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متخصص پاتولوژی

استادیار دانشگاه علوم پزشکی گلستان

- Evaluation of thyroid functions consist of:
- Anatomical (ultrasonography, scintigraphy);
- physiological (total T4 [TT4], total T3 [TT3], free thyroxine [FT4], free triiodothyronine [FT3], thyrotropin [thyroid-stimulating hormone [TSH] and thyroglobulin [Tg]); immunological (TPOAb, TSH receptor antibodies [TRAb], and anti Tg antibodies [TgAb]);
- pathological assessment (fine needle aspiration)

- Over last 50 years, laboratory evaluation of thyroid disorder has undergone sea of change.
- There has been gradual improvement in sensitivity and specificity of diagnostic tests for thyroid.
- In the 1950s, only indirect estimate of the TT4 (free and protein-bound) concentration, using the protein-bound iodide technique was available.
- The development of competitive immunoassays in the early 1970s and more recently, noncompetitive immunoradiometric assay methods have progressively improved the specificity and sensitivity of thyroid hormone testing.
- Presently, serum-based tests are available for measuring the concentration of both the total (T4 and T3) and free (FT4 and FT3) thyroid hormones in the circulation by radioimmunoassay and chemiluminescence assay

- In addition, measurements of the thyroid hormone binding plasma proteins are available.[
- Improvements in the sensitivity of assays to measure the pituitary TSH from first generation assays to fifth generation assays with sensitivity to detect TSH levels as low as <0.004 mIU/l, allow TSH to be used for detecting both hyper- and hypo-thyroidism.
- As a result, TRH stimulation test and T3 suppression test have become obsolete.
- Furthermore, measurement of the thyroid gland precursor protein, Tg as well as the measurement of calcitonin in serum, have become important tumor markers for managing patients with differentiated and medullary thyroid carcinomas (MTCs), respectively.
- Furthermore, there has been development of more sensitive and specific tests for TPOAb, TgAb, and TRAb.

- All these advances and high prevalence of thyroid disorders implies that thyroid function tests (TFTs) are performed commonly.
- Most of the times, interpretation of TFT is easy, indicating euthyroidism (normal FT4 and TSH), hypothyroidism (low FT4 or FT3 with high TSH), or thyrotoxicosis (high FT4 or FT3 with low TSH).
- the normal ranges reflect two standard deviations around the mean.
- Hence, 2.5% of the population may show minor abnormalities on both side of normal range in spite of being euthyroid.

- Sometimes interpretation becomes difficult when there is an alteration in relation between thyroid hormones and TSH.
- These pitfalls in investigations will cause dilemma in physicians and patients mind alike.
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- Variation or errors in hormonal evaluation can be preanalytical, analytical, and postanalytical. Out of these, we will discuss pitfalls in preanalytical and analytical factors

- **Variation or errors in hormonal evaluation can be :**
- preanalytical
- Analytical
- Postanalytical

Preanalytical variations

Preanalytical variations are related to :

age, pregnancy, medications, systemic, and genetic diseases

Physiological variables, and biological differences can affect TFTs evaluation.

circadian pattern

- TSH secretion follows a circadian pattern,

with the nadir in the late afternoon and peak between midnight and 4 am

Age

- The hypothalamo-pituitary-thyroid unit matures from fetal life until the end of puberty

- Both TSH and FT4 concentrations are higher in children, especially in the 1st week of life and throughout the 1st year.

- Failure to recognize this could lead to missing and/or under-treating cases of congenital hypothyroidism.

- Age-related normal reference limits should be used for all TFTs. .

Age

- **Older Age :**
- TSH tends to increase with age
- This is due to alterations in thyroid metabolism and a gradual resetting of the hypothalamic-pituitary-thyroid axis

- Diagnosing “real” hypothyroidism is challenging, as is distinguishing disease-specific symptoms from those of aging.

- For mildly elevated TSH levels (4–10 mIU/L) without elevated TPO-Ab, watchful waiting may be reasonable.

pregnancy

- Normal changes in thyroid physiology during pregnancy and the postpartum period can make TFT interpretation very challenging

- Knowledge of these changes is needed for effective patient management

pregnancy

- Owing to the structural homology between hCG and TSH, high levels of hCG during early pregnancy stimulate TSH receptors, resulting in 10–20% enlargement of the thyroid gland, 30% increase in thyroid hormone production, and a decrease in TSH levels
- The hyper-estrogenic state in pregnancy also increases hepatic TBG production, thus increasing the total thyroid hormone levels

pregnancy

- It results in a shift in the TT4 and TT3 reference range to approximately 1.5 times the nonpregnant level by 16 weeks of gestation

- When hCG levels fall after the first trimester, free thyroid hormones decrease and TSH increases.
- TBG remains high until delivery.

pregnancy

- higher cut-offs for T4, T3 and lower cut-offs for TSH are suggested during pregnancy, which should be standardized in local laboratory.

- **These dynamic changes require trimester-specific TFT reference ranges for the accurate assessment of thyroid status during pregnancy.**

- If such reference ranges are not available, the American Thyroid Association (ATA) previously recommended using TSH ranges of 0.1–2.5, 0.2–3.0, and 0.3–3.0 mIU/L for the first, second, and third trimesters[1]

- In patients with unstable thyroid function, such as the first trimester of pregnancy, during the early course of treatment for hypo- or **hyper-thyroidism FT4 measurement is a more reliable indicator of thyroid status than TSH**.

medications

- Drugs can cause both *in vitro* as well as *in vivo* effects on TFTs estimation
- Apart from medications that affect TBG levels mentioned above, numerous other commonly used medications can cause altered thyroid function in other ways

- .Drugs such as **estrogen** can increase TBG levels leading to falsely high TT4 levels though free thyroid hormone levels and TSH stay normal.
- **Glucocorticoid**s can lead to suppressed TSH levels and reduced conversion of T4 to T3 leading to lower T3 levels.
- **Metformin and dopamine** too suppresses TSH secretion.[
- **Propranolol** also inhibits deiodinase enzyme leading to lower T3 levels and may be associated with a mild increase in TSH because of low T3 levels.

- **Iodine and iodine-containing drugs such as amiadarone** can affect TFTs and cause both hyperthyroidism as well as hypothyroidism.[
- **Lithium** can also cause both hypothyroidism as well as hyperthyroidism.
- Drugs such as **phenytoin, carbamazepine, furosemide, and heparin** , **salicylates** may displace free thyroid hormones from TBG leading to elevated free hormone levels.

- Many drugs increase the metabolism of thyroxin and may increase requirements such as **phenytoin, carbamazepine, rifampicin, imatinib, and sunitinib**.

Non thyroidal illness(NIT)

- Non-Thyroidal Illness (NTI) Interpretation of thyroid function tests can be confounded by several factors in critically ill patients depending on the onset, severity, and duration of the critical illness [Understanding changes in thyroid hormones during illness will avert unnecessary testing and treatment.
- **During critical illness, FT3 is the first to fall, typically within the first 24 hours. With time, FT4 also starts to fall, followed by a decrease in TSH.**
- **During recovery from the illness, TSH increases first and can often exceed the normal range.**
- Normalization of free thyroid hormones will ensue.

- These changes in thyroid hormones in critical illness are believed to be brought about by several factors, such as reduced deiodinase activity, reduced thyroid hormone-binding protein concentrations, increased circulating pro-inflammatory cytokines, and concurrent use of certain medications, such as glucocorticoids.

Non thyroidal illness(NIT)

- In nephrotic syndrome, there is increases loss of T4 which is bound to albumin and TBG, may lead to low T4 with normal or raised TSH.

- **In addition to pregnancy and neonatal period, increased TBG levels due to systemic diseases such as acute intermittent porphyria, acute hepatitis, biliary cirrhosis, HIV infection, and hereditary TBG excess will lead to increased TT4 and normal TSH levels.**
- A genetic mutation in albumin (familial dysalbuminemic hyperthyroxinemia) and TTR (TTR associated hyperthyroxinemia) which avidly bind to T4 - also alters the relation between T4 and TSH similarly.

- A rare condition Allen Herndon Dudley syndrome due to mutation in monocarboxylate transporter-8 gene, which is required for thyroid hormone transportation into various cells, leads to raised T3, low T4, and normal or elevated TSH levels. A similar hormonal profile with raised T3, low T4, and normal TSH levels has been reported in patients with resistance to thyroid hormone due to mutation in thyroid receptor-α.[27] Rarely, serum and urinary measurement of monoiodothyronine and diiodothyronine is used to detect rare genetic condition - iodotyrosine deiodinase deficiency, which can also have raised T4, normal/low T3, and normal TSH levels.[26]

- Noncompliant patients may exhibit discordant serum TSH and FT4 values (high TSH/high FT4) because of persistent disequilibrium between FT4 and TSH. Noncompliant patients may consume thyroxin intermittently leading to normal or near normal T4, T3 levels but persistently raised TSH values. Noncompliance can be evaluated by simultaneous oral and intravenous administration of the thyroxin labeled with two different iodine isotope tracers.

- Normally, approximately 80% of the T4 and 95% of the T3 administered orally are absorbed.
- Patients with absorption defects due to interfering substances such as cholestyramine, calcium, iron, or small bowel bypass or resection, celiac disease, helicobacter pylori infection, atrophic gastritis, achlorhydria, and isolated thyroxin absorption defect can be evaluated by the administration of a single oral dose of 100 μg of L-T3 or 1 mg of levothyroxine (L-T4), followed by their measurement in blood sampled at various intervals and values plotted on graph and compared

Discrepancy	Causes
Normal FT3 or FT4 and low TSH	Subclinical hyperthyroidism Pregnancy, hyperemesis gravidarum Recent treatment of hyperthyroidism (anti-thyroid drugs or radioiodine therapy) (up to 3 months) Drugs: Dopamine, steroids Nonthyroidal illness: Acute psychosis, systemic illness
TSH	Poor compliance with T4 treatment Malabsorption of T4: Coeliac disease, atrophic gastritis, iron, calcium or multivitamin tablets, sucralfate, sevelamer, proton pump in Increased T4 metabolism: Phenytoin, rifampicin, carbamazepine, Imatinib, motesanib, sunitinib Recovering subacute thyroiditis Drugs: Amiadarone Nonthyroidal illness: Recovery phase Partial resistance to thyroid hormone Adrenal insufficiency Nephrotic syndrome Assay interference with endogenous antibodies Macro-TSH
High FT3 or FT4 and normal TSH	T4 replacement therapy Increased TBG: Pregnancy, neonatal period, drugs (estrogen therapy, tamoxifen, oral contraceptives), acute intermittent porphyria, hepatitis, biliary cirrhosis, HIV infection, hereditary TBG excess Drugs: Amiadarone, heparin Familial dysalbuminemic hyperthyroxinemia Transthyretin associated hyperthyroxinemia De-iodinase deficiency Allen-Herndon-Dudley syndrome (MCT-8 mutation) Resistance to thyroid hormone due to mutation in TR- Nonthyroidal illness: Acute psychiatric illness
High FT3 or FT4 and high TSH	TSH secreting pituitary adenoma Resistance to thyroid hormone
Low FT3 or FT4 and low TSH	Nonthyroidal illness Central hypothyroidism Congenital TSH deficiency
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FT4: Free thyroxine

Analytical variations

For estimation of TFTs, serum is preferred specimen and ideally whole blood samples should be allowed to clot for more than 30 min and then centrifuged and separated.

Serum can be stored at 4-8°C for up to 7 days. Storage at −20°C is recommended if the assay is to be delayed for more than 1 week.

Collection of serum in barrier gel tubes does not affect the results of most TFTs.

Generally, thyroid hormones are quite stable whether stored at room temperature, in refrigerator or frozen.

Furthermore, TSH and T4 in dried whole blood spots used to screen for neonatal hypothyroidism are also stable for months when stored with a desiccant.

Similarly, hemolysis, hyperlipidemia, and hyperbilirubinemia do not produce interference in hormone estimation by different assays

- Highly sensitive third-generation immunometric assays (sandwich or non-competitive assays), which are capable of detecting TSH levels <0.01 mIU/L, have been widely used since the late 1980s
- Equilibrium dialysis is gold standard for measurement of free hormone assay.
- However, this assay is complex and not widely available.
- Most laboratories use indirect measurements by competitive immunoassays.
- .

Laboratory Assay Interferences

- Many factors can interfere with laboratory tests, and TFTs are no exception.

Biotin

- The use of high-dose biotin (100–300 mg/day) for multiple sclerosis and inherited metabolic disorders has attracted the attention of laboratorians and clinicians, as it can cause inexplicable thyroid test results
- . In addition, biotin is touted for healthy nails and hair, and it may be present in supplements for this purpose in doses of up to 10 mg per tablet.
- most multivitamins for adults in the market contain only a small amount of biotin (<1–3 mg per tablet) and will not cause assay interference
- In these patients, possible interference can be confirmed by asking the patient directly about their medical history and consumption of supplements or retesting on biotin-free automated immunoassays

Biotin

- Streptavidin and biotin are commonly employed in immunoassay platforms to capture antigens (e.g., TSH and FT4) or antibodies (e.g., TRAb) onto a solid phase.

- Metod dependent

Biotin

- . **In competitive assays (e.g., FT4 assayThis will produce a falsely high FT4 result .**
- **In sandwich assays (e.g., for TSH), This will produce a falsely low TSH result**
- The combination of low TSH and high FT4 gives a false impression of hyperthyroid results on the ruthenium chemiluminescence assay system.

- Moreover, the confounding effect of biotin will be even stronger when accompanied by a falsely high TRAb result, which is a competitive assay format.

Heterophile antibodies

- Another important issue is the presence of heterophile antibodies in serum which can lead to falsely high or low TFTs.
- Heterophile antibodies are antibodies induced by external antigens (heterophile antigens) that cross-react with self-antigens.
- The best-known heterophile antibodies are human anti-mouse antibodies (HAMA), which can react with the mouse monoclonal antibodies that are used in many immunometric assays, such as in TSH estimation where if they are present they may lead to erroneously high or low values of TSH.
- Manufacturers are currently employing various approaches to deal with the HAMA issue with varying degrees of success, including the use of chimeric antibody combinations and blocking agents to neutralize the effects of HAMA on their methods.[]

Heterophile antibodies

- The presence of human anti-animal antibodies in patient serum may interfere with TSH measurement
- . If the antibodies block TSH binding to capture or detection antibodies in the assays, negative interference will occur, leading to falsely low TSH levels.
- By contrast, if the antibodies cross-link with capture and detection antibodies, positive interference will occur, resulting in falsely elevated TSH levels.
- **Heterophile antibodies, such as rheumatoid factor, may lead to similar assay interferences**.
- Interfering auto-antibodies to T4 have also been reported to falsely elevate FT4 levels problem

Macro-TSH

- Macro-TSH is a rare condition where serum contains antibodies against TSH (anti-TSH Ig) which binds to TSH and neutralizes its activity, but leaves open epitope to interact with assay antibodies leading to spuriously high value.
-

Macro-TSH

- This can be detected by:
  Linearity test:
- Serum is tested with serial dilution. In normal subjects, it shows a linear pattern (decreasing TSH concentration with increasing dilution), but in the presence of heterophile antibodies or macro-TSH it shows nonlinear pattern (increase in TSH concentration with dilution).

Macro-TSH

- **Polyethylene glycol (PEG) precipitation:** Same amount of PEG is added to serum of patient and normal subject. It is centrifuged, and TSH is measured again.

- In normal subjects, the decrease in TSH is proportional to the dilution, whereas in patients TSH levels decrease drastically due to the precipitation of antibodies.

- TSH sequestration test:
- This is done to detect the presence of anti-TSH antibodies.
- Patient's serum is mixed with serum of hypothyroid patient with known high TSH in 1:1 ration and incubated for 4 h. TSH is re-measured. In the absence of anti-TSH antibodies, the TSH values will be average of these two.
- However, in presence of anti-TSH antibodies this value will decrease, because of sequestration of TSH by anti-TSH antibodies

- Gel filtration chromatography:
- High molecular weight of anti-TSH antibodies can be demonstrated by gel filtration chromatography revealing TSH peak fraction belongs to molecular weight of immunoglobulin.

OTHER

- Antiquagulant :
- EDTA in vidas and immulite :decresed TSH

- Dialysis: increased TSH

Postanalytical variations

Postanalytic errors are relatively less in frequency when compared with the preanalytical and analytical variations.

- Wrongful data entry — Reporting a correct value in different patient.
- Wrong entry of the units — mg/L instead of mg/dL.
- Data entry errors — 20 instead of 2.0.
- Normal range not derived from the local population.
- Delayed access of the test results due to excessive turnaround time.
- Underutilization of the test results for lack of understanding.
- Failure to interpret correctly (high T4 is normal in pregnancy).
- Failure to understand the physiology (delay in TSH rise after antithyroid drugs).

Calcitonin

Calcitonin is secreted by “C” cells of thyroid.

Currently, it is measured with chemiluminescent immunometric assays, which is highly specific and not affected by interfering substance such as procalcitonin, which is raised in many physiological and pathological conditions.

Calcitonin level is affected by age and sex.

Serum calcitonin levels are <40 ng/L in children <6 months of age, <15 ng/L in children between 6 months and 3 years, and <10 ng/L above 3 years and adults.

It is raised in MTC.

It is used to screen multiple endocrine neoplasia 2, planning treatment, and follow-up after treatment for MTC.

Mild elevation of calcitonin has been observed in 3-10% normal adults, C-cell hyperplasia, autoimmune thyroiditis, chronic renal failure, and mastocytosis. Elevated calcitonin levels may also occur from nonthyroidal neuroendocrine neoplasms and heterophile antibodies.

Thyroglobulin

Tg is a protein exclusively produced by thyroid follicular cells.

Most laboratories currently use immunometric assays to measure serum Tg

One of the caveats of using immunometric Tg assays is potential interference by thyroglobulin antibodies (Tg-Ab), which are present in up to 25% of patients with DTC .

Elevated Tg-Ab can lead to falsely low levels of serum Tg.

Hence, serum Tg should always be measured together with Tg-Ab during follow-up for DTC

Serum Tg levels are elevated in most of thyroid diseases and are insensitive in the diagnosis of thyroid dysfunction.

Thyroglobulin

- Tg levels basal and stimulated should be undetectable after treatment for thyroid cancer.
- Serum Tg levels <0.5 ng/ml after recombinant TSH stimulation commensurate with 99% chances of disease-free state.
- However, poorly differentiated thyroid cancer may still exist with low serum Tg levels. A cut-off of <1.0 ng/ml levels of serum Tg on thyroxin suppressive dose and >2.0 ng/ml after TSH stimulation has been suggested for persistent disease

Immunological test

- **Tg-Ab:**
- is a marker of thyroid autoimmunity.
- Since serum Tg-Ab is elevated in 10% of the general population (especially in women), it is not as sensitive or specific as a thyroid biomarker compared with thyroid peroxidase antibodies (TPO-Ab) or TSH receptor antibodies (TRAb)
- . In the absence of TPO-Ab, Tg-Ab is not significantly associated with thyroid disease
- . The main clinical utility of the Tg-Ab test is to ensure the reliability of the serum Tg test in the follow-up of patients with DTC. For patients with elevated Tg-Ab (which renders serum Tg unreliable as a tumor marker), Tg-Ab itself can serve as a surrogate tumor marker for DTC

Immunological test

Anti-TRAb:

can be useful to diagnosis in suspected thyrotoxicosis during pregnancy to differentiate gestational thyrotoxicosis and Grave's disease to predict neonatal thyrotoxicosis in mother's with present or past Grave's disease.

Immunological test

- **TPO-Ab:**
- is found in 5–20% of the general population
- nearly always elevated in patients with Hashimoto's thyroiditis
- Apart from aiding in the diagnosis of Hashimoto's thyroiditis, TPO-Ab may play a role in the management of subclinical hypothyroidism

Thyroid cancer

- 1% of all cancer in U.S., 0.2% of all cancer deaths
- Often estrogen receptor positive, which may explain female predominance of tumors in reproductive years (for other ages, incidence in males and females is similar,
- More common in U.S. whites than blacks, for unknown reasons
- Increased risk for children exposed to ionizing radiation

- **Prognostic factors**
- 20 year survival is 90%, because most are indolent papillary carcinomas
- **Good prognostic factors**:
  - Men under age 40 years or women under age 50 / 60
  - Favorable histologic types
- **Poor prognostic factors**:
  - Men age 41+ or women 51+
  - Large tumor size, nuclear pleomorphism, tumor necrosis, vascular invasion, increased mitotic activity, higher stage, unfavorable subtypes)

- Death usually from undifferentiated, poorly differentiated, Hürthle cell or medullary carcinoma, due to distant metastases

- Poor survival in those with bone metastases (5 year: 29%, 10 year: 13%

Molecular / cytogenetics description

- Rearrangements of RET-PTC in 40% of papillary carcinomas,
- rearrangements of PAX8-PPARy in 40% of follicular carcinomas,
- BRAF mutations in 40 - 60% of papillary carcinomas

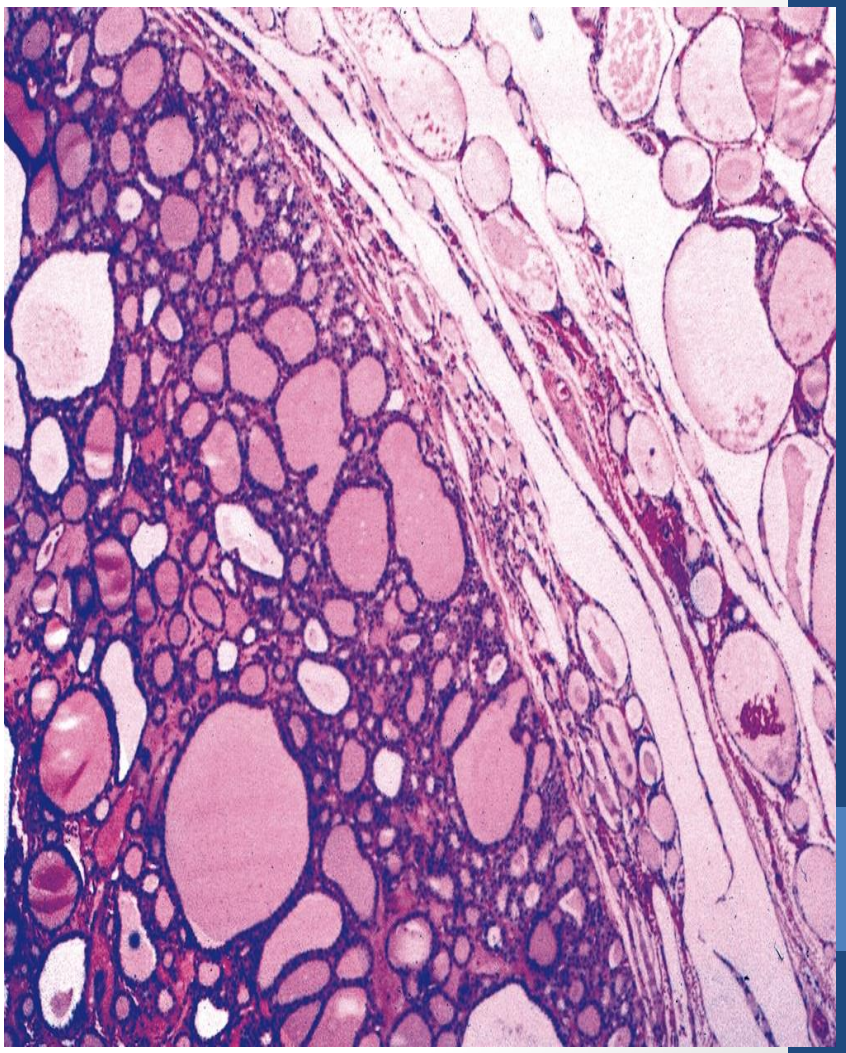
- Tumours of the thyroid gland**ICD 0 codes**

- Follicular adenoma8330/0
- Hyalinizing trabecular tumour8336/1
- Other encapsulated follicular patterned thyroid tumours
  - Follicular tumours of uncertain malignant potential8335/1
  - Well differentiated tumour of uncertain malignant potential8348/1
  - Noninvasive follicular thyroid neoplasm with papillary-like nuclear features8349/1
- Papillary thyroid carcinoma
  - Papillary carcinoma8260/3
  - Follicular variant of PTC8340/3
  - Encapsulated variant of PTC8343/3
  - Papillary microcarcinoma8341/3
  - Columnar cell variant of PTC8344/3
  - Oncocytic variant of PTC8342/3
- Follicular thyroid carcinoma (FTC), NOS8330/3
  - FTC, minimally invasive8335/3
  - FTC, encapsulated angioinvasive8339/3
  - FTC, widely invasive8330/3
- Hürthle (oncocytic) cell tumours
  - Hürthle cell adenoma8290/0
  - Hürthle cell carcinoma8290/3
- Poorly differentiated thyroid carcinoma8337/3
- Anaplastic thyroid carcinoma

Follicular adenoma

- Benign tumor that shows evidence of follicular differentiation but lacks evidence of capsular and vascular invasion and lacks papillary carcinoma nuclear features
- Presents with long standing solitary thyroid nodule

Follicular adenoma



Follicular Carcinoma

- Thyroid carcinoma with follicular differentiation but no papillary nuclear features)
- Comprises 6 - 10% of thyroid carcinomas
- Insufficient dietary iodine is a risk factor
- Usually solitary "cold" nodule on radionuclide scan
- Extensive sampling of capsule is recommended

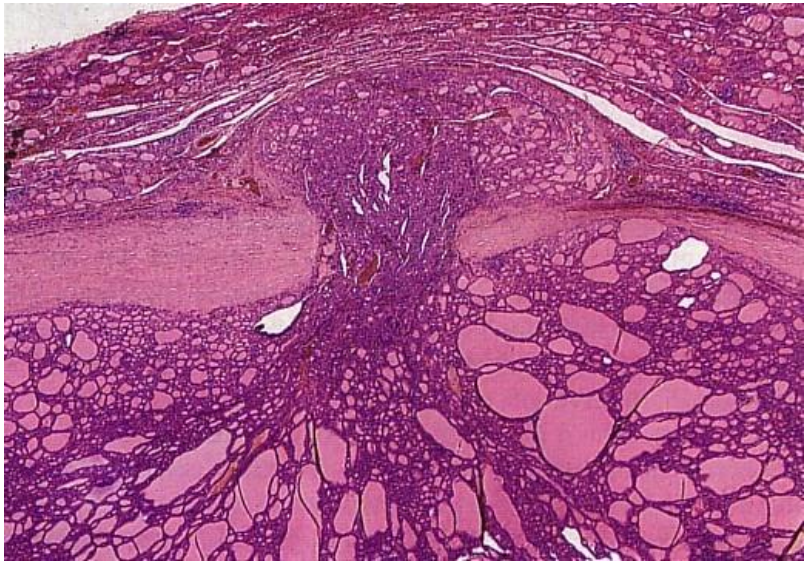
Epidemiology

- 75% women
- Older age than papillary carcinoma, peak age: 40 - 60
- Rarely in children

Gross description

- Tan to brown solid cut surface, can have cystic changes and hemorrhage
- Minimally invasive: usually single encapsulated nodule, with thickened and irregular capsule
- Widely invasive: extensive permeation of capsule or no capsule
- All capsule with adjacent tissue needs to be submitted for histological evaluation

Follicular Carcinoma



- Solitary round or oval nodule
- Thick capsule
- Composed of follicles
- Capsular invasion or vascular invasion within our outside capsular wall

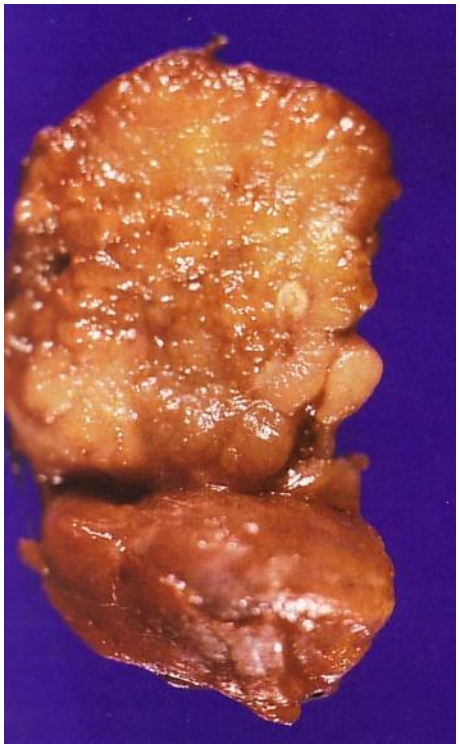
Papillary thyroid carcinoma

- **Etiology**
- Ionizing radiation is the best established risk factor, including:
  - Iatrogenic (e.g., radiation for head and neck cancer)
  - Post Chernobyl nuclear accident (Endocr Pathol 2006;17:307)
  - Post atom bomb
- Can be familial in 4.5% of cases

- Classic variant of papillary thyroid carcinoma is characterized by two cardinal features:
  - The presence of true papillae defined as papillae with a central vascular core
  - Nuclear features in the overlying epithelial cells defined by nuclear enlargement, nuclear membrane irregularity and a distinct chromatin pattern

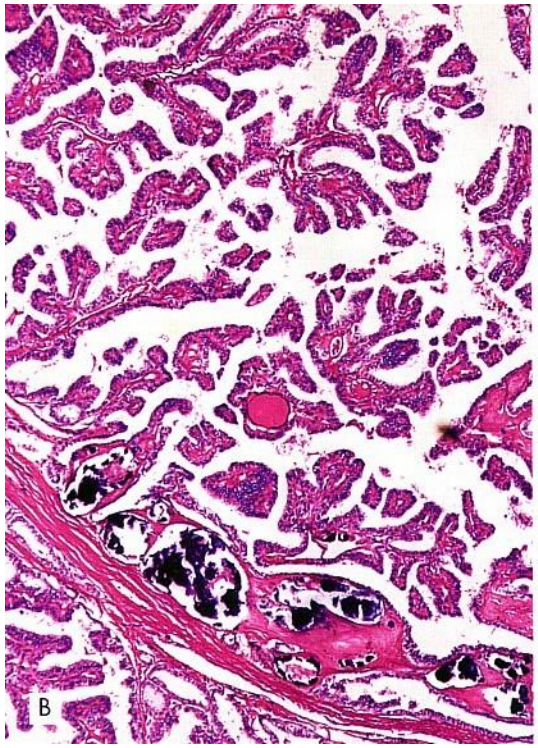
- **Clinical features**
- Painless palpable thyroid mass
- **Diagnosis**
- Typically, the diagnosis is first rendered on ultrasound guided pre-operative fine needle aspiration cytology
- Surgical pathology report of a resected specimen provides further information about the subtyping (i.e., variant) and microstaging

Papillary Carcinoma



- Variable size (microscopic to several cm)
- Solid or cystic
- Infiltrative or encapsulated
- Solitary or multicentric (20%)

Papillary Carcinoma



- Papillae
- Psammoma bodies

- NUCLEAR FEATURES***

- Noninvasive follicular thyroid neoplasm with papillary-like nuclear features:
- :
- Excluded factors:
- Capsular invasion
- Vesseles invasion
- Tumor necrosis