

# Thyroid Function Tests (TFT)



**Gandham.Rajeev**

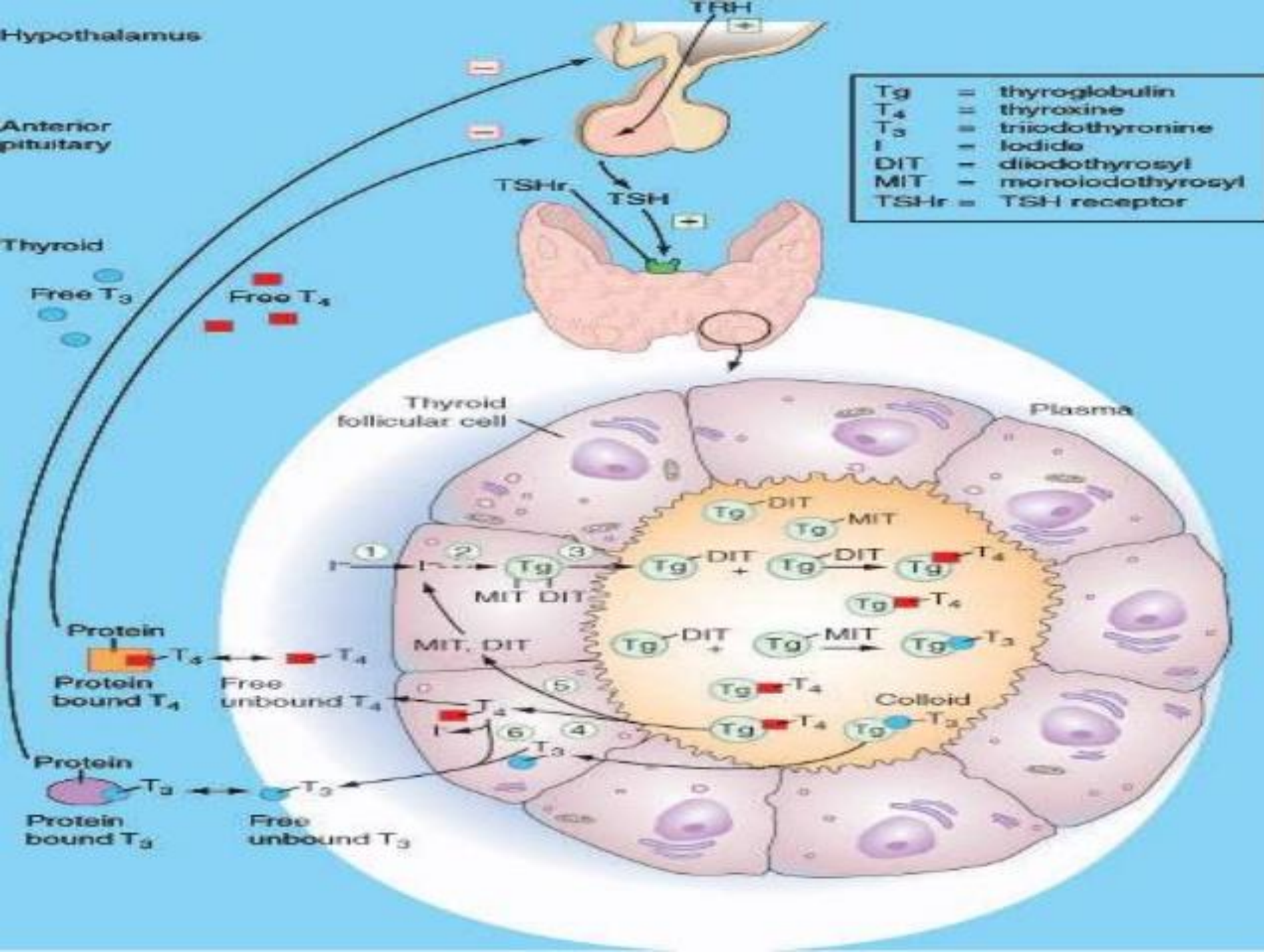
**Email: [gandhamrajeev33@gmail.com](mailto:gandhamrajeev33@gmail.com)**

Hypothalamus

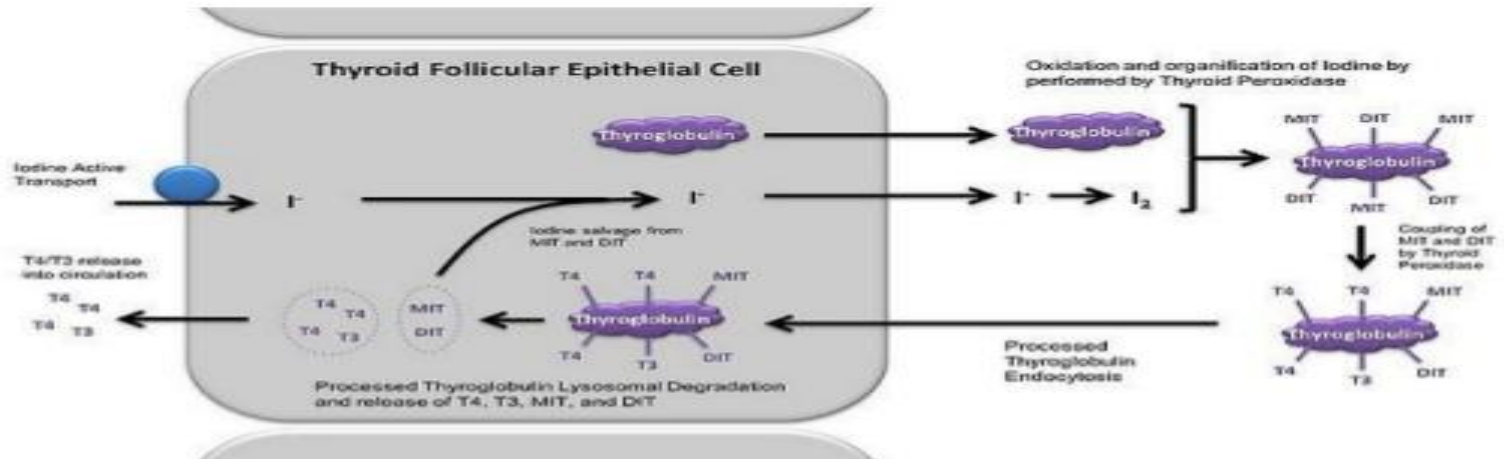
Anterior pituitary

Thyroid

- Tg = thyroglobulin
- T<sub>4</sub> = thyroxine
- T<sub>3</sub> = triiodothyronine
- I = iodide
- DIT = diiodothyrosyl
- MIT = moniodothyrosyl
- TSHr = TSH receptor



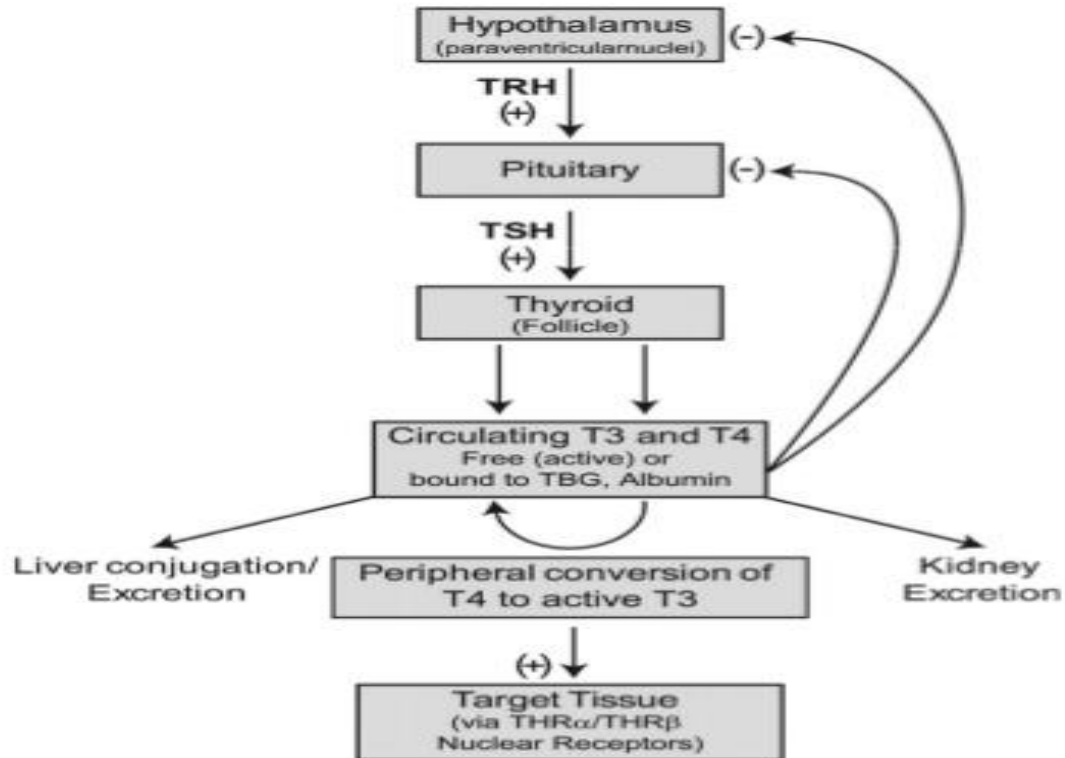
**\*Biosynthesis of thyroid hormones:-**



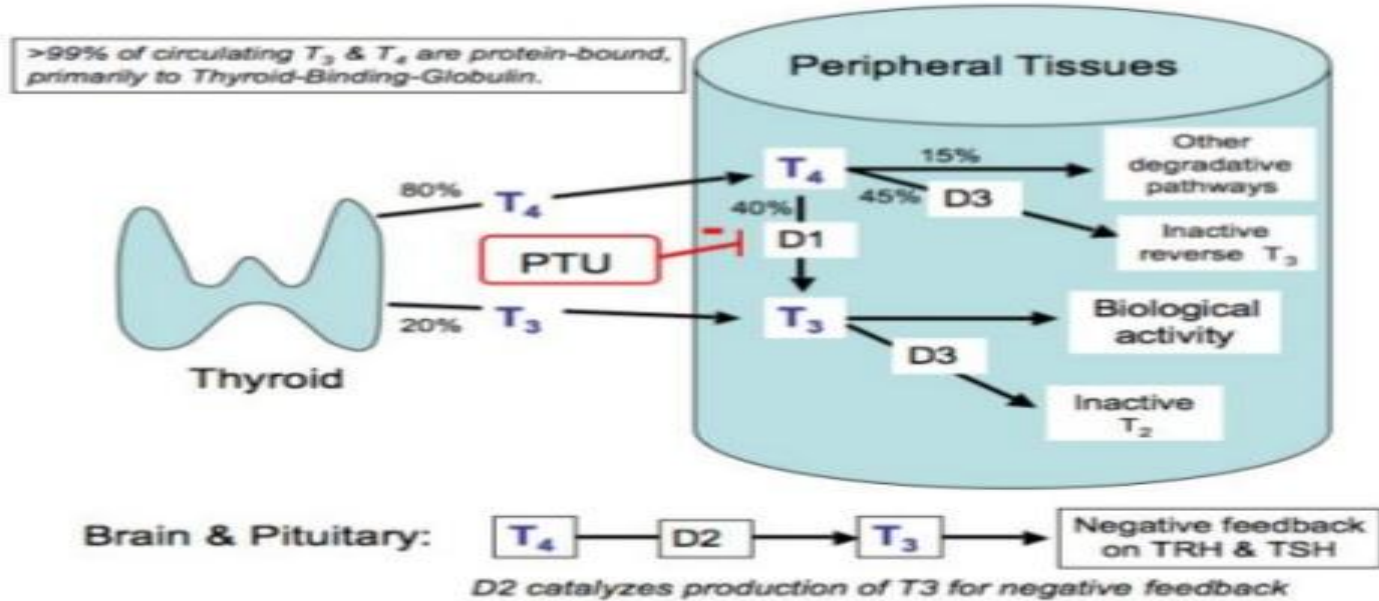
**Steps:**

1. Iodide (I<sup>-</sup>) enters the thyroid cell via sodium iodide symporter
2. It enters the colloid through pendrin receptor
3. It is oxidized into Iodine (I<sup>0</sup>) by peroxidase enzyme
4. Then it is organified into MIT and DIT (mono and di iodo thyronine)
5. Then after coupling it forms T<sub>3</sub> (Tri iodo thyronine) and T<sub>4</sub> (Thyroxine)
6. T<sub>3</sub> and T<sub>4</sub> conjugate with TBG (thyroid binding globulin)
7. conjugated TBG is stored in colloid till required
8. While releasing into blood stream, it is first endocytosed into thyroid cell and then de-coupled to form, T<sub>3</sub> and T<sub>4</sub> with MIT and DIT
9. MIT and DIT can be reutilized for coupling
10. T<sub>3</sub> and T<sub>4</sub> are released into the blood stream

**\*Regulation of thyroid hormone production**



**What happens to thyroid hormones after release**



**Action of thyroid hormone on the body:**

# HPT axis

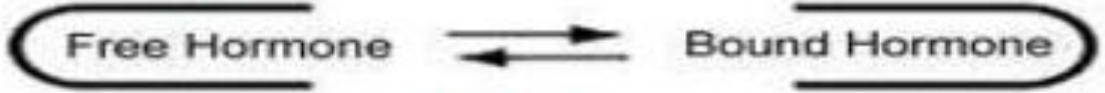


Hypothalamus

TRH Stimulation

Inhibition

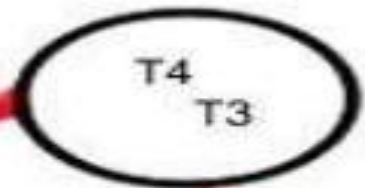
Pituitary



Liver



Thyroid Binding Proteins



TSH Stimulation



Thyroid

# COMMON TESTS TO EVALUATE THE THYROID

- Blood:
  - TSH
  - Free T4, T3 (rarely T4, T3U, FTI)
- Radiology:
  - Radionuclear - thyroid function vs. imaging
  - Other imaging: ultrasound, CT, MRI
- Pathology:
  - FNA biopsy

# CLASSIFICATION OF TESTS BASED ON FUNCTIONS OF THYROID

## **❑ Tests measuring blood levels of thyroid hormones:**

- Sr PBI
- Circulating T3 and T4 level
- Circulating TSH level
- Plasma tyrosine level

## **❑ Tests based on primary function of thyroid viz substrate input & hormone synthesis:**

- RIU (Radioiodine uptake studies)
- PBI<sup>131</sup>
- T3 suppression test
- TSH stimulation test
- TRH stimulation test

## **❑ Tests based on metabolic effects of thyroid hormones**

- BMR
- Sr cholesterol
- Sr creatine level
- Sr uric acid level
- Sr CK enzyme





## ❑ Scanning of thyroid gland

## ❑ Immunological tests to detect autoimmune diseases of thyroid gland:

- Agar gel diffusion test(PPT test)
- TRCH test: tanned red cell haemagglutination test
- Complement fixation test



# Indications of TFT

- Diagnosing thyroid disorder in symptomatic person
- Screening newborns for hypothyroidism
- Monitoring thyroid replacement therapy in hypothyroidism patients
- Diagnosis & monitoring female infertility patients
- Screening adults for thyroid disorders



# Hypothyroidism

## Causes:

### **PRIMARY: (high TSH)**

- **Autoimmune** : Hashimoto`s, Atrophic
- **Iatrogenic**: I<sup>131</sup> t/t, subtotal/total thyroidectomy, external irradiation of neck for Ca
- **Drugs**: I excess, lithium, antithyroid drugs, p- aminosalicylic acid, Interferon
- **Congenital hypothyroidism**: absent/ectopic thyroid gland, dyshormonogenesis, TSHR mutation
- **Iodine deficiency**
- **Infiltrative disorders**

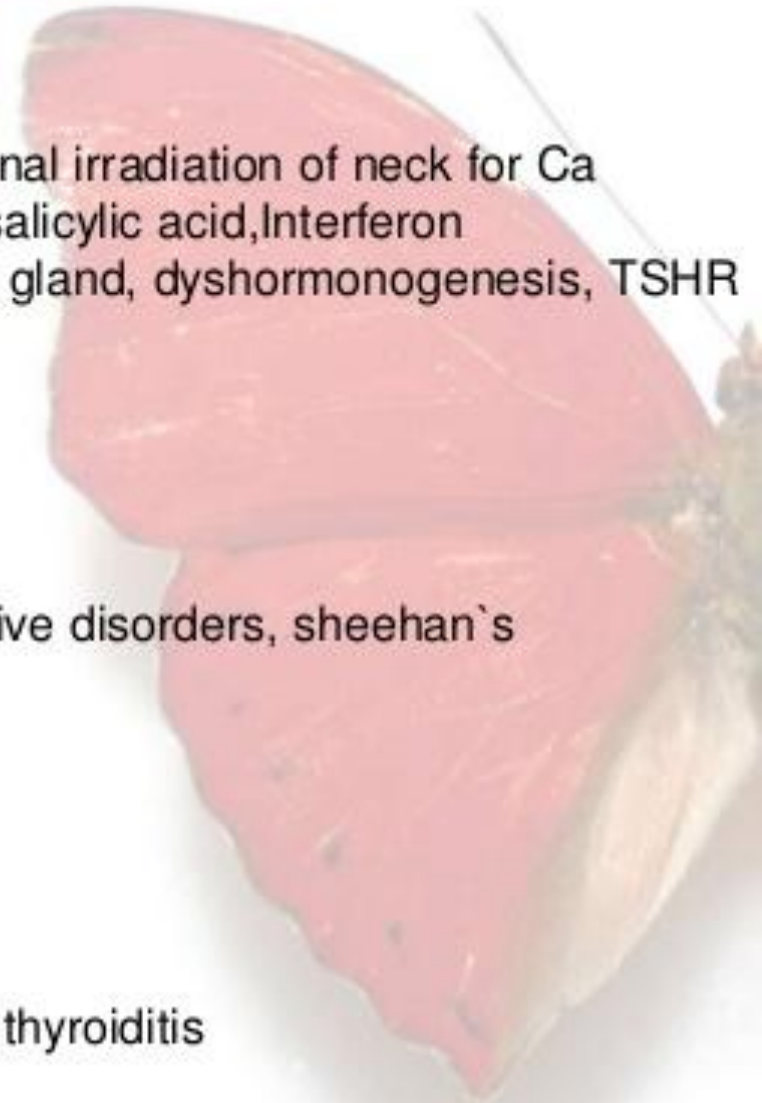
### **SECONDARY: (Low TSH)**

- **Hypopituitarism**: tumors, surgery, irradiation ,infiltrative disorders, sheehan`s syndrome,trauma
- **Isolated TSH deficiency or inactivity**

### **TERTIARY : (LOW TSH ,Low TRH)**

Diseases of hypothalamus

**TRANSIENT**: silent / postpartum thyroiditis, subacute thyroiditis



# Clinical Features of Hypothyroidism

Tiredness

Forgetfulness/Slower Thinking

Moodiness/ Irritability

Depression

Inability to Concentrate

Thinning Hair/Hair Loss

Loss of Body Hair

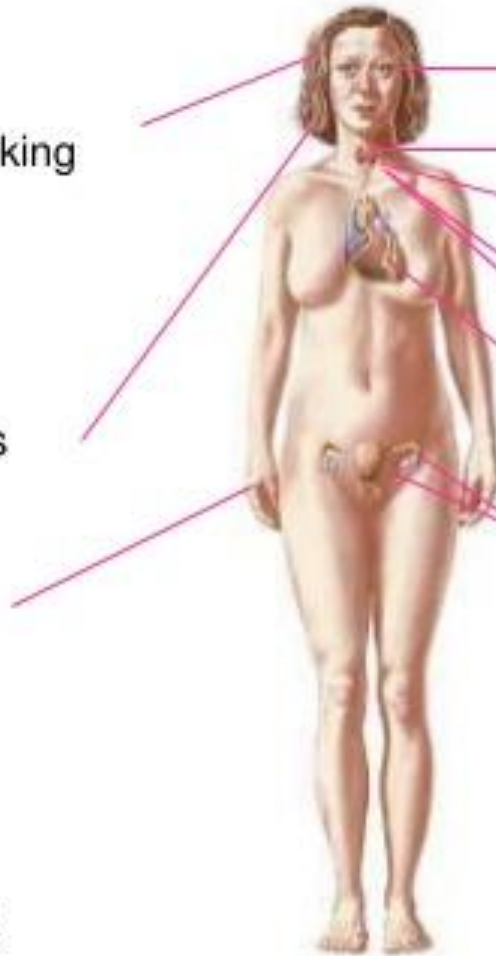
Dry, Patchy Skin

Weight Gain

Cold Intolerance

Elevated Cholesterol

Family History of Thyroid Disease or Diabetes



Puffy Eyes

Enlarged Thyroid (Goiter)

Hoarseness/  
Deepening of Voice

Persistent Dry or Sore Throat

Difficulty Swallowing

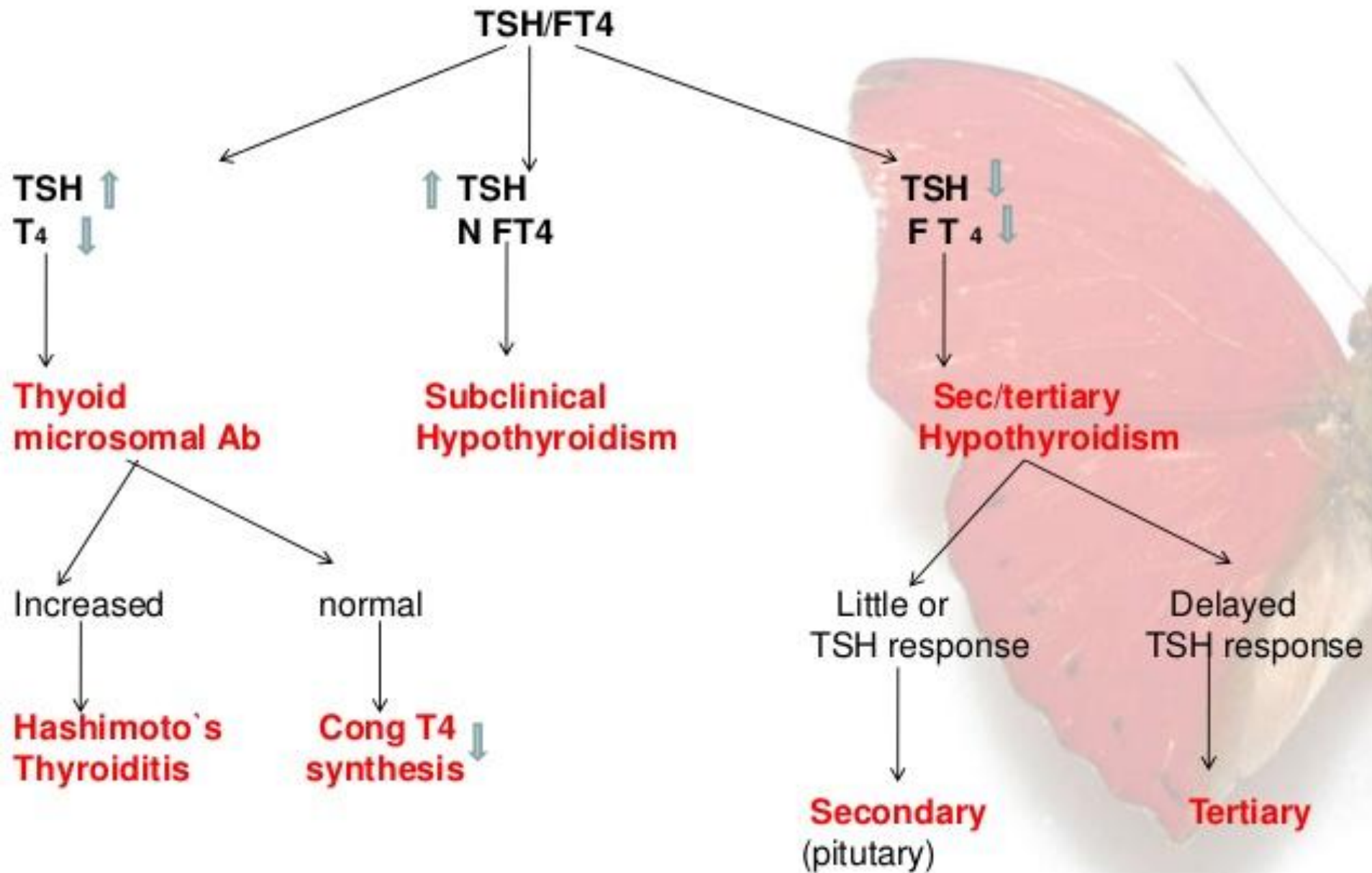
Slower Heartbeat

Menstrual Irregularities/  
Heavy Period

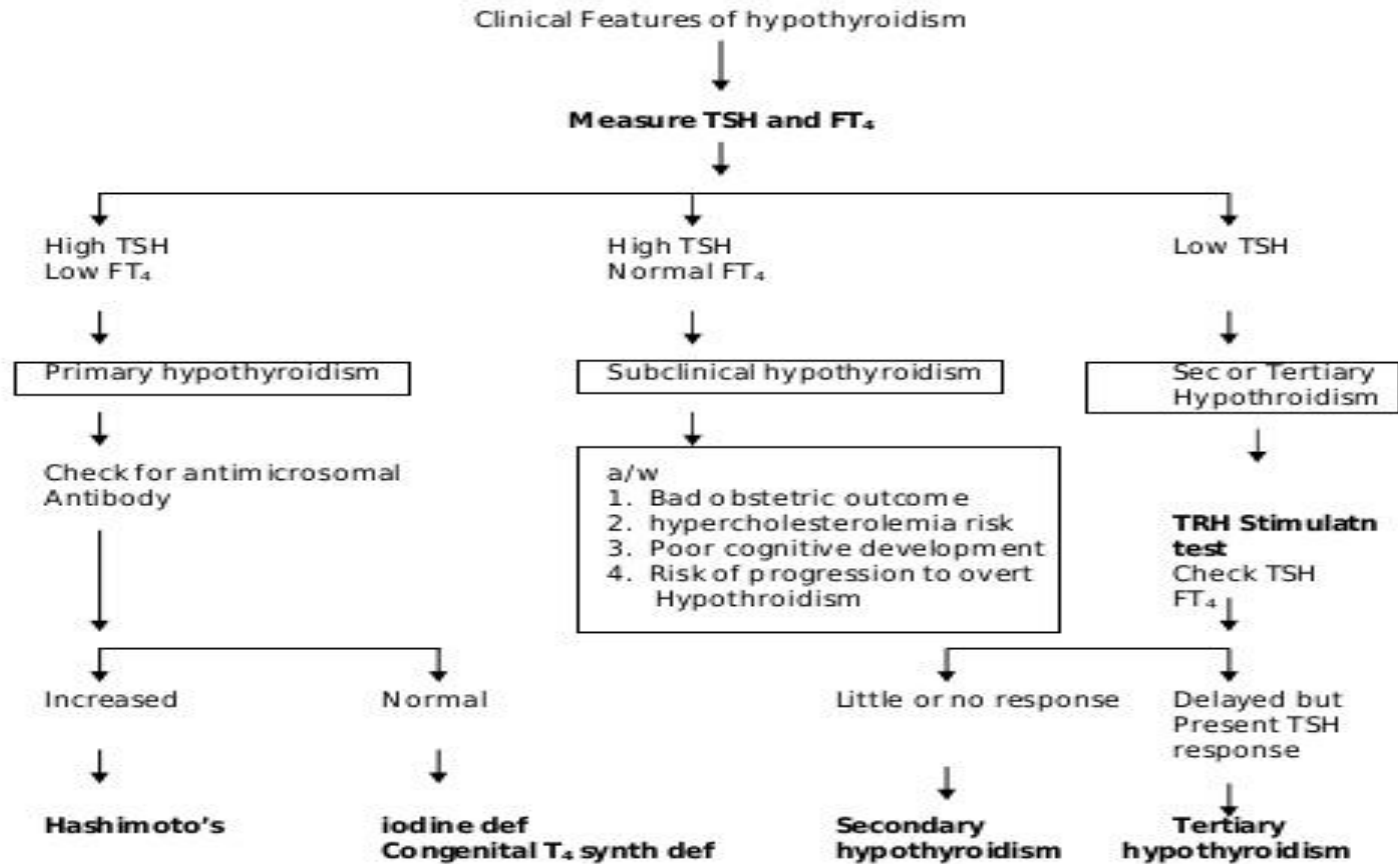
Infertility

Constipation

Muscle Weakness/  
Cramps



**Laboratory features:**



# Hyperthyroidism

## Causes:

### **PRIMARY HYPERTHYROIDISM**

- Grave`s disease
- Toxic MNG
- Toxic Adenoma
- Functioning thyroid carcinoma mets
- Activating mutation of TSH receptor
- Struma ovarii
- Drugs: iodine excess(Jod basedow phenomenon)

### **SECONDARY HYPERTHYROIDISM**

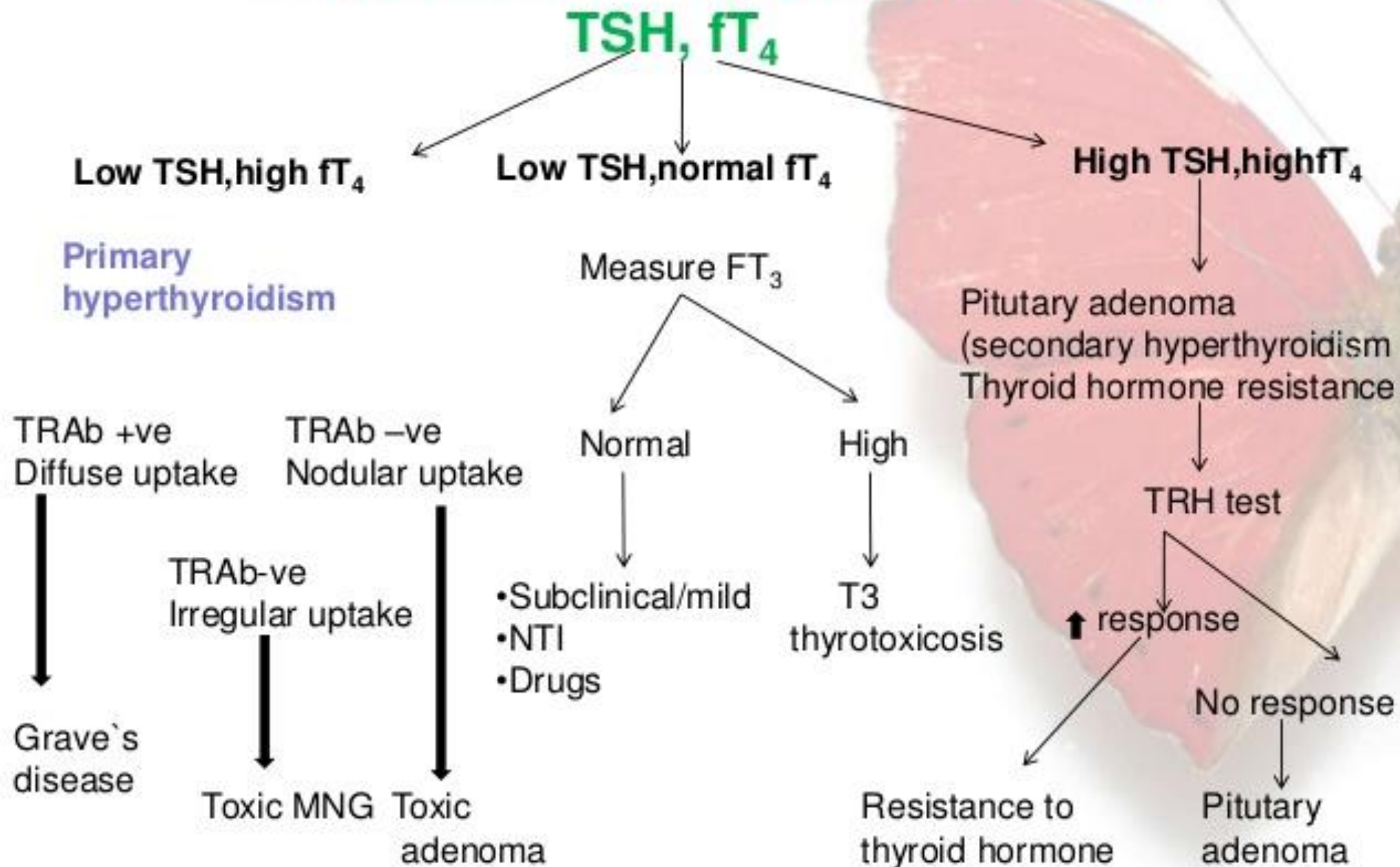
- TSH secreting pituitary adenoma
- TRH syndrome
- Chorionic gonadotropin secreting tumors
- Gestational thyrotoxicosis

### **THYROTOXICOSIS WITHOUT HYPERTHYROIDISM**

- Subacute thyroiditis
- Silent thyroiditis
- Other causes of thyroid destruction: amiodarone, radiation, infarction of adenoma
- Ingestion of excess thyroid hormone



# Evaluation of hyperthyroidism



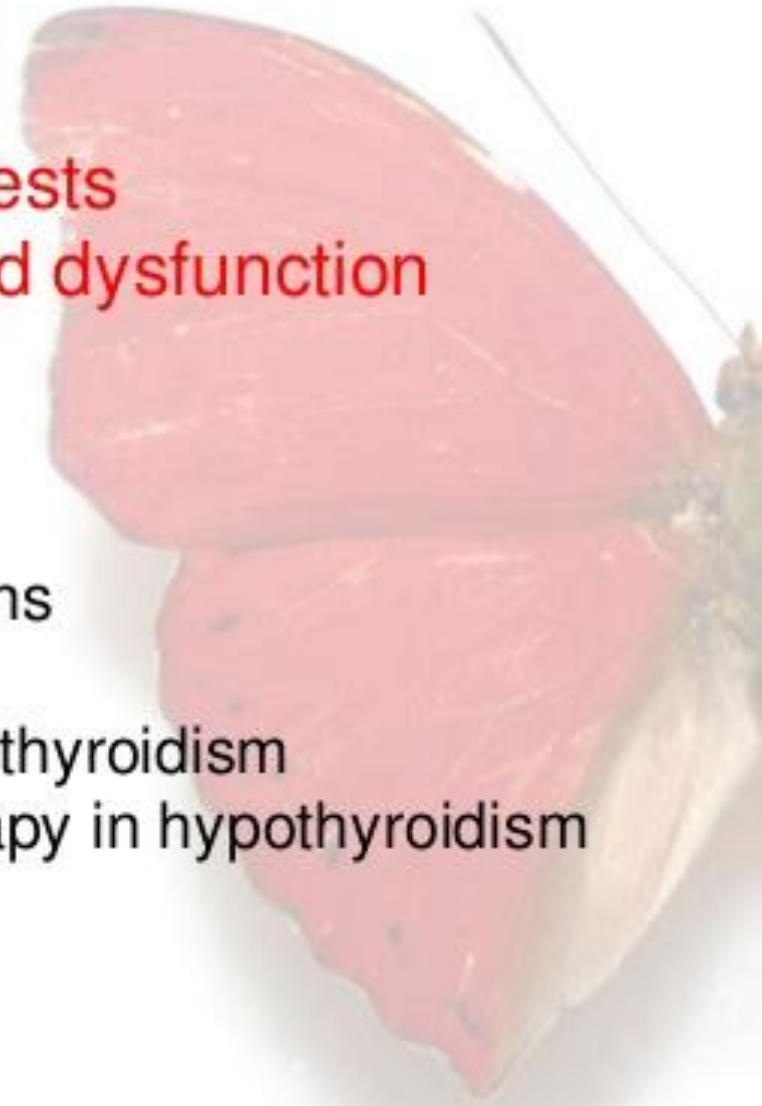


# TSH

- First line test in Thyroid function tests
- Normal TSH level excludes thyroid dysfunction


## Uses:

- Screening for euthyroidism
- Screening of hypothyroidism in newborns
- Diagnosis of 1 & 2 hypothyroidism
- Diagnosis of clinical & subclinical hyperthyroidism
- Follow up of T3 & T4 replacement therapy in hypothyroidism



# TSH

## Increase

- 
- Primary hypothyroidism
  - Addison`s disease
  - Anti TSH antibodies
  - PreEclampsia
  - Hypothermia,fasting state
  - Pitutary adenoma
  - Postoperatively
  - Acute psychiatric illness
  - Thyroiditis
  - Drugs:Amiodarone,bensarazide ,clomiphene, iopanoic acid, lithium, methimazole, metoclopramide,morphine,propylthiouracil,radiographic dyes

## Decrease

- 
- Primary hyperthyroidism
  - Hashimoto`s thyroiditis
  - Hypothyroidism(2 or 3) sometimes
  - Organic brain syndrome
  - Drugs: ASA,heparin,ketoconazole,T3, dopamine, glucocorticoids,octreotide

# Methods of TSH estimation

- Radioimmunoassay
- Immunometric assay
- Chemiluminiscent & fluorescent techniques (3<sup>rd</sup> gen)

• **Normal values: TSH 0.4 to 4mU/L**



# Concept of FT3 and FT4

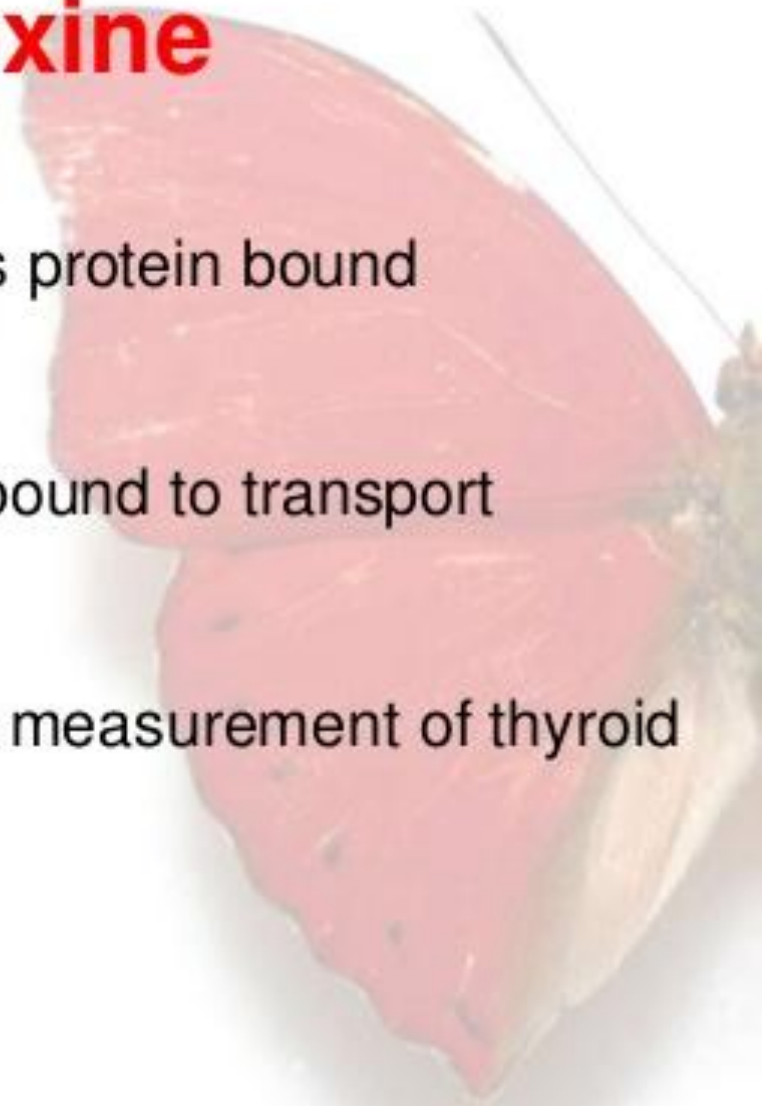
- Out of the total T3 and T4 in circulation, most of it remains bound to thyroid binding globulin \*, thyroid binding prealbumin and Thyroid binding albumin. (\*note – this is not thyroglobulin)
- Only about 0.05% of each T3 and T4 remains free in circulation. This is FT3 and FT4.
- These are better indicators for thyroid function than total T3 and Total T4. (total=bound+free).
- For example in pregnancy, level of thyroid binding globulin rises; hence though total T3 and total T4 remains same, level of FT3 and FT4 decreases. Notes on Renal function tests.

# Differences between T3 and T4

	<b>T3</b>	<b>T4</b>
<b>Secretion</b>	<b>30 microgram /day</b>	<b>80 microgram /day</b>
<b>Source</b>	<b>20 – 25% by gland 75 - 80% by conversion</b>	<b>Solely by gland</b>
<b>Half-life</b>	<b>1 day</b>	<b>7 days</b>
<b>Potency</b>	<b>3-4 times more potent than T4</b>	<b>Potent</b>
<b>Binding</b>	<b>0.2% in unbound</b>	<b>0.02% in unbound</b>

# Total thyroxine

- Total thyroxine includes free as well as protein bound thyroxine.
- Normal levels: 5 to 12.5ug/dL, largely bound to transport protein esp TBG.
- T4 combined with TSH gives the best measurement of thyroid function.



# Thyroxine



## Increase

- Hyperthyroidism
- Factitious hyperthyroidism
- Pituitary TSH secreting pituitary tumor
- Raised TBG



## Decrease

- Primary hypothyroidism
- Secondary/pituitary hypothyroidism
- Severe non thyroidal illness
- Decrease TBG

## Free T<sub>4</sub>

- Small fraction of total T<sub>4</sub> unbound to protein
- Metabolically active form
- 0.05% of total T<sub>4</sub>
- Do not get affected by TBG levels or NTI
- Measurement useful in conditions where TBG levels are affected
- Normal levels: 0.89- 1.76ng/dL





# TBG(Thyroid binding globulin)

Main sr.carrier protein for both T4 & T3  
(13-39ug/dL)



## Increase

- Drugs:Clofibrate,estrogen,O.c Pills,Heroin.methadone
- Genetic
- Acute & chronic hepatitis
- Pregnancy
- Acute intermittent porphyria
- Angioneurotic edema
- Hyperproteinemia



## Decrease

- Drugs:Androgens ,glucocorticoids,phenytoin,larg e doses of salicylates
- Malnutrition
- Hypoproteinemia,nephrotic syndrome
- Acromegaly,cushing`s syndrome
- Liver failure
- Sepsis

# Thyroglobulin



Synthesised & secreted by thyroid follicles(30ng/ml)

Reflects throid mass,thyroid injury & TSH receptor stimulation

Tg ↑ Grave`s disease  
Thyroiditis  
Nodular goitre

Indications :Monitoring recurrence of certain variants of thyroid Ca  
Thyroid dysgenesis in Congenital hypothyroidism  
Follow up of patients with thyroid malignancy

# Thyroid Autoantibodies

- Diagnosing autoimmune diseases
- Autoantibodies : Tg, Thyroid microsomal Ag, TSH receptor, non Tg colloid antigen, TSH, T4

## Anti Tg antibodies

Methods: Agar gel diffusion precipitation test

Tanned red cell haemagglutination tests (TRCH test)

ELISA

Immunofluorescence of tissue section

RIA

**Positive:** Hashimoto's thyroiditis, Grave's disease, myxedema, nontoxic goitre, thyroid ca, pernicious anaemia



# Thyroid tests

**Detect antibodies to thyroid tissue (Anti thyroid antibodies)**

**Found in Hashimoto's thyroiditis (95% of patients) and Grave's disease (55% of patients)**

**Adults without thyroid disease (10% of adults)**

**In Grave's disease, hyperthyroidism is caused by antibodies activating TSH receptors**

**In thyroiditis, hypothyroidism is caused by antibodies competitively binding to TSH receptors thus blocking the TSH from eliciting the response**

## Radioiodine uptake studies

Correlates with functional activity of thyroid gland

- Tracer dose of  $I^{131}$  orally followed by measurement of amount of radioactivity over thyroid gland at 2 hrs and again at 24hrs
- Normal radioactive uptake 20 to 40 % of administered dose at 24 hr

### • Increased Uptake :

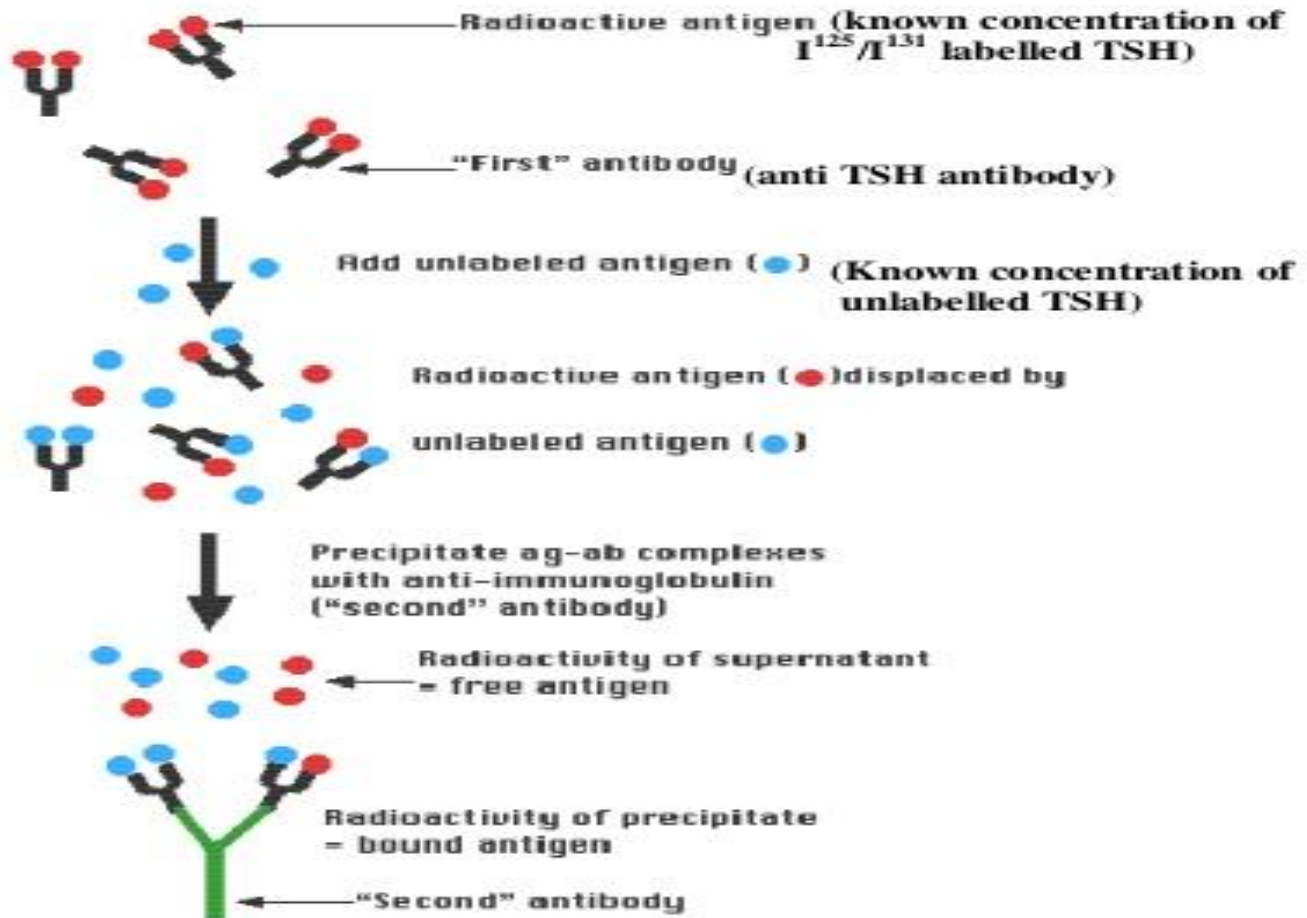
- hyperthyroidism due to grave`s disease,
- toxic MNG,
- toxic adenoma,
- TSH secreting tumor

### Decreased uptake:

- hypothyroidism
- subacute thyroiditis,
- large  $I_2$  doses, thyroid hormone
- factitious hyperthyroidism

### Method: (Radioimmunoassay)(RIA)

The technique was introduced in 1960 by Berson and Yalow as an assay for the concentration of insulin in plasma. It represented the first time that hormone levels in the blood could be detected by an in vitro assay.



- A mixture is prepared of
  - radioactive antigen
    - Because of the ease with which iodine atoms can be introduced into tyrosine residues in a protein (TSH here), the radioactive isotopes  $^{125}I$  or  $^{131}I$  are often used.
  - antibodies ("First" antibody) against that antigen.
- Known amounts of unlabeled ("cold") antigen (known unlabelled TSH) are added to samples of the mixture. These compete for the binding sites of the antibodies.

## **(v) Antithyroid antibodies**

**Antibodies used:**

<b>Anti microsomal antibody</b>	<b>Hashimoto</b>
<b>Anti thyroid peroxidase antibody (anti TPO)</b>	
<b>Anti TSH receptor antibody</b>	<b>Grave's</b>

**Uses:**

For diagnosis and monitoring of autoimmune thyroid disorders

## TRH stimulation tests

**Uses:** Confirms diagnosis of secondary hypothyroidism  
Evaluation of suspected hypothalamic disease

### **Procedure:**

TRH injected iv(200 or 500ug) followed by measurement of serum TSH at 20 & 60 min

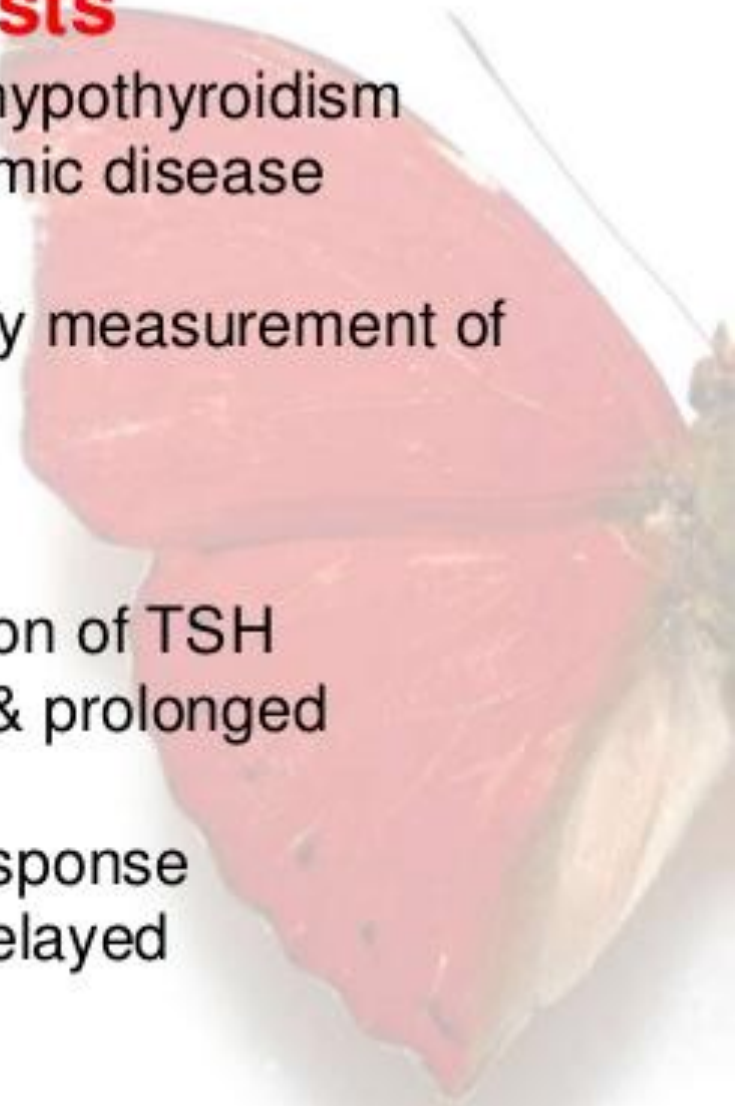
### **Interpretation:**

Peak response in **normal** 4 times elevation of TSH

**Primary hypothyroidism:** exaggerated & prolonged response

**Secondary hypothyroidism:** blunted response

**Tertiary hypothyroidism:** response is delayed





# TSH stimulation test

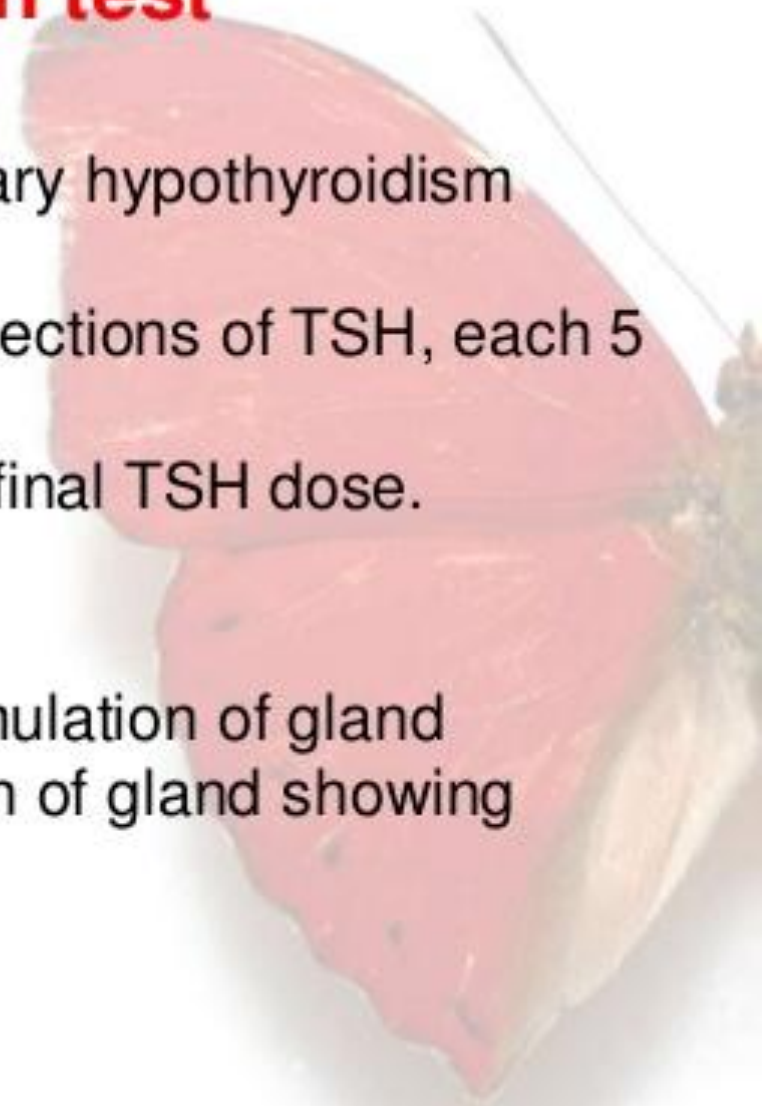
**Use:** Differentiates primary from secondary hypothyroidism

**Procedure:** After 24hr RIU studies, 3 injections of TSH, each 5 USP units are given at 24 hrs interval  
24hr RIU is measured after 42 hrs after final TSH dose.

## **Interpretation:**

In primary hypothyroidism, failure of stimulation of gland

In secondary hypothyroidism, stimulation of gland showing increased RIU.



# Tests based on metabolic effects of thyroid hormones

## **BMR:**

Between 5% & 20% normal

- Euthyroid state : -10% to 10% of normal
- Hyperthyroidism: 50% to 75%
- Hypothyroidism: < -20%

## **Sr. CHOLESTROL LEVEL:**

- 260mg% hypothyroidism

## **Sr. CREATINE LEVEL**

- 0.6mg% hyperthyroidism

## **Sr. URIC ACID LEVEL:**

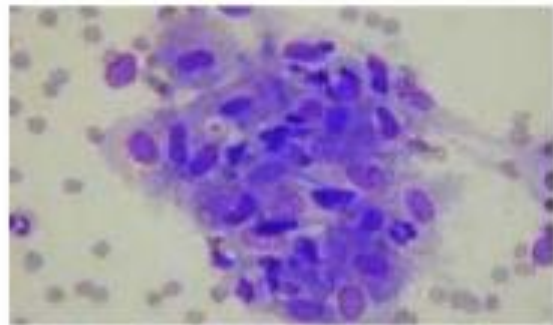
Myxedema 6.5 to 11mg%

## **Sr. CK LEVELS↑ & HYPERCALCEMIA**



# Summary

Disease	Total serum T4	Total serum T3	T3 resin uptake	Free T4 index	Radioactive iodine uptake test	TSH
Hypothyroidism	↓	↓	↓	↓	↓	↑ 1° ↓ 2°, 3°
Hyperthyroidism	↑	↑	↑	↑	↑	↓
T3 toxicosis	No change	↑	No change	No change	No change	↓
Euthyroid sick syndrome	No change	↓	↑	variable	No change	No change



## FNAC thyroid

### **Indications:**

- Diagnosis of diffuse non toxic goitre
- Diagnosis of solitary or dominant thyroid nodule
- Confirmation of clinically obvious malignancy
- To obtain material for special laboratory investigations aimed at defining prognostic parameters.

**Main limitation:** Inability to distinguish between between follicular adenoma & carcinoma.

**Contraindications:** No

**Complications:**

- Local h`age & haematoma.
- Transient laryngeal nerve paresis.
- Tracheal puncture
- Rarely, needling causes formation of a hot nodule



# Materials



- Syringes & syringe holder(pistol)
- 22-25 guage needle
- Cotton Swabs
- Alcohol bottles for wet fixation

# FNAC aspiration technique

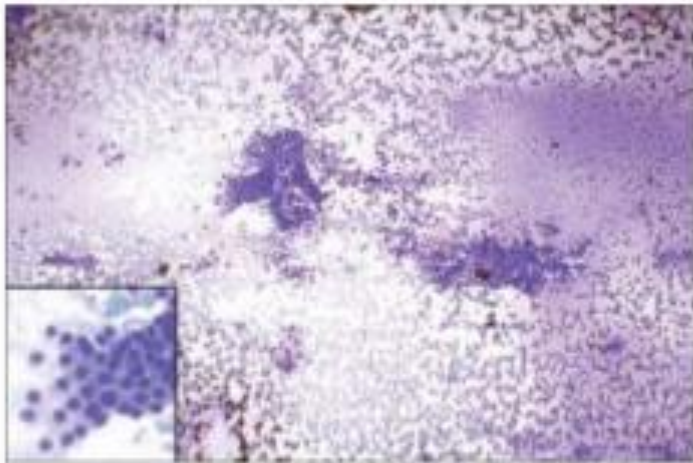


## FNAC non aspiration technique





## Sample adequacy



- Six groups of follicular cells, each containing 10 to 20 cells on two separate slides
- Presence of colloid indicates benign nature



# Bethesda system of reporting FNAC thyroid

## 1. Non diagnostic/Unsatisfactory

Cyst fluid only

Virtually acellular specimen

Other (obscuring blood, clotting artifacts)

## 2. Benign

Consistent with Benign follicular nodule (adenomatous, colloid nodule)

Consistent with lymphocytic (hashimoto's thyroiditis) with proper clinical context

Consistent with granulomatous (subacute thyroiditis)

Other

## 3. Atypia of undetermined significance/Follicular lesion of undetermined significance

## 4. Follicular neoplasm or suspicious for follicular neoplasm

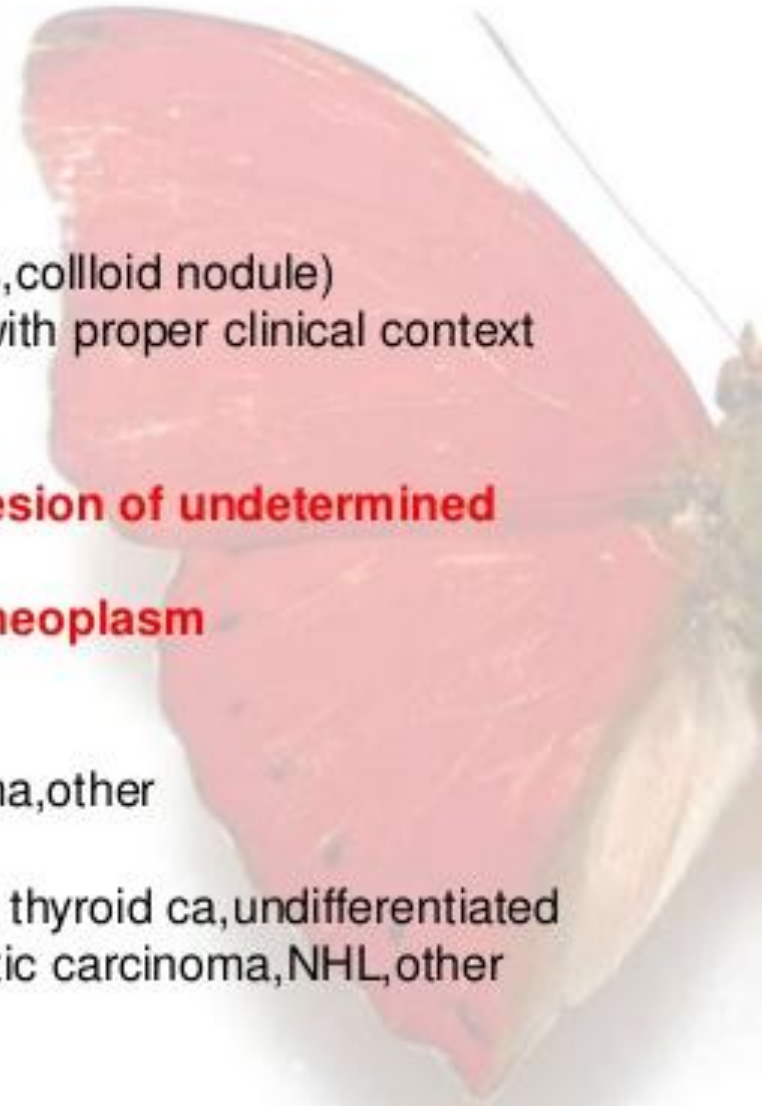
Specify if hurthe (oncocytic type)

## 5. Suspicious for malignancy

Suspicious for papillary, medullary, metastatic, lymphoma, other

## 6. Malignant

Papillary thyroid Ca, poorly differentiated ca, medullary thyroid ca, undifferentiated ca, Squamous cell ca, Ca with mixed features, metastatic carcinoma, NHL, other



# Approach to a case of thyroid enlargement

## DIFFUSE

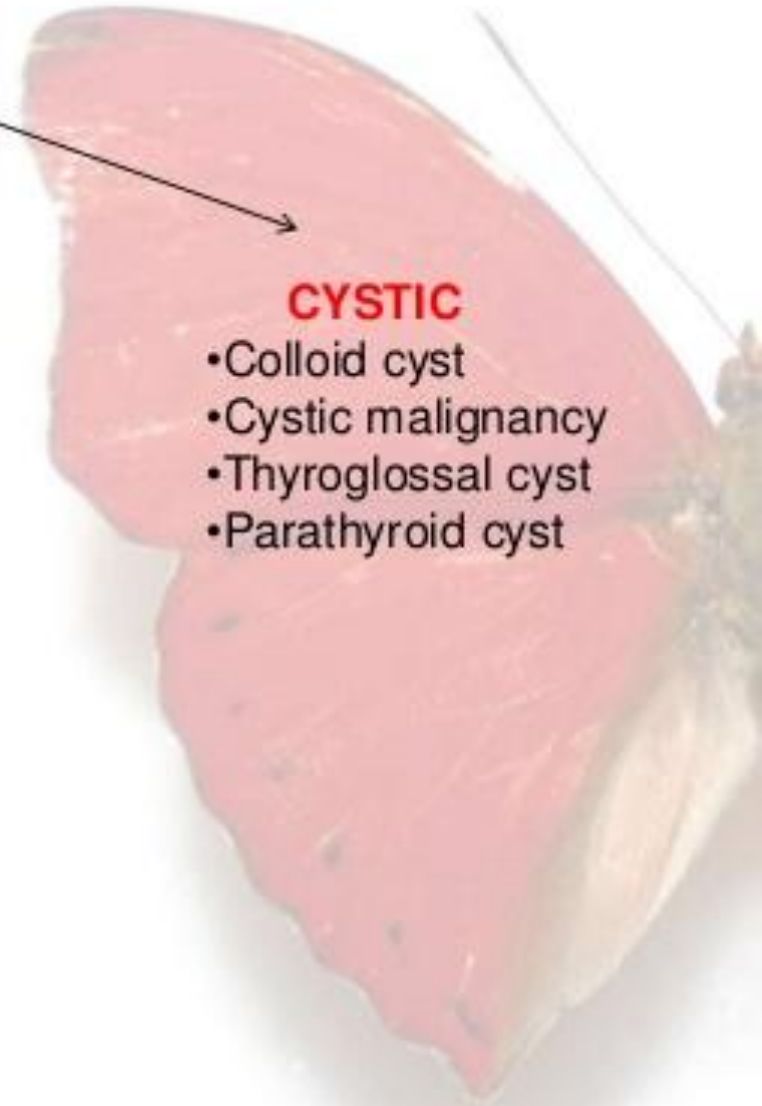
- Acute suppurative thyroiditis
- Subacute thyroiditis
- Hashimoto's thyroiditis
- Adenomatous /colloid goitre
- Painless /silent thyroiditis
- Grave's disease
- Invasive fibrosis

## NODULAR

- Follicular
- Medullary
- Papillary
- Toxic nodule

## CYSTIC

- Colloid cyst
- Cystic malignancy
- Thyroglossal cyst
- Parathyroid cyst



## References

- Fauci, Braunwald, Kasper et al. Harrison's principles of Internal Medicine. 17<sup>th</sup> ed. Boston: Mc Graw Hill; 2008; p2224 to 2246.
- Richard .A. McPherson, Mathew R Pincus. Henry's Clinical Diagnosis And Management by Laboratory methods. 21ed. USA: Saunders An imprint of Elsevier, 2008 p. 263 to 279.
- Leopald G. Koss, Myron R. Melamed's Koss' Diagnostic Cytology and its histopathologic bases. 5<sup>th</sup> ed. New York: Lippincott & Williams & Wilkins; 2006; p1148-1185. vol 2.
- Svante R Orell, Gregory F Sterrett, Darell Whitaker's Fine Needle Aspiration Cytology. 4<sup>th</sup> ed. Australia: Churchill Living An Imprint of Elsevier, 2005; p125-164.
- Edmund S. Cibas & Syed Z. Ali. The bethesda system for reporting thyroid cytology. American journal of clinical pathology 2009; 132:658-665.
- Chatterjee MN & Rana Shinde, Textbook of medical biochemistry, 7<sup>th</sup> ed. JAYPEE; 2009 p638-646.

Thank you 😊

