic ulcer. It is currently less frequently employed. Suppression of a ***Cortisol*** following dexamethasone administration occurs in patients with pituitary dependent Cushing's disease. Dexamethasone does not interfere with the measurement of serum ***Cortisol***.

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Pheochromocytoma

Introduction: Catecholamine-secreting tumours that arise from chromaffin cells of the adrenal medulla and the sympathetic ganglia are referred to as "pheochromocytomas" and "catecholamine-secreting paragangliomas" ("extra-adrenal pheochromocytomas"), respectively. Because the tumours have similar clinical presentations and are treated with similar approaches, many clinicians use the term "pheochromocytoma" to refer to both adrenal pheochromocytomas and catecholamine-secreting paragangliomas. However, the distinction between pheochromocytoma and paraganglioma is an important one because of implications for associated neoplasms, risk for malignancy, and genetic testing.

Classic Triad: The classic triad of symptoms in patients with a pheochromocytoma consists of episodic headache, sweating, and tachycardia. About half have paroxysmal hypertension; most of the rest have what appears to be essential hypertension or normal blood pressure. Most patients with pheochromocytoma do not have the three classic symptoms, and patients with essential hypertension may have paroxysmal symptoms.

1. Sustained or paroxysmal hypertension is the most common sign of pheochromocytoma, but approximately 5 to 15 percent of patients present with normal blood pressure. The frequency of normotension is higher in patients with adrenal incidentaloma or in those undergoing periodic screening for familial pheochromocytoma.
2. Headache, which may be mild or severe, and variable in duration, occurs in up to 90 percent of symptomatic patients.
3. Generalized sweating occurs in up to 60 to 70 percent of symptomatic patients. Other symptoms include forceful palpitations, tremor, pallor, dyspnea, generalized weakness, and panic attack-type symptoms (particularly in pheochromocytomas that produce epinephrine)

Diagnostic Testing:

The diagnosis is typically confirmed by measurements of urinary and plasma fractionated metanephrines and catecholamines.

Interfering medications — Although it is preferred that patients not receive any medication during the diagnostic evaluation, treatment with

all antihypertensive medications may be continued. Tricyclic antidepressants interfere most frequently with the interpretation of 24-hour urinary catecholamines and metabolites. To effectively screen for catecholamine-secreting tumors, treatment with tricyclic antidepressants and other psychoactive agents (but not selective serotonin reuptake inhibitors) should be tapered and discontinued at least two weeks before any hormonal assessments.There are certainly clinical situations for which it is contraindicated to discontinue certain medications (e.g., anti-psychotics) and if case-detection testing is positive, then computed imaging (e.g., CT scan of the abdomen and pelvis) would be needed to exclude a catecholamine-secreting tumor. Furthermore, catecholamine secretion may be appropriately increased in situations of physical stress or illness (e.g., stroke, myocardial infarction, congestive heart failure, obstructive sleep apnea). Therefore, the clinical circumstances under which catecholamines and metanephrines are measured must be assessed in each case. Levodopa is the most common and only pharmacotherapeutic agent that causes markedly abnormal levels of dopamine.

24-hour urine fractionated catecholamines and metanephrines — The 24-hour urine collection for fractionated metanephrines and catecholamines should include measurement of urinary creatinine to verify an adequate collection.A positive case-detection test for a catecholamine-secreting tumor includes one or more of the following findings:

1. Norepinephrine >170 mcg/24 hour
2. Epinephrine >35 mcg/24 hour
3. Dopamine >700 mcg/24 hour
4. Normetanephrine >900 mcg/24 hour or metanephrine >400 mcg/24 hour.

Plasma fractionated metanephrines — measuring plasma fractionated metanephrines is a first-line test when there is a high index of suspicion for pheochromocytoma. Plasma fractionated metanephrines are also a good first-line test for children because obtaining a complete 24-hour urine collection is difficult.

24-hour urinary fractionated catecholamines and metanephrines should be the first test in patients with a somewhat lower index of suspicion for pheochromocytoma. This includes patients with:

1. Resistant hypertension
2. Hyperadrenergic spells (e.g., self-limited episodes of nonexertional palpitations, diaphoresis, headache, tremor, or pallor)

3. An incidentally discovered adrenal mass that does not have imaging characteristics consistent with pheochromocytoma

Other Tests:

Clonidine suppression test — The high false-positive rate for plasma catecholamines and fractionated metanephrines triggered the development of a confirmatory test, the clonidine suppression test. This test is intended to distinguish between pheochromocytoma and false-positive increases in plasma catecholamines and fractionated metanephrines. Clonidine is a centrally acting alpha (2)-adrenergic receptor agonist that normally suppresses the release of catecholamines from neurons but does not affect the catecholamine secretion from a pheochromocytoma. Clonidine (0.3 mg) is administered orally, and plasma catecholamines or fractionated metanephrines are measured before and three hours after the dose. In patients with essential hypertension, plasma catecholamine concentrations decrease (norepinephrine + epinephrine <500 pg/mL or >50 percent decrease in norepinephrine) as do plasma normetanephrine concentrations (into normal range or >40 percent decrease). However, these concentrations remain increased in patients with pheochromocytoma. With this criterion, the test is 92 percent accurate.Patients also should not be taking diuretics, beta-adrenergic blockers, or tricyclic antidepressants, but alpha-adrenergic blockers do not interfere with the test.Clonidine suppression tests should **NOT** be performed in hypovolemic patients because of the risk of a marked reduction in blood pressure, or in patients with normal plasma catecholamine values because the results are often inaccurate.

Plasma catecholamines — Because of poor overall accuracy in testing for pheochromocytoma, measurement of plasma catecholamines no longer has a role.

Neuropeptide Y — Plasma neuropeptide Y levels are increased in 87 percent of patients with pheochromocytoma [5], but they also lack the accuracy of 24-hour urinary fractionated metanephrines and catecholamines.

Vanillylmandelic acid — The 24-hour urinary vanillylmandelic acid (VMA) excretion has poor diagnostic sensitivity and specificity compared with fractionated 24-hour urinary fractionated metanephrines.

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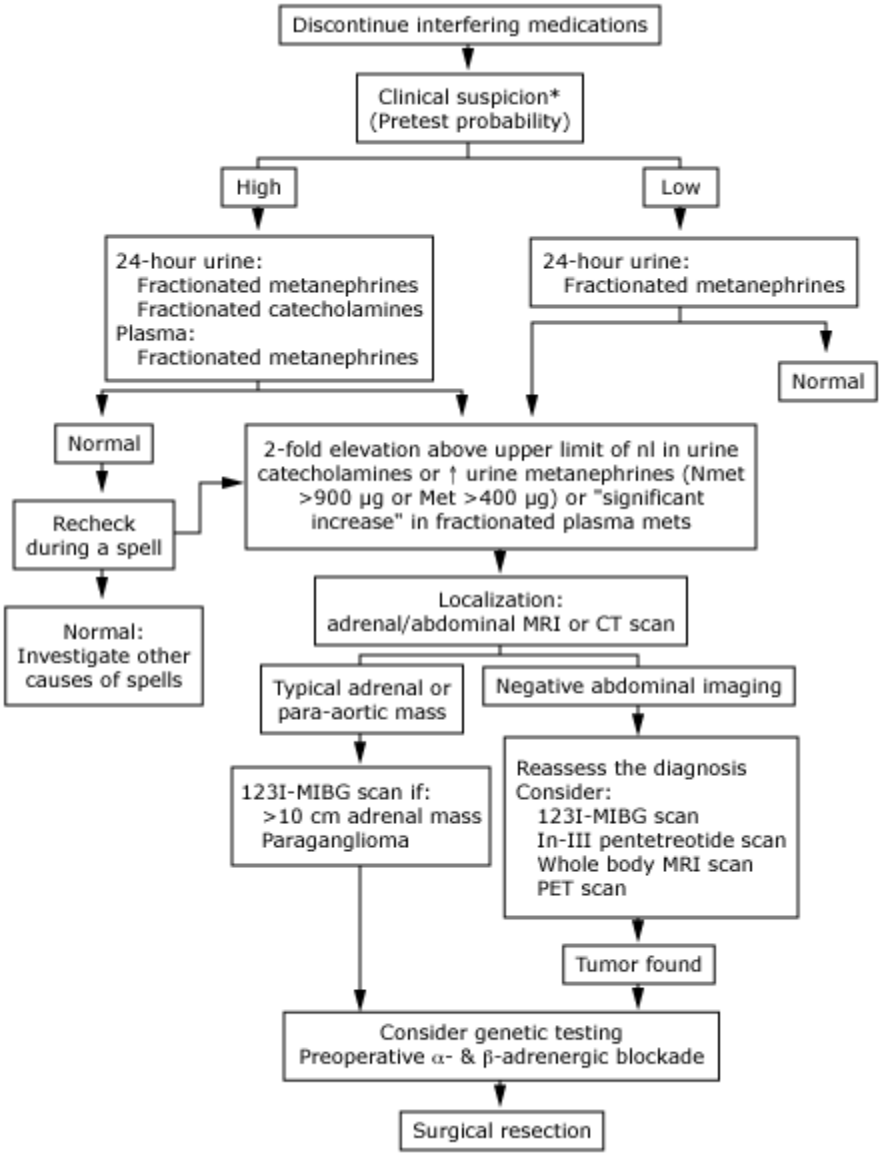
Diagnostic approach to suspected pheochromocytoma



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Renin Aldosterone Disorders

Primary Hyperaldosteronism: Non-suppressible (primary) hypersecretion of aldosterone is an underdiagnosed cause of hypertension.

The classic presenting features

1. Hypertension
2. Hypokalemia:Normokalaemia, however, may be more common than hypokalemia in patients diagnosed with primary aldosteronism.

Common causes

1. Aldosterone-producing Adenomas - (High aldosterone, low PRA)
2. Bilateral adrenal Hyperplasia – (Idiopathic Hyperaldosteronism-responds to posture)
3. Primary adrenal hyperplasia
4. Familial Hyperaldosteronism type I (glucocorticoid-remediable aldosteronism) or type II. (rare ones)

Detection of cases

We recommend 2008 Endocrine Society Guidelines, for detection of Primary Hyperaldosteronism in patients with:

1. Hypertension and hypokalemia
2. Severe or resistant hypertension
3. Hypertension and an adrenal incidentaloma
4. Hypertension and a family history of early-onset hypertension or cerebrovascular accident at a young age (<40 years)
5. Hypertension and first-degree relatives with documented primary hyperaldosteronism

The Initial Evaluation should consist of:

1. Documenting that the active renin mass concentration (ARC) or plasma renin activity (PRA) is reduced (typically undetectable), and
2. The plasma aldosterone concentration (PAC) is inappropriately high for the PRC or PRA

3. Aldosterone Renin Ratio (ARR) (PAC/ARC) greater than 20 to 50 (depending upon the laboratory reference values)

Measurement of ARR: a suggested approach

Preparation for aldosterone-renin ratio (ARR) measurement*:*

1. Attempt to correct hypokalemia, after measuring plasma potassium in blood collected slowly with a syringe and needle [preferably not a Vacutainer to minimize the risk of spuriously raising potassium], avoiding fist clenchingduring collection, waiting at least 5 seconds after tourniquet release (if used) to achieve insertion of needle, andensuring separation of plasma from cells within 30 minutes of collection.
2. Encourage patient to liberalize (rather than restrict) sodium intake.
3. Withdraw agents that markedly affect the ARR (48) for at least 4 weeks:
a. Spironolactone, eplerenone, amiloride, and triamterene
b. Potassium-wasting diuretics
c. Products derived from liquorice root (e.g., confectionary licorice, chewing tobacco)
4. If the results of ARR off the above agents are not diagnostic, and if hypertension can be controlled with relatively non interfering medications, withdraw other medications that may affect the ARR for at least 2 weeks:
a. Beta-adrenergic blockers, central alpha-2 agonists (e.g., clonidine, alpha-methyldopa), nonsteroidal anti-inflammatory drugs
b. Angiotensin-converting enzyme inhibitors, angiotensin receptor blockers, renin inhibitors, dihydropyridine calcium channel antagonists
5. If necessary to maintain hypertension control, commence other antihypertensive medications that have lesser effects on the ARR (e.g., verapamil slow-release, hydralazine [with verapamil slow-release, to avoid reflex tachycardia],prazosin, doxazosin, terazosin;.
6. Establish oral contraceptive (OC) and hormone replacement therapy (HRT) status, as estrogen-containing medications may lower direct renin concentration (DRC) and cause false positive ARR when DRC (rather than plasma renin
activity) is measured. Do not withdraw OC unless confident of alternative effective contraception.

Conditions for collection of blood

1. Collect blood mid-morning, after the patient has been up (sitting, standing, or walking) for at least 2 hours and seated for 5-15 minutes.
2. Collect blood carefully, avoiding stasis and hemolysis
3. Maintain sample at room temperature (and not on ice, as this will promote conversion of inactive to active renin)during delivery to laboratory and prior to centrifugation and rapid freezing of plasma component pending assay.
4. Blood is collected for PRA in tubes containing EDTA and centrifuged at room temperature.

Factors to take into account when interpreting results

1. Age: in patients aged >65 years, renin can be lowered more than aldosterone by age alone, leading to a raised ARR
2. Time of day, recent diet, posture, and length of time in that posture
3 Medications
4. Method of blood collection, including any difficulty doing so
5. Level of potassium
6. Level of creatinine (renal failure can lead to false positive ARR)

Interpretation:

Morning Normal Renin values : Normal, morning plasma renin activity for seated subjects ranges from about 1 to 4 ng/mL per h (0.8 to 3.0 nmol/L per h). Corresponding active renin concentrations are 8 to 35 mU/L
Among the various assays available PRA (Plasma Renin Activity) rather than PRC (Plasma Renin concentration) is preferred method because ARR is heavily dependent on PRA and dependence is highest at the low end of the assay. One must use a reliable laboratory method with documented performance and sufficient assay sensitivity at the low range.
Morning serum (and plasma) aldosterone values: range from 5 to 30 ng/dL (140 to 830 pmol/L) in seated normal subjects with unrestricted salt intakes

ARR Ratio:Although ARR is generally recommended as the best screening test, there are no universally accepted trough values that define ratio levels. Attention should be paid to the following

1. **Units**: Most published cutoff values refer to aldosterone expressed as ng/dl and PRA expressed in ng/mL/with this presently most popular unit system ,most groups use ratios exceeding 20 to 40 as the cut off value requiring further diagnostic workup for primary aldosteronism (PA) . Less often PRC,(mU/L) rather than PRA is measured and aldosterone levels are provided in SI units (pmol/L),and suggested critical ratios, under these circumstances, range from 70 to 130. Care must be taken to avoid derivatization of ratios on "mixed units" other than the combinations specified here.
2. **Effect of drugs**: Beta-adrenergic blockers, central alpha-2 agonists (e.g., clonidine, alpha-methyldopa), nonsteroidal anti-inflammatory drugs suppress PRA and aldosterone and may lead to a false positive ARR. Angiotensin-converting enzyme inhibitors, angiotensin receptor blockers, renin inhibitors, dihydropyridine calcium channel antagonists increase PRA and may lead to false negative ARR.
3. **Effect of age:** PRA declines with age more than aldosterone, leading to high ARR
4. **CRF:** islinked to low PRA and high ARR
5. **Aldosterone levels**: In the absence of hypokalemia <9 ng/dl are inconsistent with PA. On the other hand strict Plasma aldosterone results in a significant rate of false negative cases. Even if aldosterone levels ≥15 ng/dl are set as a requirement for diagnosis in the context of increased ARR, Some patients unduly excluded.

An elevated PAC/ARC ratio alone is not diagnostic of Primary Hyperaldosteronism.

We recommend confirming the diagnosis

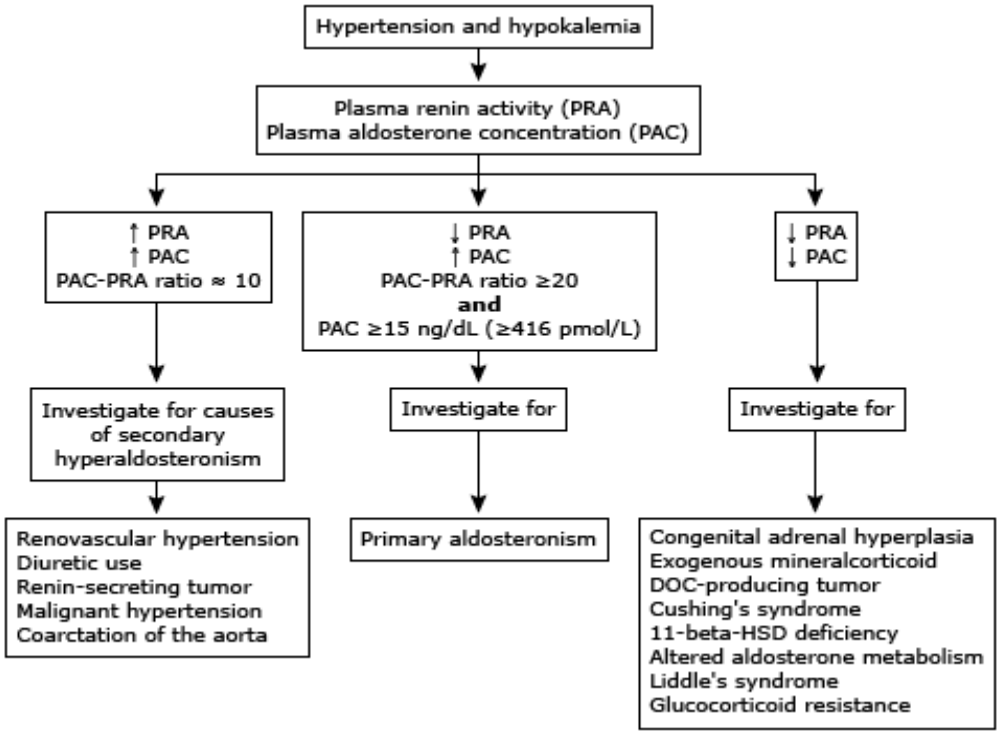
1. By demonstrating inappropriate aldosterone secretion.
2. Aldosterone suppression testing can be performed with orally administered sodium chloride (an Oral salt loading test) and Measurement of Urinary Aldosterone Excretion
3. Or with Intravenous sodium chloride loading (Saline infusion Test) and Measurement of the plasma Aldosterone concentration

4. Other tests include Captopril test, Fludrocortisone suppression test

To distinguish between aldosterone-secreting adenoma and bilateral hyperplasia.

1. Adrenal CT Scan which will also exclude adrenocortical carcinoma
2. Adrenal CT Scan in case if normal then We recommend adrenal venous sampling to confirm unilateral disease.

Algorithm for Investigating Hyperaldosteronism



Diabetes Insipidus

Diabetes Insipidus (DI)is defined as the passage of large volumes (>3 L/24h) of dilute urine. (Osmolality <300 mOsm/kg)

CLASSIFICATION

Primary Polydipsia (also called psychogenic polydipsia), characterized by a primary increase in water intake.

Cranial or Central Diabetes Insipidus, due to deficient secretion of ADH.

Nephrogenic DI, characterized by normal ADH secretion but varying degrees of renal resistance to its water-retaining effect

Differential diagnosis Other conditions that may result in the complaint of polyuria include nocturia due to prostatic hypertrophy, and a solute (osmotic) diuresis due, for example, to hyperglycemia or relief of urinary tract obstruction. As a general rule, salt wasting nephropathies do not cause sufficient sodium wasting to induce true polyuria. A solute diuresis can be differentiated from diabetes insipidus based upon the following:

- The urine osmolality in a solute diuresis is usually above 300 mOsm/kg, in contrast to the dilute urine in a water diuresis.
- Total solute excretion is normal with a water diuresis (600 to 900 mOsmol/day) but markedly increased with an osmotic diuresis.

Diagnosis. The cause of polyuria (water diuresis) is often suggested from the history (e.g., age of onset, rate of onset, eliciting the possible presence of the different causes of DI, family history), and by the plasma sodium concentration.

Even if the history and/or plasma sodium concentration and urine osmolality appear to be helpful, we recommend confirming the diagnosis in most patients by

- examining the response (urine volume and osmolality) to water restriction
- and, if appropriate, administration of exogenous ADH once the plasma osmolality reaches 295 to 300 mOsmol/kg or the plasma sodium is 145 meq/L or higher. We prefer desmopressin to aqueous vasopressin.

Plasma ADH levels if the response to the water restriction test is equivocal. Urine ADH testing may be performed if high sensitivity plasma ADH assays are not available.

We recommend not performing a water restriction test in patients with dilute urine who are strongly suspected of having nephrogenic DI (e.g., newborns

and very young infants and long-term lithium use in adults). In these settings, testing for a lack of response to desmopressin can be performed without prior water restriction. The response to water restriction and desmopressin helps establish the diagnosis

A submaximal increase in urine osmolality in response to water deprivation (but usually to ≥300 mOsmol/kg), with desmopressin resulting in a rise in urine osmolality of more than 100 percent in complete central DI and 15 to 50 percent in partial central DI.

1. Submaximal rise in urine osmolality in response to water restriction (but to well below 300 mOsmol/kg), with desmopressin producing little or no elevation in urine osmolality in complete nephrogenic DI, and a small (<45 percent) elevation in urine osmolality with partial nephrogenic DI.
2. Primary polydipsia will be associated with a rise in urine osmolality, usually to above 500 mOsmol/kg, and no response to desmopressin since endogenous release is intact.

If the history and water restriction test provide equivocal results, plasma samples collected at baseline and following water deprivation (prior to the administration of ADH) should be sent for measurement of ADH.

1. Nephrogenic DI is excluded if there is an appropriate relationship between the rise in urine osmolality and plasma ADH
2. Central DI is excluded if there is an appropriate rise in plasma ADH with the rise in plasma sodium or osmolality.

Water or Fluid Deprivation Test

1. Start the test at 0800 h. Weigh the patient and check urine and plasma osmolality. If the urine osmolality is less than 750 mOsm/kg and plasma osmolality is less than 300 mOsm/kg start fluid deprivation.
2. Take samples of plasma and urine after every hour to measure osmolality and monitor weight and urine output.
3. Observe the patient for the entire duration of the test, not only to prevent surreptitious drinking but also to be certain that the results are not confused by non-osmotic stimulants of AVP secretion—e.g., smoking, postural hypotension, vaso-vagal reactions, or other episodes of nausea or hypotension.
4. Fluid restriction should be stopped if:
   a. The urine osmolality reaches >600 mOsm/kg with plasma osmolality< 295 mOsm/kg indicating that both ADH release and effect are intact and patient is a case of primary polydipsia.
   b. Plasma osmolality increases to >300 mOsm/kg
   c. These levels indicate a severe dehydration of the patient
   d. Rise in urine osmolality is < 30 mOsm/kg over 3 successive urine samples
5. Now administer DDAVP 20 µg intra-nasally or 2 µg IM. Patient may now eat and drink freely but not more than twice the urine volume he passed during water deprivation to avoid acute dilutional hyponatraemia.
6. Continue to measure hourly urine volumes and take samples for osmolality from each hourly sample.
7. Weight of the patient must also be determined at every hour.

Dose

Subcutaneous injection: 5 units of aqueous vasopressin

Intranasal spray:

Adults:4 puffs

Children: 2 puffs

Method

Urine and Plasma osmolality is measured by osmometry.

Interpretation

The test should be terminated if the urine osmolality rises to750 mOsm/kg.

Post fluid restriction		Post DDAVP (Desmopressin)	Diagnosis
Plasma osmolality	Urine osmolality	% increase in Urine osmolality	
283 – 293	$\geq$ 800	-	Normal
<293	>600	-	Primary polydipsia
>293	< 300	100%	Complete Central DI
>293	300 - 600	50%	Partial central DI
>293	<300	0%	Complete nephrogenic DI
>293	<300	15-50%	Partial nephrogenic DI

Variables:

Interpretation is not always this simple. Anomalous results may occur in a number of situations. The following are the most common.

a. Patient surreptitiously drinks water during the test.
b. The bladder is not completely emptied at each hourly interval.
c. A nephrogenic diabetes insipidus response may occur in compulsive water drinkers because the medullary solutes have been washed out and the osmolar gradient across the collecting duct wall is defective.
d. For intranasal spray, nasal mucosa & dose transferred by spray may also vary.

Contraindications for this Test: Do not perform the water deprivation test in patients with renal insufficiency, uncontrolled diabetes mellitus, and hypovolemia of any cause or uncorrected deficiency of adrenal or thyroid hormone.The test is not required if there is evidence for the ability to concentrate urine e.g. spot urine osmolality > 750 mmol/kg.

PSCP Guidelines 14/2015

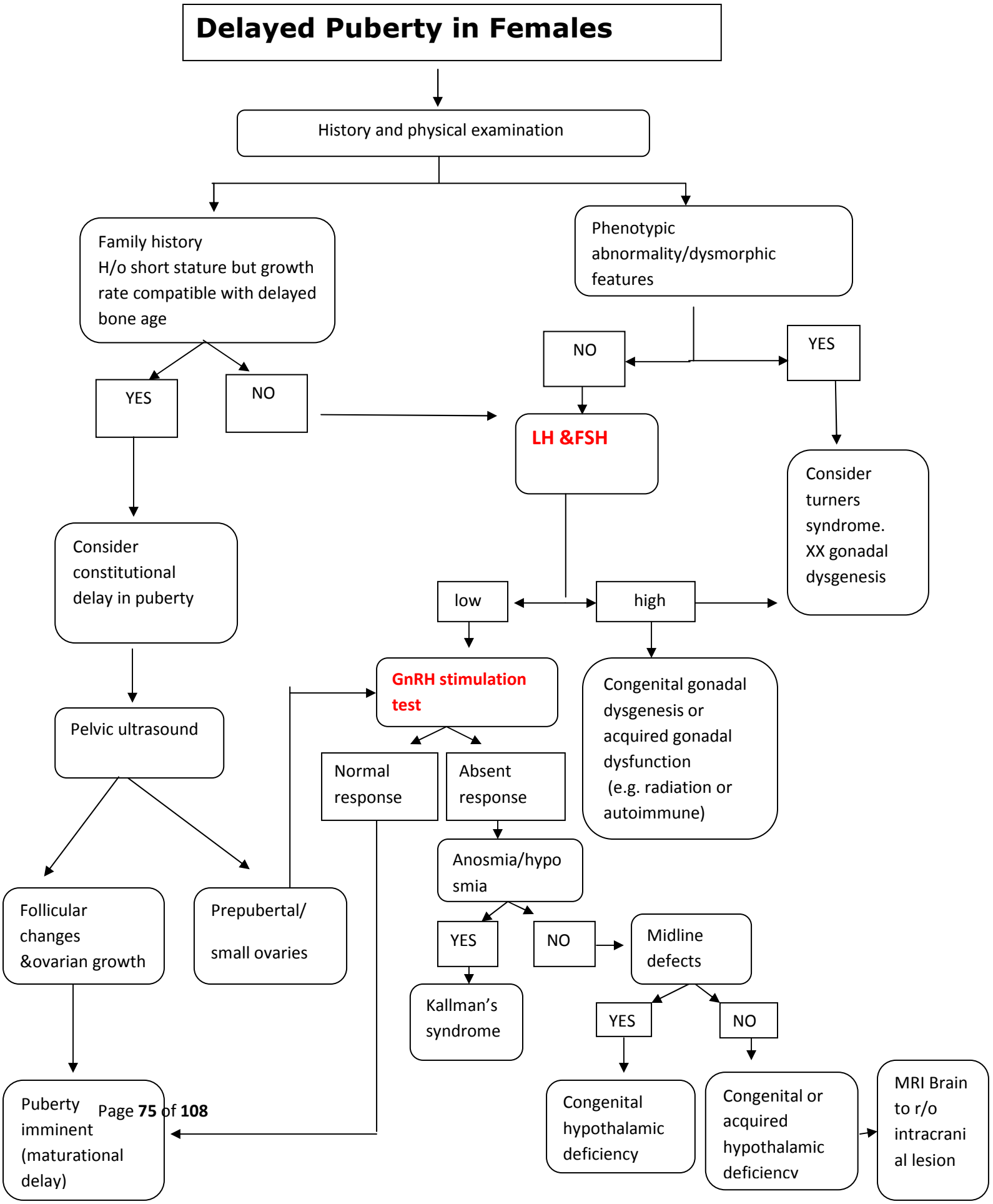
Delayed Puberty

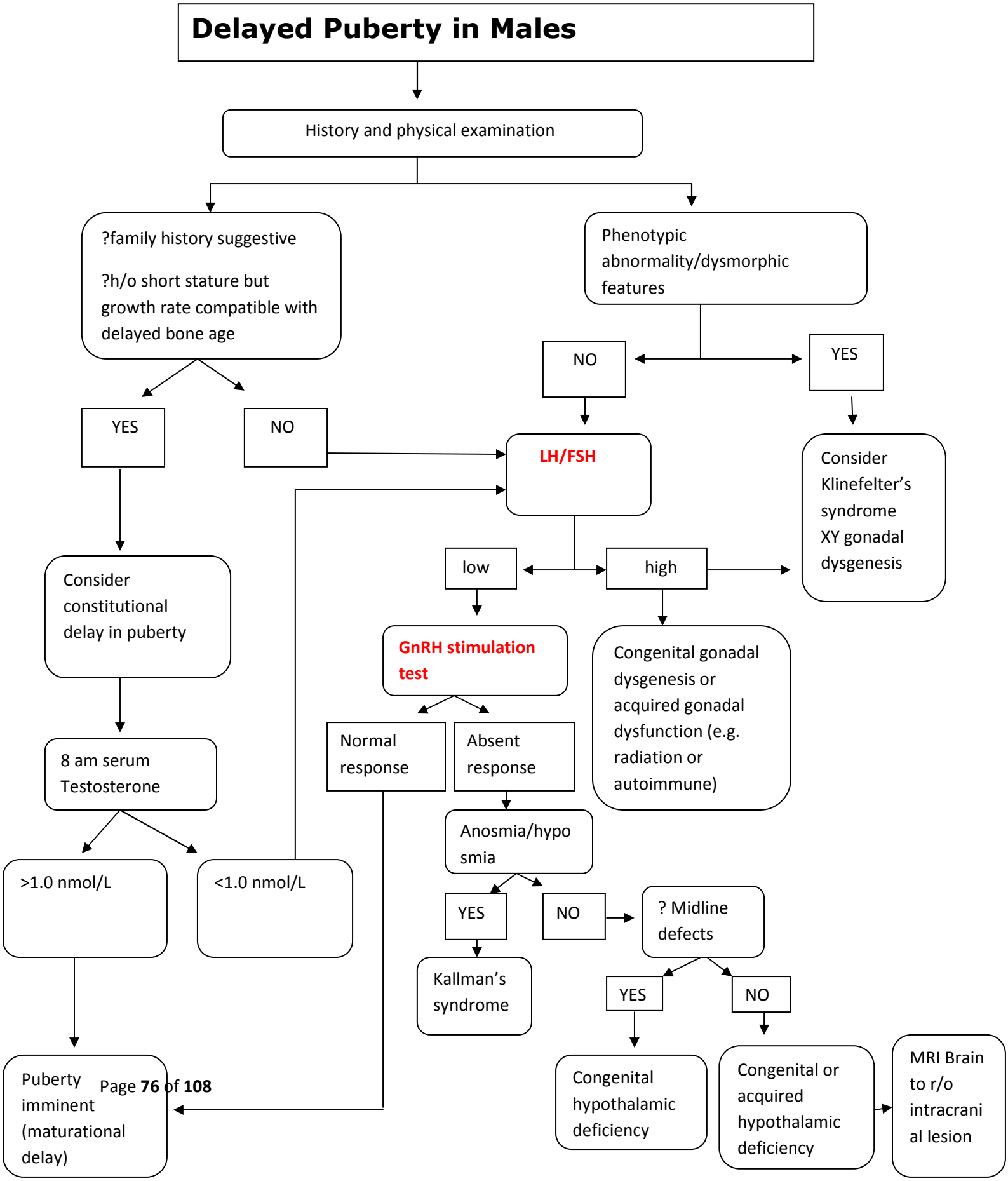
Definition:A commonly accepted definition of delayed puberty is the absence of secondary sexual development at an age 2 to2.5 SD later than the population mean-an age at which 95 percent of normal children have already entered puberty (traditionally, 14 years in boys and 13 years in girls).

Evaluation Protocol:

1. **Clinical History**:A detailed history including family history of delayed puberty and any systemic illness in the patient should be taken. Emphasis should be given on specific disorders (e.g., celiac disease, thyroid disease, and anorexia) that may cause temporary delay of puberty (functional hypogonadotropic hypogonadism), as well as medication use, nutritional status, and psychosocial functioning.
2. **Physical examination**: It should include height, weight, /phenotypic anomaly, any stigmata of a systemic illness, any dysmorphic features, presence of secondary sexual characteristics, upper to lower segment ratio, breast and areola size and stage in females and testicular and penile size in males and pubic hair staging must be determined and recorded.
3. **Exclude Constitutional Delay:** It is one of the commonest cause of delayed sexual development other common causes such as systemic or chronic illnesses need to be ruled out. Children with constitutional delay have slow physiological development reflected by delayed bone age. They are usually thinner and shorter than average (third to fifth percentile) but grow at a normal rate for their bone age.
4. **Determination of gonadotropin levels:**After a thorough clinical history and a detailed physical examination and in the absence of physical characteristics of syndromes, the first step is to determine the gonadotrophin levels. If the FSH/LH levels are low the differential diagnosis is between constitutional delay in puberty or hypothalamic GnRH or pituitary LH/FSH deficiency.

5. **GnRH stimulation test**: It is the next step in case of low gonadotropin levels.
    a. A normal response indicates endocrine onset of puberty in a patient with constitutional delay who is on the verge of physical development. (Also in a female a pelvic ultrasound which will show follicular changes and ovarian growth and in a male A 08:00 serum testosterone concenteration will be >1.0nmol/L if puberty is imminent).
    b. **Hypogonadotrophic hypogonadism** :Absent response may be seen in situations termed as "hypogonadotropic hypogonadism" such as idiopathic isolated gonadotropin deficiency,combined genetic syndromes such as Kallman's syndrome, hypothalamic lesions like intracranial tumours. Eating disorders may significantly delay the onset of puberty and extreme maturational delay can occur.
    c. **Hypergonadotropic hypogonadism:**If the initial FSH/LH levels are raised,"hypergonadotropic hypogonadism" they indicate primary ovarian failure or testicular failure such as seen in congenital gonadal dysgenesis in conditions like turner's syndrome,Noonan's syndrome, XX/XY gonadal dysgenesis, or acquired gonadal dysfunction(for example radiation or autoimmune mediated).


Delayed Puberty in Females
History and physical examination
Family history
H/o short stature but growth rate compatible with delayed bone age
Phenotypic abnormality/dysmorphic features
YES
NO
NO
YES
LH &FSH
Consider turners syndrome. XX gonadal dysgenesis
Consider constitutional delay in puberty
low
high
GnRH stimulation test
Congenital gonadal dysgenesis or acquired gonadal dysfunction (e.g. radiation or autoimmune)
Pelvic ultrasound
Normal response
Absent response
Anosmia/hypo smia
Follicular changes &ovarian growth
Prepubertal/ small ovaries
YES
NO
Midline defects
Kallman's syndrome
YES
NO
Puberty imminent (maturational delay)
Congenital hypothalamic deficiency
Congenital or acquired hypothalamic deficiencv
MRI Brain to r/o intracrani al lesion


Delayed Puberty in Males
History and physical examination
?family history suggestive
?h/o short stature but growth rate compatible with delayed bone age
Phenotypic abnormality/dysmorphic features
NO
YES
YES
NO
YES
NO
LH/FSH
Consider Klinefelter's syndrome XY gonadal dysgenesis
Consider constitutional delay in puberty
low
high
GnRH stimulation test
Congenital gonadal dysgenesis or acquired gonadal dysfunction (e.g. radiation or autoimmune)
8 am serum Testosterone
Normal response
Absent response
Anosmia/hypo smia
>1.0 nmol/L
<1.0 nmol/L
YES
NO
? Midline defects
Kallman's syndrome
YES
NO
Puberty imminent (maturational delay)
Congenital hypothalamic deficiency
Congenital or acquired hypothalamic deficiency
MRI Brain to r/o intracrani al lesion

PSCP Guidelines 15/2015

Precocious Puberty

Introduction: Sexual precocity is the general term for early puberty. Isosexual precocity refers to a girl who feminizes or a boy who virilizes early. Central precocious puberty or true precocious puberty is a term reserved for children with gonadotropin-releasing hormone (GnRH)-dependent early puberty that follows the normal pubertal pattern and the normal control mechanisms through GnRH. The only difference from normal puberty is the earlier age at onset. Central precocious puberty can be idiopathic or caused by organic conditions such as a brain tumor or a hamartoma of the tuber cinereum. GnRH-independent isosexual precocity is caused by excessive estrogen secretion in girls or androgen secretion in boys from sources other than the GnRH-gonadotropin axis (such as the gonads, the adrenal glands, ectopic human chorionic gonadotropin (hCG) secretion, and exogenous sources of sex steroids). Gonadotropins are suppressed by negative feedback in all forms of GnRH-independent isosexual precocity because sex steroid secretion is autonomous. Contrasexual (or heterosexual) precocity refers to girls who virilize and boys who feminize.

Onset of puberty among Caucasian girls as early as 7 years of age or among African American girls as early as 6 years of age can be considered normal if there are no neurologic symptoms or signs of increased intracranial pressure, if there is not a rapid advancement in pubertal development or bone age, and if menses is after at least age 9 years. If there is a family history of a pattern of early pubertal development, the child is even more likely to have a variant of normal puberty rather than a pathologic condition causing sexual precocity. Among boys the earliest limit of normal puberty is 9 years of age. Onset earlier than these limits is considered sexual precocity

Assessment / Evaluation of Precocious Puberty

The aim is to exclude a life-threatening disease and identify the need for urgent management to prevent deleterious effect on growth, reproduction and behavior. The key questions to be addressed include the following:

Is this precocious puberty?Following points should be kept in mind while selecting patient for lab investigations:

1. Confirmation of precocious puberty is mandatory to avoid unnecessary investigations and treatment. A significant proportion of children presenting with concerns of early pubertal development represent physiological variations that do not require treatment.
2. The differentiation of lipomastia from thelarche is particularly important in obese girls.
3. Inspection of vaginal mucosa is a reliable indicator of estrogenic status, with red, glistening mucosa suggesting pre-pubertal state and pale mucosa indicating estrogen exposure.
4. Bone age and uterine ultrasound are vital in confirming the progressive nature of precocious puberty. Tubular uterus with no visible endometrial stripe is suggestive of pre-pubertal state, while pubertal state is characterized by pear-shaped structure and endometrial thickness greater than 3 mm.
5. Estradiol levels above 10 pmol/L and testosterone levels in the pubertal range are indicative of pubertal development in boys and girls, respectively.

Is this complete or incomplete precocious puberty?

1. This is particularly relevant in girls as incomplete variants are common. **Isolated thelarche** is characterized by normal growth, isolated FSH elevation with prepubertal LH levels, age-appropriate skeletal maturation and small ovarian cysts on ultrasound.
2. Onset before 3 years of age is frequently associated with regression over 1-3 years. Later onset usually represents slowly progressive form of precocious puberty.
3. **Isolated pubarche** is a benign condition requiring no treatment. The condition, however, needs to be differentiated from other causes of androgen excess, including non-classical congenital adrenal hyperplasia and androgen producing adrenal or ovarian tumors.
4. **Isolated vaginal bleeding** without significant breast development is unlikely to be due to an endocrine cause and should prompt evaluation for local pathology including infection, foreign body, abuse and rarely tumors.

Is this gonadotropin-dependent or -independent precocious puberty?

1. Testicular volume is the most important indicator for etiology of precocious puberty in boys. Boys with gonadotropin-dependent precocious puberty have pubertal testicular volume (more than 4 mL),

while pre-pubertal testicular volume is characteristic of GIPP. Boys with isolated "apparent LH excess" [human chorionic gonadotropin (HCG) secreting tumor, GIPP] have smaller testes for the same pubertal status compared to those with gonadotropin-dependent precocious puberty.

2. Discordant pubertal development (vaginal bleeding within 1 year of breast development) indicates hyperestrogenic state due to ovarian cysts, McCune Albright syndrome or hypothyroidism.
3. **Basal Gonadotropin Levels:**GnRH-stimulated gonadotropin level remains the gold standard for differentiating gonadotropin-dependent and -independent precocious puberty. The development of third-generation assays for gonadotropin levels has prompted the use of basal gonadotropin levels in diagnosing gonadotropin-dependent precocious puberty. LH is a better indicator of pubertal status compared to FSH as it shows greater increase during puberty. ***Basal LH of more than 0.6 IU/L and LH to FSH ratio of more than 1 are suggestive of gonadotropin-dependent precocious puberty***. Recently, basal LH levels greater than 0.1 IU/L were shown to have sensitivity of 94% and specificity of 88% for gonadotropin dependent precocious puberty. The specificity was increased to 100% using a cutoff of 0.3 IU/L although at the cost of lower sensitivity.
4. **GnRH Stimulation Test**is required if baseline gonadotropin levels are inconclusive. Different protocols are available for the test measuring 2-7 samples after injection of intravenous or subcutaneous GnRH (100 g). Pubertal LH levels (>5 U/L) and LH to FSH ratio of more than 0.9 are diagnostic of central precocious puberty. Blunted response is pathognomonic of peripheral precocious puberty. The difficulties in procuring GnRH have led to the development of GnRH agonist test in the assessment of pubertal disorders. Recently, the test has been found to have good diagnostic accuracy with the use of single sample after administration of GnRH agonist, Triptorelin (100 µg subcutaneously). The role of allopregnenolone and kisspeptin as markers of gonadotropin-dependent precocious puberty remains speculative at the moment.

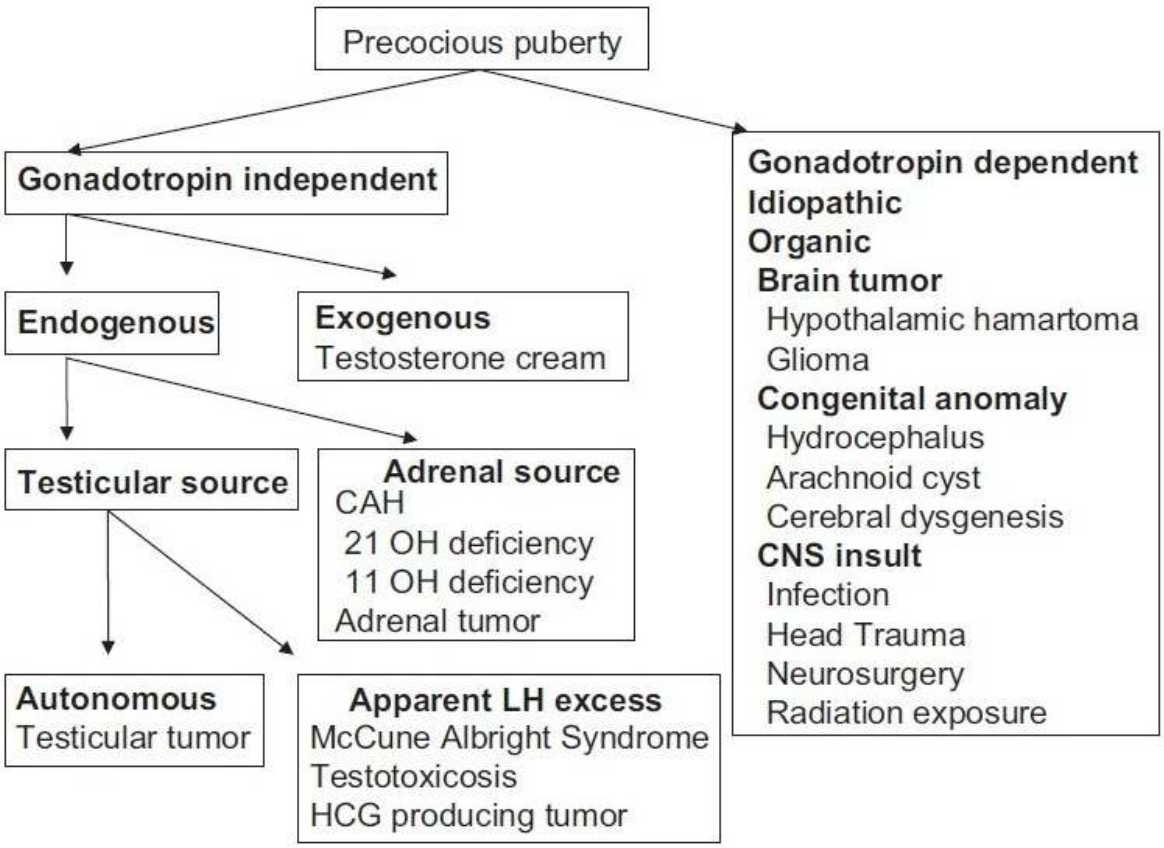
<u>Is there a serious underlying cause for precocious puberty?</u>

1. The main aim of evaluation of gonadotropin-dependent precocious puberty is the identification of an underlying organic etiology.
2. High resolution magnetic resonance imaging **(MRI)** of the hypothalamic-pituitary region is desirable; however, computerized tomography scan may be considered if MRI is not feasible. Currently,

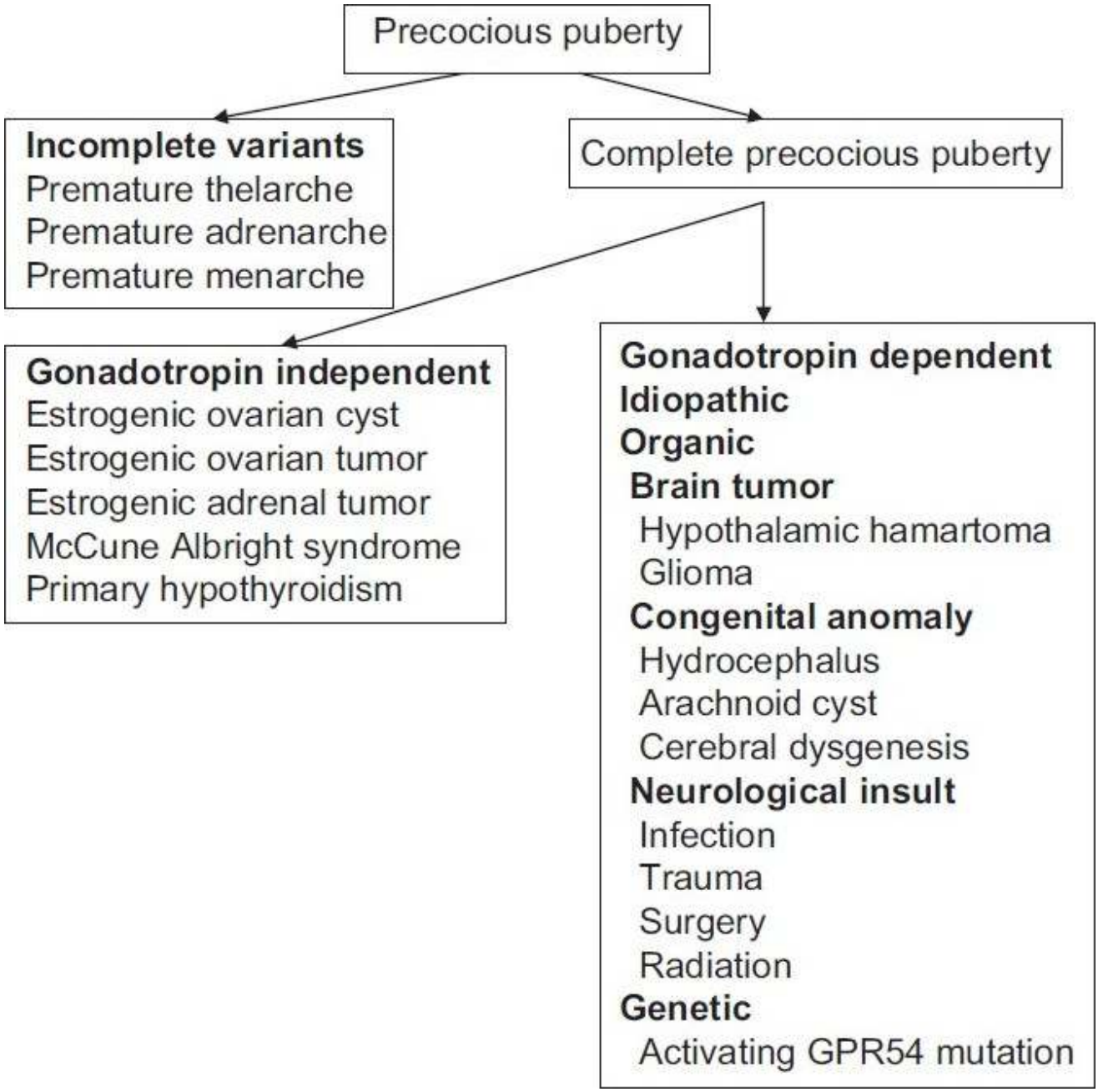
CNS imaging in central precocious puberty (CPP) is recommended in girls with the onset of pubertal changes before the age of 6 years. Studies have, however, indicated that neurogenic etiology may be present in girls with pubertal onset, between 6 and 8 years of age. The need for CNS imaging should therefore be individualized according to the age at onset, rate of progression and neurological features. CNS imaging is mandatory in boys with CPP where the likelihood of organic pathology is very high.

3. Thyroid profile and ovarian and adrenal imaging should be done in girls with GIPP. In boys with pre-pubertal LH levels, imaging for adrenals and estimation of 17 hydroxyprogesterone (17-OHP) and 11 deoxycortisol (11-OHDOC) should be done. Blood HCG levels should be estimated if these investigations are non-contributory. Testotoxicosis should be considered in boys presenting with peripheral precocity at an early age after exclusion of adrenal pathology or HCG secreting tumor.

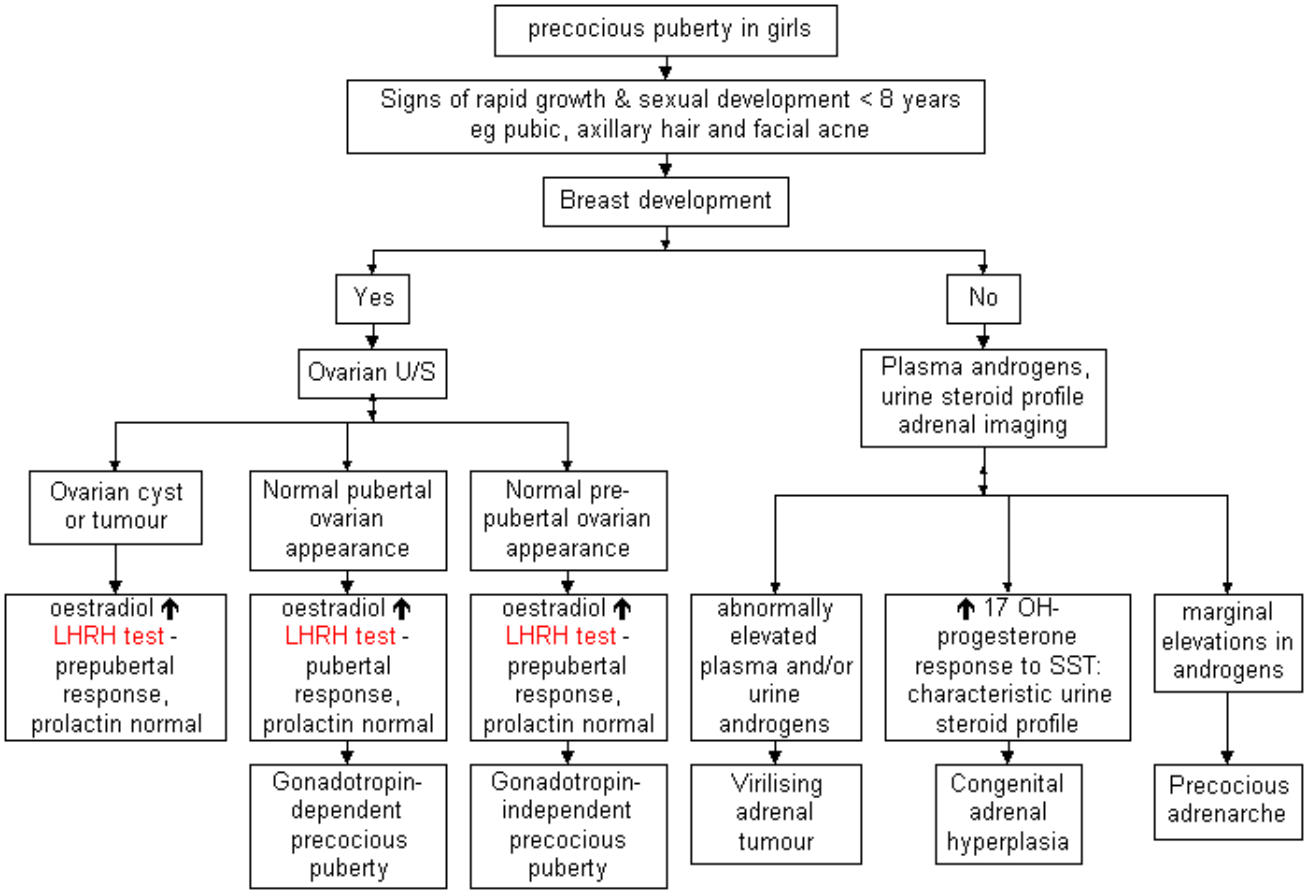
Etiology of Precocious Puberty in Boys



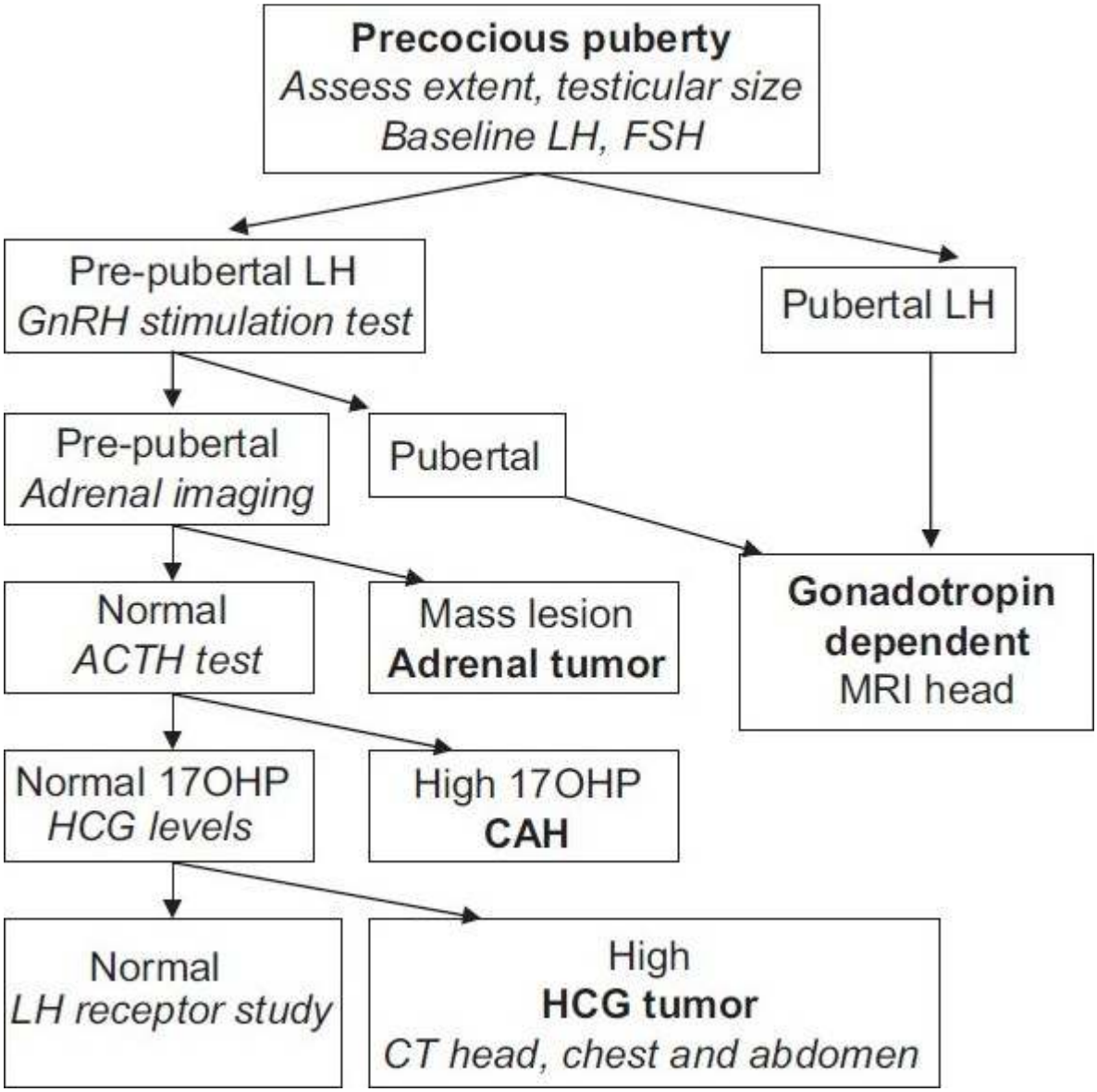
Etiology of Precocious Puberty in Girls



Approach to a Girl with Precocious Puberty



Approach to a Boy with Precocious Puberty



GnRH Stimulation Test

Indication: To diagnose hypothalamic pituitary disease in precocious and delayed puberty in both sexes in children with low basal gonadotropins concentrations.

Principle: GnRH (gonadotropin releasing hormone) is a decapeptide secreted by the hypothalamus which stimulates the production and secretion of LH and FSH by anterior pituitary.

Side Effects: GnRH May Rarely Cause Nausea, Headache And Abdominal Pain.

Preparation: No Specific Patient preparation is required.

Requirement:

1. Plain tubes.
2. 100ug GnRH preparation. The dose for children is 2.5 µg/kg body weight to a maximum of 100ug (0.1mg).

Procedure:

1. 0 min: Take 3ml blood for LH and FSH. Immediately give GnRH i.v. as a bolus(dose as above)
2. 30 min: Take 3ml blood for LH and FSH.
3. 60 min: Take 3ml blood for LH and FSH

Interpretation:

In delayed Puberty:

In women: The normal response varies in relation to menstrual cycle.

1. In Follicular Phase; LH peak should be increased at least 2 fold over baseline or a net change of at least 10 IU/L and FSH peak should be at least 1.5 fold over baseline or a net change of at least 2 IU/L.
2. In luteal phase; LH peak should be increased at least 8 fold over baseline or a net change of 20 IU/L and FSH should be increased at least 1.5 fold over baseline or a net change of 2 IU/L.

In men: The response may be considered normal if the basal values are in the reference range and there is at least a doubling at 30 min for both FSH/LH

(An exaggerated response in seen in primary and secondary gonadal failure)

HypogonadotropicHypogonadism:

1. Increase in Serum Gonadotropins> 10 mIU/ml over baseline is normal.
2. Little to no increase in gonadotrophic pituitary disease is likely.

In Central Precocious Puberty:

LH response will be greater than FSH response with absolute value of > 5 U/L. FSH response will be greater than LH response with value of > 5 U/L. The levels of FSH, LH would be more at 30 minutes as compared to 60 minutes.

In Peripheral Precocious Puberty:

In patients with precocious puberty who failed to respond, indicate peripheral precocious puberty.

PSCP Guidelines 16/2015

Infertility

Infertility is the failure to conceive (regardless of cause) after 1 year of unprotected intercourse. This condition affects approximately 10-15% of reproductive-aged couples

History and Physical Examination;

Workup should begin with a detailed history of the couple and should address the following points;

1. Medical records /review of systems to identify any endocrinologic or immunologic issue that may be associated with infertility. Female partner's menstrual history as well as history of weight changes, hirsutism, frontal balding and acne , Male partner's medical history, including previous semen analysis results, history of impotence any previous pregnancy in female partners/ existence of offspring from previous female partners.
2. The type of infertility (primary or secondary) and its duration
3. Previous pregnancies and their outcomes
4. Previous infertility evaluation/treatment
5. Couple's history of sexually transmitted diseases (STDs); surgical contraception (e.g., vasectomy, tubal ligation); lifestyle; consumption of alcohol, tobacco, and recreational drugs (amount and frequency); occupation; and physical activities

Examination for infertility should include the following

1. Records of blood pressure, pulse rate, temperature & Height/weight findings to calculate body mass index
2. Evidence of systemic illnesses/ venereal diseases/chromosomal anomalies/ previous surgeries
3. Assessment of secondary sex characteristics, their degree and stage of development in both partners

Diagnostic Tests:

Male Patient

Semen Analysis

1. Semen analysis is one of the most important tests conducted in the workup of the infertile couple. Studies show that approximately 47% of couples with infertility have a male factor component.
2. It is impossible to characterize a man's semen quality from evaluation of a single semen sample. It is therefore helpful to examine two or three samples to obtain baseline data.
3. Sperm cells in the semen sample collected are evaluated with respect to their count, morphology, motility etc. A total sperm count of > 39M sperms /ejaculate, over 4% of which have normal morphology following strict criteria & 32% having rapid progression within 60 min of ejaculation is considered normal.
4. **WHO Lower Reference Limits:** 5^{th} percentile (lower reference limits and 95% confidence intervals in parentheses), derived from a study of over 1900 men whose partners had a time-to-pregnancy of ≤12 months.
    a. Volume: 1.5 mL (95% CI 1.4-1.7)
    b. Sperm concentration: 15 million spermatozoa/mL (95% CI 12-16)
    c. Total sperm number:39 million spermatozoa per ejaculate (95% CI 33-46)
    d. Morphology: 4 per cent normal forms (95% CI 3-4), using "strict" Tygerberg method
    e. Vitality: 58 per cent live (95% CI 55-63)
    f. Motility:
        (1) Progressive motility — 32 per cent (95% CI 31-34)
        (2) Total (progressive + non-progressive motility) — 40 per cent (95% CI 38-42)

Endocrine Evaluation

Serum Testosterone &Gonadotropins(FSH, LH)

1. A low serum Te with a concomitant low/normal gonadotropins is considered compatible with HypogonadotropicHypogonadism. A pituitary or hypothalamic cause can be differentiated by carrying out **GnRH stimulation test**.
2. A low serum Te with an associated high level of gonadotropinsis consistent with Hypergonadotropic Hypogonadism. This is suggestive of primary testicular disease /leydig cell dysfunction. Leydig cell reserve is evaluated in such cases by **CG stimulation**. Males with hypogonadism have decreased Te response to this test.

3. Elevated serum Te with low/normal gonadotropins may result from adrenal or testicular tumors which require imaging & localization investigations.
4. An elevated Te &Gonadotropin levels can be due to Androgen insensitivity (Partial /Complete) or hCG secreting tumours. β-hCG levels can help differentiate the two conditions.

Antimullerian Hormone

AMH measurements are commonly used to evaluate testicular presence and function in infants with intersex conditions or ambiguous genitalia, and to distinguish between cryptorchidism (testicles present but not palpable) and anorchia (testicles absent) in males.

Serum Estradiol

Evidence of feminization in an infertile male signifies high concentration of Estrogens. Certain adrenal or testicular tumors can produce high levels of serum estrogens. Imaging studies are helpful in such cases to assist localization. Exogenous estrogen administration may also be considered.

Serum Prolactin

Hyperprolactinaemia causes infertility by inhibiting GnRH secretion. The treatment consists of identification and elimination of the cause.

Karyotyping

Patient's karyotype is indicated when a chromosomal anomaly is suspected e.g., 47 XXY or Klinefelter's syndrome.

Female Patient

It is useful to divide female patients with infertility into those with and without menstrual problems.

Assessment of Ovulatory Function

1. A progesterone level drawn on day 21 or approximately seven days after the date of suspected ovulation can be an indicator of ovulatory function and used to evaluate strategies relating to fertility treatment. A level greater than 30 nmol/L usually indicates that ovulation has occurred and that adequate progesterone is being made to support an early pregnancy.
2. Patients having inadequate progesterone secretion after ovulation indicate luteal phase defect.

Assessment of endocrine parameters

Gonadotropins(FSH, LH) with Ovarian Hormones

The most commonly used tests to assess the ovarian function/reserves are the basal Gonadotropins (FSH, LH) with ovarian hormones. An elevated level of serum FSH &LH with inappropriately normal /low ovarian hormones, estradiol /progesterone indicates primary ovarian failure. On the other hand a low normal serum Gonadotropins with low ovarian hormones indicates Hypogonadotropic hypogonadism .This suggests pituitary or hypothalamic cause that can be identified by GnRH test.

Anti-müllerian Hormone (AMH)

Because of the gender differences in AMH concentrations, its changes in circulating concentrations with sexual development, and its specificity for granulosa cells, measurement of AMH has utility in the assessment of gonadal function, fertility, and as a gonadal tumor marker. Since AMH is produced continuously in the granulosa cells of small follicles during the menstrual cycle, it is superior to the episodically released gonadotropins and ovarian steroids as a marker of ovarian reserve.

Androgens

Infertility in androgen excess, including PCOS and other similar disorders, generally results from the lack of regular ovulation. (Women with PCOS do not ovulate in a normal fashion and as a result their endometrium is not developed properly). However, other mechanisms may also play a role.Patients with non-classic adrenal hyperplasia often have very elevated levels of progesterone and/or 17-hydroxyprogesterone, which may lead to an excessively thin endometrium lining, making it difficult for the pregnancy to implant (adhere to the endometrium) and grow.

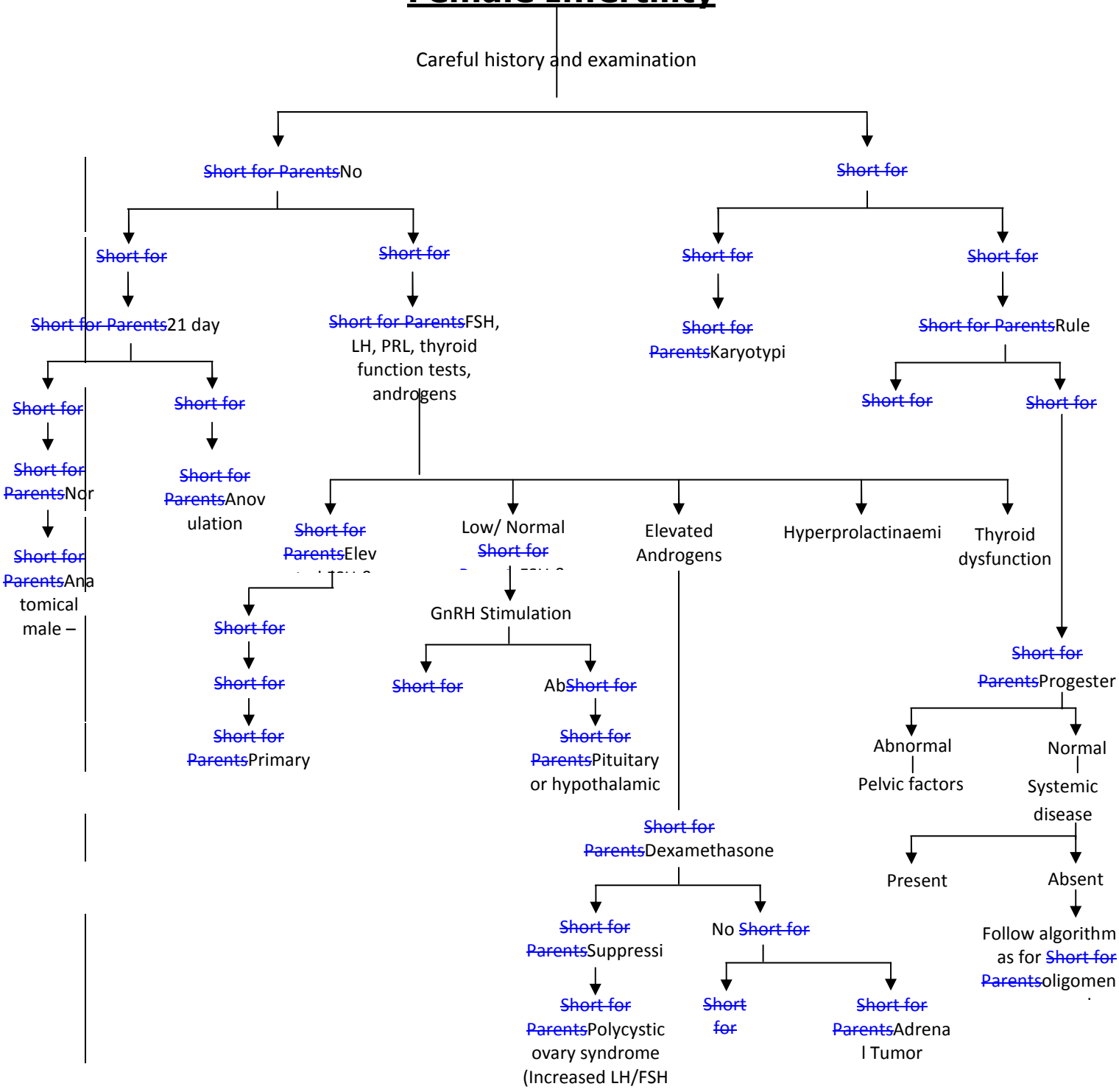
Imaging studies

These are conducted to rule out any mechanical causes that can be a cause for infertility. Hysterosalpingogram and assessment of fallopian tube patency.

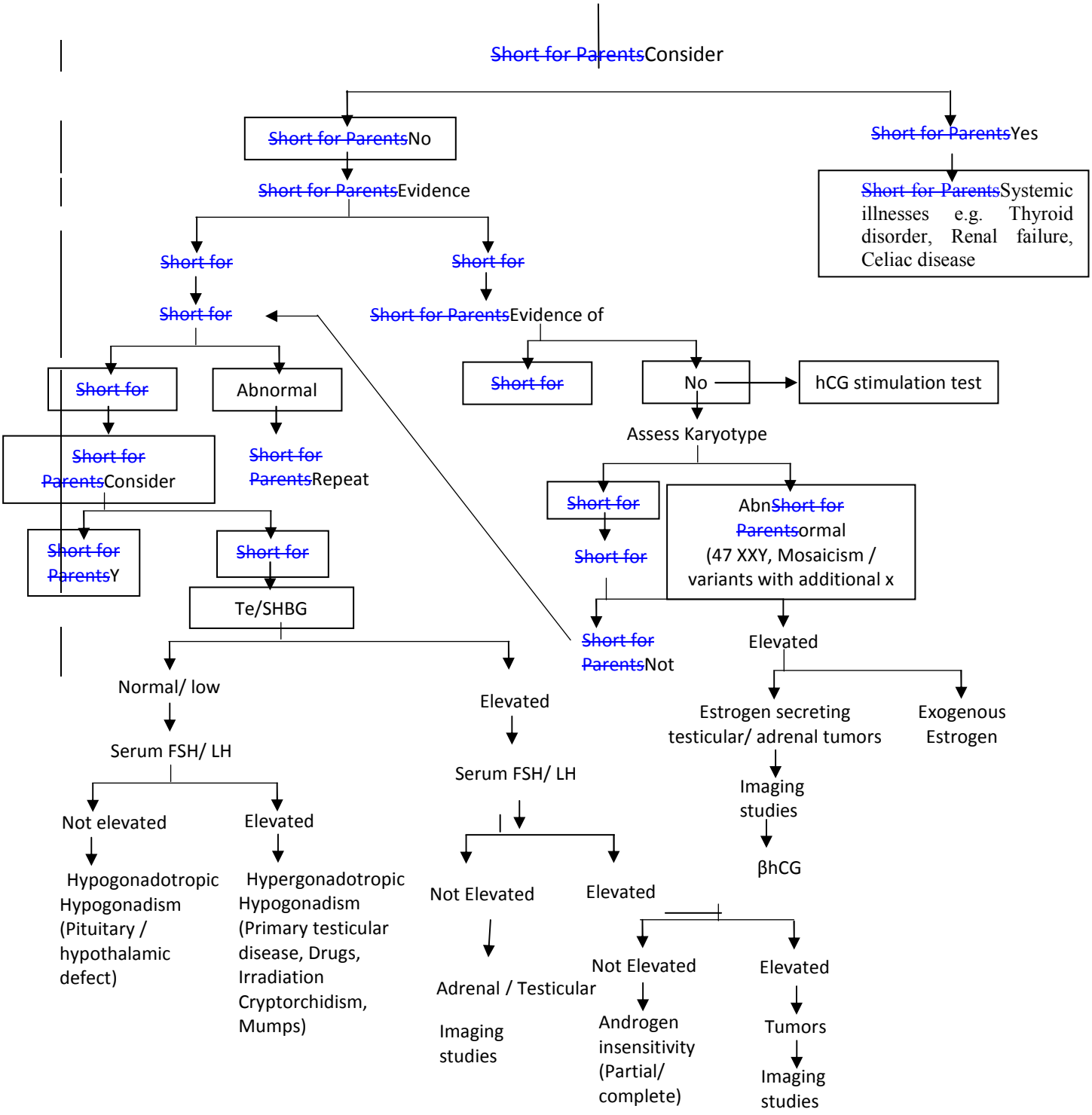
Ruling out Infectious Agents

Among the most common microorganisms involved in sexually transmitted infections, interfering with female fertility, there are Chlamydia trachomatis and Neisseriagonorrhoea. C. trachomatis is considered the most important cause of tubal lacerations and obstruction, pelvic inflammatory disease (PID) and adhesions. N.gonorrhoea, even though its overall incidence seems to decline, is still to be considered in the same sense, while bacterial vaginosis should not be ignored, as causative agents can produce ascending infections of the female genital tract.

Female Infertility



Male Infertility



PSCP Guidelines 17/2015

Hirsutism

Background: Hirsutism is a common disorder affecting up to 8 percent of women

Definition: Hirsutism is commonly defined as *androgen* mediated hair growth in a woman on her body or faces which manifests in same temporal development as males and in the same pattern. Hirsutism is one of the most common endocrine disorders and differs significantly from hypertrichosis which is a uniform growth of hair over the entire body.

Role of laboratory evaluation: The diagnosis of hirsutism is usually a clinical one and practically speaking the main usefulness for laboratory hormone testing in hirsutism is to help differentiate benign causes from tumours (occult ovarian or adrenal neoplasm) or other conditions such as polycystic ovary syndrome, late-onset adrenal hyperplasia, and Cushing's syndrome.

Causes:

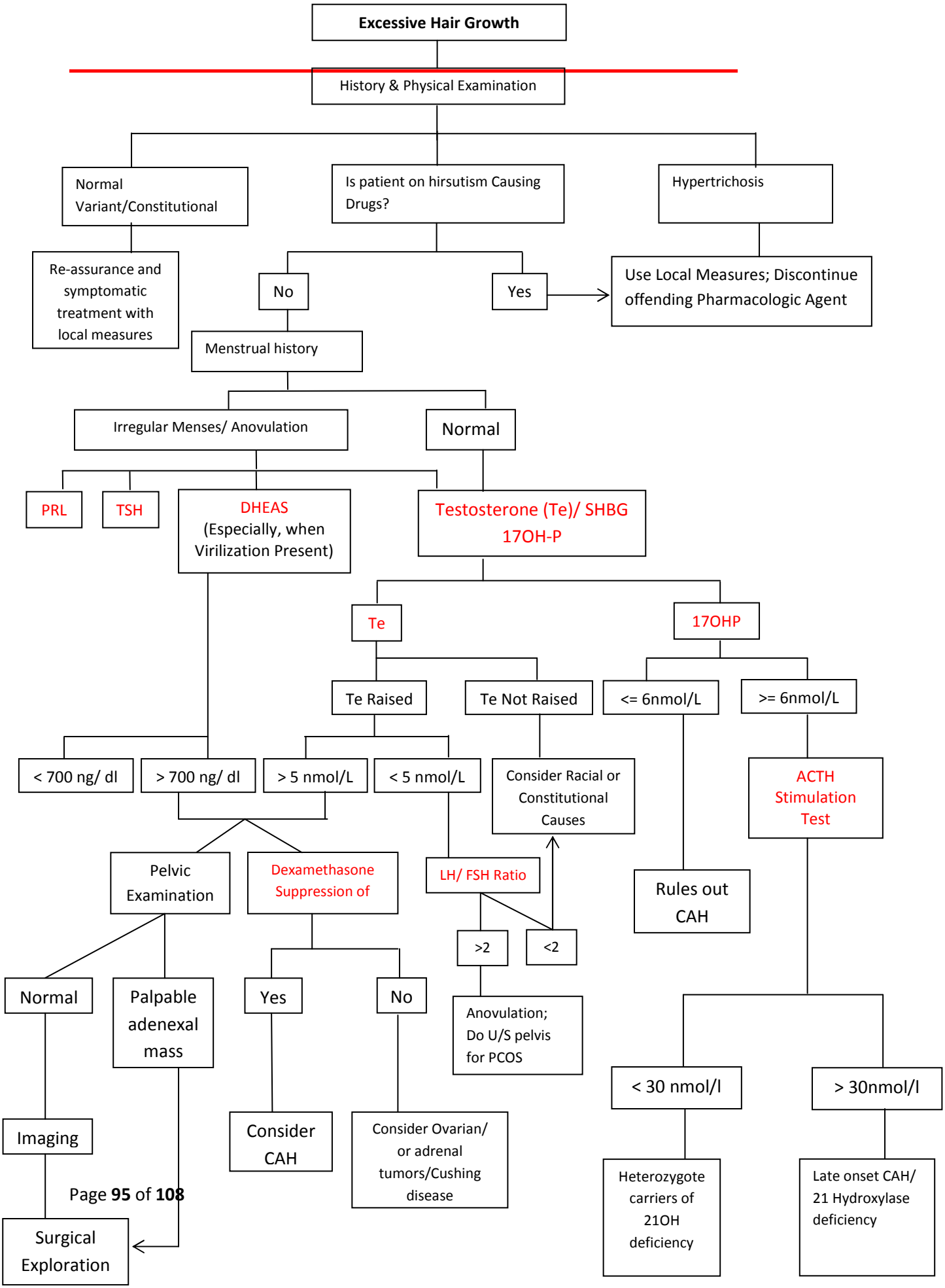
1. **Combined adrenal and ovarian**(Idiopathic hirsutism;PCOS)
2. **Adrenal** (CAH,Cushing's disease,Androgen secreting adrenal tumors)
3. **Ovarian Causes**(Androgen secreting ovarian tumours,Insulin resistance syndromes)
4. **Exogenous Androgens**(Anabolic steroids,Danazol,Norgesterolcontaining oral contraceptives)

Investigation Protocol: A thorough history and physical examination are essential to evaluate women with hirsutism to determine which patients need additional diagnostic testing

1. **Clinical history**: It should include history of onset and rapidity of development of hirsutism, growth/sexual development, menstrual, fertility, family history and history of intake of drugs like anabolic steroids, phenytoin, danazol, and oral contraceptives containing levonorgestrel etc.
2. **Physical examination**: Evaluation of BMI/fat distribution, blood pressure estimation, assessment of hair growth (Ferriman Gallway score), acne, features of virilization (Clitoromegaly, increased muscle mass). At the same time one should also look for features of other

endocrine disorders like cushingoid features,Galactorrhoea, acanthosis nigricans and skin tags etc.

3. **Exclude Constitutional causes**: Idiopathic/constitutional cause is a diagnosis of exclusion and such patients generally have normal menses and normal levels of testosterone, 17α-hydroxyprogesterone (17-OHP), and Dehydroepiandrosterone sulfate (DHEAS). They require reassurance and symptomatic treatment with local measures.
4. **Hormone levels**: For diagnostic purposes, serum levels of testosterone, SHBG and 17-OHP are usually sufficient. For patients with irregular menses, anovulation and idiopathic hirsutism, prolactin levels and thyroid function tests may be considered. Hirsutism outside of the perimenarchal period, rapid progression of hirsutism, or signs of Cushing's syndrome or virilization should indicate the possibility of an ovarian or adrenal neoplasm. Diagnostic testing should examining levels of serum DHEAS as well in such cases.
5. A serum testosterone of >7nmol/L and DHEAS >700 µg/dl should be thoroughly investigated to see if it is suppressible by dexamethasone.
6. **Dexamethasone suppression test:** Adrenal suppression is carried out with dexamethasone 0.5mg orally 6 hourly for 5 days with a repeat DHEAS measurement performed on 5^{th} day. If DHEAS is suppressed to <170ug/dl an adrenal tumour is excluded and diagnosis of adrenal hyperplasia is made. Such high levels or rapid virilization also indicate the need for pelvic examination and ultrasound. If pelvic ultrasound does not disclose an ovarian mass, MRI or CT Scan of the ovaries and CT Scan of the adrenals are indicated.
7. **ACTH Stimulation test**: The most common cause of adrenal hyperplasia is 21 hydroxylase deficiency(complete or partial). If 17OHP ie elevated (>6 nmol/L), further evaluation requires an ACTH stimulation test. A post stimulation value of >30 nmol/L indicates late onset CAH.
8. PCOS is a relatively common cause of hirsutism. Serum testosterone is often elevated and is usually suppressible by dexamethasone, with an increased LH/FSH ratio (>2) and also moderatehyperprolactinaemia. Pelvic ultrasound should be carried out to aid in confirmation of the diagnosis.


Excessive Hair Growth
History & Physical Examination
Normal Variant/Constitutional
Is patient on hirsutism Causing Drugs?
Hypertrichosis
Re-assurance and symptomatic treatment with local measures
No
Yes
Use Local Measures; Discontinue offending Pharmacologic Agent
Menstrual history
Irregular Menses/ Anovulation
Normal
PRL
TSH
DHEAS (Especially, when Virilization Present)
Testosterone (Te)/ SHBG 17OH-P
Te
17OHP
Te Raised
Te Not Raised
<= 6nmol/L
>= 6nmol/L
< 700 ng/ dl
> 700 ng/ dl
> 5 nmol/L
< 5 nmol/L
Consider Racial or Constitutional Causes
ACTH Stimulation Test
Pelvic Examination
Dexamethasone Suppression of
LH/ FSH Ratio
Rules out CAH
>2
<2
Normal
Palpable adenexal mass
Yes
No
Anovulation; Do U/S pelvis for PCOS
< 30 nmol/l
> 30nmol/l
Imaging
Consider CAH
Consider Ovarian/ or adrenal tumors/Cushing disease
Heterozygote carriers of 21OH deficiency
Late onset CAH/ 21 Hydroxylase deficiency
Surgical Exploration

PSCP Guidelines 18/2015

Polycystic Ovary Syndrome (PCOS)

Background: Polycystic Ovary Syndrome (PCOS) affects 1-8% of women of reproductive age group and is the most common endocrinopathy and the commonest cause of anovulatory infertility in this age group. Advances in medical literature have carried PCOS beyond the realm of gynecology and infertility, and focuses on its long-term metabolic health implications.

Clinical Features: PCOS is characterized by myriad of symptoms and signs that include

1. Menstrual Disturbance,(Oligo/Amenorrhea),
2. Hyperandrogenism (Hirsutism, Acne, Male Pattern Balding)
3. Obesity,
4. Infertility,
5. Insulin Resistance And
6. Presence Of Polycystic Ovaries On Ultrasound.

Metabolic and other health implications: The consequences of PCOS extend beyond the reproductive axis. There has been an increasing awareness of the metabolic abnormalities associated with the syndrome. These include

1. Impaired glucose tolerance and type 2 diabetes
2. Dyslipidemia
3. Metabolic syndrome
4. Hypertension
5. Coronary and other vascular disease

The diagnosis of PCOS has lifelong implications with increased risk for metabolic syndrome, type 2 diabetes mellitus, and possibly cardiovascular disease and endometrial carcinoma.

Diagnostic Criteria: An Rotterdam ESHRE/ASRM-Sponsored PCOS consensus workshop group in 2003 proposed that the syndrome can be diagnosed if at least two of the following are present, (after the exclusion of other medical conditions that cause irregular menstrual cycle and androgen excess):

1. Oligo-ovulation or anovulation
2. Clinical or biochemical evidence of androgen excess

3. Polycystic ovaries as defined by ultrasonography (presence of 12 or more follicles 2-9mm in diameter and/or increased ovarian volume>10ml(without a cyst or dominant follicle in either ovary)

a. Thus according to the diagnostic criteria polycystic ovaries need not to be present to make a diagnosis of the PCOS, and conversely, the presence of polycystic ovaries is not necessarily associated with other symptomatology.

Laboratory Evaluation:

1. PCOS is a diagnosis of exclusion and biochemical and/or radiologic studies must be done to ascertain the diagnosis. There are a number of conditions that need to be excluded before making a diagnosis of PCOS(See PCOS workup flowchart)
2. The ratio of LH to FSH is greater than 3:1 in about 30 percent of women with PCOS. However a single measurement of LH and FSH provides little diagnostic sensitivity since gonadotropin concentrations vary over the menstrual cycle and are released in a pulsatile fashion into the circulation. Thus, in routine clinical practice, the LH/FSH ratio has little diagnostic utility for PCOS.
3. Checking for testosterone levels can test androgen excess. An elevated free Te level is a sensitive indicator of androgen excess. Elevated levels of DHEAS and androstenedione also might help establish hyperandrogenaemia.
4. Late onset adrenal hyperplasia due to 21-hydroxylase deficiency, which is present in 1 to 6 percent of hirsute women, can be ruled out by determining 17-hydroxyprogesterone levels.
5. Conditions like Cushing's syndrome and prolactin excess may be excluded by the determination of 24-hour urine sample for free cortisol and serum prolactin levels, respectively.
6. Amenorrhea of primary ovarian insufficiency is accompanied with symptoms of estrogen deficiency including hot flashes and urogenital symptoms. Serum FSH levels are elevated with low estradiol levels.
7. Because nearly 30 percent of women with PCOS have IGT, determinations of fasting plasma glucose, glucose tolerance and insulin resistance are of paramount importance.

References:

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PCOS workup

Hyper Prolactinaemia. Raised serum prolactin levels

Thyroid Dysfunction. Deranged thyroid function tests

Adrenal/Ovarian Tumors. Clinical Features of hirsutism, amenorrhea, virilisation.Testosterone level >7 nmol/L. DHEA-S level > 700ug/dL

Functional Hypothalamic Amenorrhoea- Clinical History,FSH,LH (low to low N), Estradiol low

Pregnancy-Amenorrhea/other signs & symptoms,beta hCG

Cushing Syndrome. Clinical suspicion. Raised levels of urinary free cortisol

Late Onset CAH. Raised 17-hydroxy progesterone level

Acromegaly.Clinical suspicion. Raised Serum IGF-1

Primary Ovarian Insufficiency. SerumFSH raised, Estradiol

Exclude

Suspected PCOS

Oligo/amenorrhea (beta hCG negative)

Serum LH and FSH levels

LH/ FSH ratio>3

BMI >30 KG/M^2, Strong family history of type 2 diabetes, age >40 years

Fasting plasma glucose /75 g OGTT, lipid profile, fasting insulin levels

Imaging studies (if the pelvic examination is inadequate, patient has abdominal pain, testosterone levels are unusually high, needed to support diagnostic criteria)

Transvaginal ovarian ultrasonography and measurement of endometrial thickness

CT scan and MRI if tumor suspected

Infertile patient

Detailed workup of couple to rule out other causes of infertility

Disorders of Sexual Development

Congenital Adrenal Hyperplasia (CAH):

CAH refers to any of several autosomal recessive disorders resulting from mutations for enzymes mediating the biochemical steps of production of cortisol from cholesterol by the adrenal glands.Three major Enzymes deficiency are clinically important

1. 21-Hydroxylase
2. 11-b-Hydroxylase
3. 17-a-Hydroxylase

Clinical Presentations:

1. Ambiguous Genitalia (in XX)
2. Salt Wasting Variety (in both genders)
3. Precocious Puberty (in XY)
4. Hirsutism (non-classical CAH in young females)

Laboratory Diagnosis of CAH:

1. In classic 21-hydroxylase deficiency, laboratory studies will show **very high concentrations of 17-hydroxyprogesterone (greater than 242 nmol/L in random blood sample;** with the normal being less than 3 nmol/L at 3 days age in a full-term infant).
2. Salt-wasting patients tend to have higher 17-hydroxyprogesterone levels than non-salt-wasting patients
3. In non-classical variety, ACTH stimulation test has to be carried out with measurement of **17-hydroxyprogesterone.**

Androgen Insensitivity Syndrome:

Androgen Insensitivity Syndrome (AIS) previously called testicular feminization syndrome due to a loss-of--function mutations in the androgen receptor gene (AR; OMIM# 313700), resulting into peripheral androgen resistance. AIS has traditionally been classified into ***three clinical subgroups*** based on the genital phenotype i.e. Complete AIS, Partial AIS and Mild AIS:

1. **CAIS: Normal Female**: These patients are phenotypically females and also have breast development but internal female organs are absent or just rudimentary.
2. **PAIS:**There can several forms this disease which have also been divided into grades.

a. Predominantly Female:Phenotype Ambiguous phenotype Predominantly female phenotype with absence of pubic or axillary hair at puberty (Grade 7). Normal female genital phenotype; androgen-dependent pubic and/or axillary hair at puberty (Grade 6). Essentially female phenotype; separate urethral and vagina orifices;
b. Mild Clitoromegaly: small degree of posterior labial fusion (Grade 5).
c. Severely Limited Masculinization: phallic structure intermediate between clitoris and penis; urogenital sinus with perianal orifice and labioscrotal folds (Grade 4).
d. Predominantly Male Phenotype:perianal hypospadias; small penis; cryptorchidism and/or bifid scrotum(Grade 3).
e. Mildly Defective Fetal Masculinization: isolated hypospadias and/or micropenis (Grade 2).

3. **MAIS Normal male**:Infertility with azoospermia; reduced virilization at puberty (Grade1).

ACTH Stimulation Test(for CAH)

Indication: For diagnosis and characterization of congenital adrenal hyperplasia (CAH) due to 21-hydoxylase deficiency and other causes of adrenal hyperplasia. This test is often carried out in hyperandrogenised women to diagnose late onset CAH.

Principle: Adrenal glucocorticoid secretion is controlled by adrenocorticotrophic hormone (ACTH) released by the anterior pituitary. This test evaluates the ability of the adrenal cortex to produce cortisol after stimulation by synthetic ACTH (tetracosactide: Synacthen ®). In subjects with enzyme deficiency in the steroid synthetic pathway, cortisol may, or may not, be adequately secreted. However, there is excessive secretion of the precursor steroids before the defective enzyme. The commonest form of CAH is due to deficiency of 21-hydroxylase and in these subjects increased secretion of 17 hydoxy-progesterone(17OH-P) can be detected.

Contraindication: The synacthen test gives unreliable results in the two weeks following pituitary surgery.

Side Effects: There are rare reports of hypersensitivity reactions to Synacthen particularly in children with history of allergic disorders.

Preparation: There are no dietary restrictions for this test. This test should be performed in the morning as the cortisol responses between the morning and late afternoon may differ by as much as 100 nmol/L 30min post synacthen.

Requirement:

1. Plain tubes. And EDTA tube
2. 250mg Synacthen preparation. The dose for children is 35 µg/kg body weight to a maximum of 250ug.

Procedure:

1. 0 min: Take 3ml blood for 17OH-P and cortisol. And ACTH. Give 250µg Synacthen I/M or IV
2. 30 min: Take 3ml blood for 17OH-P and cortisol.
3. 60 min: Take 3ml blood for 17OH-P and cortisol
4. The samples are immediately transported to processing room where serum is separated.

Interpretation:

Normal Cortisol Response:A normal cortisol response would be indicated by a rise in the cortisol concentration of the 30 min sample to greater than 600 nmol/L.

Congenital adrenal hyperplasia:Marked rise in 17OH-P after ACTH stimulation,which varies according to whether the patient is homozygous or heterozygous.CAH is suggested by a base line 17-OHP > 12nmol/l.(A base line of > 100 nmol/l is diagnostic of homozygosity for 21- hydroxylase deficiency and the stimulation test is not required)

Heterozygotes for 21-OH deficiency:

Heterozygotes for 21 hydroxylase deficiency have post stimulation values of 17-OHP> 35nmol/l:

Diagnosis Of 17OH-P deficiency CAH after infancy based on 17OHP levels

	Classic Form	Non-classic form	Unaffected
Baseline 17-OHP levels	>300nmol/L	6-300nmol/L**	<6nmol/L**
17-OHP levels post ACTH Stimulation	>300nmol/L*	31-300nmol/L	<30nmol/L

***test usually not required*

*** Randomly measured 17OHP can be normal in the non-classic form*

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2. New MI, Lorenzen F, Lerner AJ, Kohn B, Oberfield SE, Pollack Ms, Dupont B, Stoner E, Levy DJ, Pang S, Levine LS. Genotyping steroid 21-hydroxylase deficiency: hormonal referencedata. J Clin Endocrinol Metab 1983; 57:320-6.

HCG Stimulation Test

Indication

1. In infants with ambiguous genitalia and palpable gonads.
2. In males with delayed puberty and/or undescended testes.
3. To confirm the presence of testes.
   Male infants usually have plasma testosterone concentrations within the low adult range(8-12 no/L) during the third and fourth months and it is very difficult to differentiate abnormally low levels from normal levels, hence ***HCG*** test has to be carried out.

Contraindications: None.

Principle:HCG is a double polypeptide hormone and shares a common subunit with LH. It stimulates testicular Leydig cells to secrete androgens via the LH receptors. A single injection of HCG is adequate as it has a long half-life (2.5 days) and produces a progressive but modest rise in plasma testosterone for 72-120 hours.

Side effects: Headache, tiredness

Preparation: No patient preparation is required.

Requirements

1. hCG injection- dose is 100 iu/kg or 1500 iu/m2 body surface area for infants or 5000 units for children over 2 years.
2. Two plain blood tubes.

Test protocol:

DAY 0:

a. The patient reports in the procedure block & blood samples for basal testosterone, FSH and LH are taken with Prior appointment.
b. Inj 1500 units (infants) or 5000 units (over 2 years) HCG i.m. or 100 IU/Kg is given intramuscularly.

DAY 3:

c. Blood sample for testosterone is taken.
d. Samples are analyzed for testosterone

Interpretation:A doubling of testosterone concentration over base line with testosterone > 5.2 nmol/l is consistent with normal leydig cell function.

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3. Forest MG, David M, Lecoq A, Jeune M, Bertrans J. Kinetics of the ***HCG*** –induced steroidogenic response of the human testis. III. Studies in children of the plasma levels of testosterone and ***HCG***: rationale for testicular stimulation test. Pediatr Res 1980;14:819-24.
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Index

Pakistan Society of Chemical Pathologists (PSCP)

(http://www.pscp.org.pk/)

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