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# Thyroid function tests: a review

G. SHIVARAJ, B. DESAI PRAKASH, V. SONAL, K. SHRUTHI, H. VINAYAK, M. AVINASH

Department of Biochemistry, J. N. Medical College, Belgaum 590010, Karnataka (India)

**Abstract. – In this paper, we review the tests that are executed to aid the diagnosis of thyroid dysfunction. Thyroid function tests provide information at physiological, pathological and anatomical levels. Along with history and physical examination they owe to many specific findings that are associated with thyroid functioning. So an attempt has been made to put forward a gist of thyroid function tests. Serum tests of thyroid function are serum total thyroxine (T4), serum total triiodothyronin (T3), free thyroxine (FT4), free triiodothyronin (FT3), reverse triiodothyronin (rT3), thyroid stimulating hormone (TSH), serum calcitonin and protein thyroglobulin (Tg). The serological tests are antithyroglobulin antibodies (ATA) and antimicrosomal antibodies (AMA). An invasive test for histologic examination is done by fine needle aspiration cytology (FNAC) and noninvasive test includes ultrasonography, magnetic resonance imaging, and positron emission tomography. Further molecular study provides molecular markers for thyroid cancer. These tests can provide greater sensitivity and specificity that enhance the likelihood of early detection of ambiguous thyroid disease with only minimal clinical findings. Lastly, *in vivo* tests are thyroidal radioiodine and iodide uptake is also done.**

*Key Words:*

Serum total thyroxine, Free thyroxine, Thyroid stimulating hormone, Serum thyroglobulin, Serum calcitonin.

## Introduction

Thyroid functions have subtle clinical features associated with some forms of thyroid dysfunction. The clinicians must decide which test is best suiting to diagnose or exclude disorder. It is emphasized that single thyroid function test (TFT) is not absolute in diagnostic accuracy and it must be thus a careful selection of such tests so that their combination can give comprehensive data that would enhance the diagnostic accuracy[1].

### *Serum Total Thyroxine ($T_4$/$TT_4$)*

The concentration of total $T_4$ in adults ranges from 5 to 12 µg/dL (64 to 154 nmol/L)[1]. The concentrations of $T_4$ below or above this range in absence of thyroid dysfunction, is as a result of an abnormal level of serum Thyroid Binding Globulin (TBG). Such abnormally high values are observed in many physiological conditions in women with hyperestrogenic state of pregnancy[1]. Hyperthyroidism and hypothyroidism can be associated with abnormal menstrual cycles[2]. Reference ranges for thyroid function tests for $TT_4$ in cord-blood is 7.4-13.1 µg/dL, 1-2 weeks is 9.9-16.6 µg/dL, 1-4 months is 7.8-16.5 µg/dL, 1-5 years is 7.3-15 µg/dL, and 5-10 years is 6.4-13.3 µg/dL[3]. Small seasonal variations and changes related to high altitude, cold and heat are also seen. The variation is also related to postural changes in serum proteins concentration and true circadian variation. There is increased binding to serum proteins in cases of Familial Dysalbuminemic Hyperthyroxinemia (FDH) which shows increased TBG[1]. Subclinical primary hypothyroidism is more common in persons with chronic kidney disease (CKD)[4].

In thyrotoxic state serum $TT_4$ concentration is elevated and said to be hyperthyroidism that can be caused by Graves' disease, Plummer's disease (toxic thyroid adenoma), early phase of acute thyroiditis, thyrotoxic factitia, struma ovarii and normal in some cases of Luft's syndrome (Hypermetabolic Mitochondrial Miopathy).

In hypothyroidism serum $TT_4$ concentration is low in case of thyroid gland failure. It can be further classified into primary, secondary and tertiary. The cause for primary hypothyroidism can be gland destruction and severe inborn error of hormonogenesis, secondary hypothyroidism is caused by pituitary failure and tertiary by hypothalamic failure. Sometimes it can be subclinical if there is thyroid transporter defect or deiodinase defect[1].

*Corresponding Author*: Shivaraj Gowda, MD; e-mail: drshivaraj@yahoo.com

### Serum Total Triiodothyronine ($T_3$/$TT_3$)

A normal serum $TT_3$ concentration in adult range from 80-190 ng/dL[1]. It reflects the functional state of peripheral tissue rather than secretory performance of the thyroid gland. Sex difference is small, but age difference is more dramatic. The decline of mean $TT_4$ is also observed in old age all though not in healthy subjects, which suggest that fall in $TT_3$, might reflect prevalence of non-thyroidal illness rather than an effect of age alone[2]. Positive co-relation between serum $TT_3$ level and body weight has been observed[5]. Hormones are iodothyronines that control growth and development, as well as brain function and metabolism. The $T_3$ and $T_4$ level were found to be significantly raised in the moderate depression as compared to the healthy controls[6].

Thyroid study on mutations in the monocarboxylate cell membrane transporter 8 (MCT8) genes, located on the X chromosome (Xq13-q21 and Xq12-q13) has established the physiological importance of MCT8 as a thyroid hormone transporter. This syndrome combines thyroid and neurological abnormalities. MCT8 gene (also known as SLC16A2 and XPCT) defect should be suspected in front of psychomotor impairment (severe developmental delay, truncal hypotomia and limb spasticity) and high serum $T_3$, low $T_4$ and $rT_3$ concentrations. However, the neurological manifestations of this syndrome cannot be explained by the thyroid function tests. The phenotype is different from that of global hormone deficiency or excess. Treatment with L-$T_4$ (physiological doses) has not corrected in several patients the phenotype. It has been recommended the use of higher doses of L-$T_4$ during pregnancy. MCT8 knockout mices have demonstrated tissue-specific TH excess and deprivation due to different tissue dependency on MCT8 for cellular thyroid hormone uptake[7-8].

The principle uses for obtaining the serum $T_3$ are to determine the severity of hyperthyroidism, and to confirm the diagnosis of suspected thyrotoxicosis in which serum $T_4$ levels are normal or equivocal[9]. In addition it may be required to carry out the test in cases of functioning thyroid adenomas, where $T_3$ toxicosis may be present and such patients may have normal or borderline elevated serum $T_4$ levels along with suppressed serum TSH levels[10]. Serum $T_3$ is misleadingly elevated in women who are pregnant or who take oral estrogen, due to the high serum levels of TBG in these conditions[11]. $TT_4$ and $TT_3$ measurements are rarely used as stand-alone tests, but are employed in conjunction with a binding protein estimate test i.e. Thyroid Hormone Binding Ratio (THBR) to form a Free Hormone Index i.e. $FT_4I$ or $FT_3I$[12].

### Free Thyroxine ($FT_4$)

The normal values for $FT_4$ in adults range from 1.0 to 3.0 ng/dL (13 to 39 pmol/L)[1]. A minute amount of thyroid hormone circulates in the blood in a free form, not bound to serum proteins. It is in reversible equilibrium with the bound hormone and represents the diffusible fraction of the hormone capable of traversing cellular membranes to exert its effects on body tissues. Although changes in serum hormone-binding proteins affect both the total hormone concentration and the corresponding fraction circulating free in the euthyroid person, the absolute concentration of free hormone remains constant and correlates with the tissue hormone level and its biologic effect[13]. Serum $FT_4$ may be suppressed in the patients with thyroidal illness and transiently rise in acute thyroidal illness, when thyroid-binding protein frequently falls[11].

### Free Triiodothyronine ($FT_3$)

The normal adult reference value is 0.25-0.65 ng/dL (3.8-10 nmol/L)[1]. Free triiodothyronine ($FT_3$) measures the very tiny amount of $T_3$ that circulates unbound. It is useful in looking for hyperthyroidism or thyroxine overplacement in women who are pregnant or taking any effective drugs that varies the TBG like estrogen[11]. More consistently, patients with a variety of non-thyroidal illnesses have low $FT_3$ levels[1]. This decrease is characteristic of all conditions associated with depressed serum $TT_3$ concentrations due to a diminished conversion of $T_4$ to $T_3$ in peripheral tissues[14].

Marked elevations in both $FT_4$ and $FT_3$ concentrations in the absence of hypermetabolism are typical of patients with resistance to thyroid hormone[15]. The $FT_3$ concentration is usually normal or even high in hypothyroid persons living in areas of severe endemic iodine deficiency and their $FT_4$ levels are, however, normal or low[16]. Information concerning this value can be the most important parameter in evaluation of thyroid function because it relates to patients status although other mechanisms exists for cell to control the active amount of the thyroid hormone by autoregulation of receptor[17] and regulation of deiodinase activity[18]. Rarely, a defect in thyroid

hormone transport in the cells would abolish the free hormone and metabolic effect co-relation[7]. The free hormone concentration is high in thyrotoxicosis, low in hypothyroidism,and normal in euthyroidism[19].

### Triiodothyronine Resin Uptake Test ($T_3$RU)

Values correlate inversely with the concentration of unsaturated TBG[1]. A high resin uptake is seen with hyperthyroidism and with chronic liver disease, nephrotic syndrome, anabolic steroid administration, and high dose corticosteroid administration, indicating low amounts of thyroid binding proteins (TBP) or high levels of $T_4$ in the patient's serum. Thyroid hormones circulate mainly bound to serum binding proteins (TBP). Changes in TBP concentrations will acutely modify the concentration of free hormones with a consequent new equilibrium. Therefore, the aim of TBP is to maintain a constant serum free hormone concentration. Three TBP are prevalent: *thyroxine-binding globulin* (a 54 KD glycoprotein synthesized by the liver whose gene resides on the long arm of the X chromosome), *transthyretin* (RET) a 55 KD synthesized by the liver and in the chorioid plexus. It is a tetramer whose every single polypeptide contains 125 amino acids. The RET gene proto-oncogene codes for a tyrosine kinase membrane receptor. Germline point mutations of the RET proto-oncogene were demonstrated as causative of MEN 2 and of nearly 50% of the sporadic Medullary Thyroid Carcinoma with a very high specificity; *albumin* (a 66.5 KD, 585-amino acid protein synthesized by the liver). A low resin uptake (high TBP) is seen with estrogen therapy, pregnancy, acute hepatitis, genetic TBP increase, and hypothyroidism. A low resin uptake with low TBP may be seen in severe illness[11].

### Free Thyroxine and Free Triiodothyronine Index ($FT_4$I, $FT_3$I)

This $FT_4/FT_3$ index can be obtained by determination of $TT_3$ and $TT_4$[1]. The $FT_4$ elevated in euthyroid patients with FDH. This is the benign autosomal dominant trait in which an abnormal albumin molecule binds $T_4$ with much greater affinity than $T_3$[11].

### Reverse Triiodothyronine ($rT_3$)

Reverse $T_3$ ($rT_3$) is principally a product of $T_4$ degradation in peripheral tissues. It is also secreted by the thyroid gland, but the amounts are practically insignificant. Thus measurement of $rT_3$ concentration in serum reflects both tissue supply and metabolism of $T_4$ and identify conditions that favor this particular pathway of $T_4$ degradation. The normal range in adult serum for $rT_3$ is 14-30 ng/dl (0.22-0.46 nmol/L)[1] although varying values have been reported. It is elevated in subjects with high TBG and in some individuals with Familial Dysalbuminemic Hyperthyroxinemia (FDH)[2]. Serum $rT_3$ levels are normal in hypothyroid patients treated with $T_4$, indicating that peripheral $T_4$ metabolism is an important source of circulating $rT_3$[20]. Values are high in thyrotoxicosis and low in untreated hypothyroidism. High values are normally found in cord blood and in newborns[21].

### Thyrotropin or Thyroid Stimulating Hormone (TSH)

Likely to all pituitary hormones, TSH is secreted pulsately and has a circadian rhythm. Serum TSH concentrations are highest in the evening at 23 hours, during the first hours of sleep. The serum TSH values vary as the age changes.

Normal range is approximately 0.5-4.5 mU/L[1]. The American Association of Clinical Endocrinologists (AACE) has revised these guidelines as of early 2003, narrowing the range 0.3-3.0 mU/L. The majority of practitioners including endocrinologists and the physicians who specialize in thyroid disease rely solely on the TSH test as the primary test, for diagnosing and managing most thyroid conditions[22]. Moreover, to minimize the cost of a TFT the study was aimed to determine if TSH or $FT_4$ alone as a first-line test would be adequate in assessing the thyroid hormone status of patients. Analyzed TFT records from January 1996 to May 2000 in the Port Moresby General Hospital was done. The biochemical status of 95% of patients will be appropriately categorized as euthyroidism, hypothyroidism or hyperthyroidism with only 5% discrepant (i.e., normal TSH with abnormal $FT_4$) results. In contrast, using $FT_4$ alone as a first-line test correctly classifies only 84% of TFTs. Euthyroid status is observed in 50% of patients and $FT_4$ assays on these samples will be excluded appropriately if a TSH-only protocol is adopted. This will save a quarter of the yearly cost of Thyroid Function Test (TFT) on reagents alone by performing TSH only. Hence TSH alone is an adequate first-line thyroid function test, when it is normal no further $FT_4$ test is necessary unless clinically indicated[23].

TSH's production is controlled by thyrotrophin-releasing hormone (TRH), a tripeptide (pyroglutamyl–Histidyl–Proline amide) produced in peptidergic neurons of the hypothalamic paraventricular nuclei. TSH release is controlled through negative feedback by the thyroid hormones. TRH test may be needed to diagnose hyperthyroidism in a hospitalized patient with a basal sensitive TSH level of less than 0.1 microU/ml because a detectable TRH response contraindicates hyperthyroidism whereas hyperthyroid patients with nonthyroidal illness have the expected absent response.

Hypothyroidism must be diagnosed on the basis of both a high TSH level and a low $FT_4I$ because an isolated high TSH value may merely reflect the recovery phase of a nonthyroidal illness. No clinical urgency exists for subclinical hypothyroidism[24]. Serum thyrotropin measurements in the community were studied that showed most abnormalities of serum thyrotropin concentrations are transient. This showed variation of TSH different at different times hence diagnosis cannot be totally relied upon value of TSH[25]. The pit falls of TSH measurements are nonthyroidal illness (*sick euthyroid syndrome*), changing thyroid status, central hypothyroidism, hyperthyroidism associated with inappropriate TSH secreation, and central resistance to thyroid hormone[9].

### Serum Thyroglobulin (Tg)

Tg is the principal iodoprotein of the thyroid gland, that is produced by normal thyroid tissue and also by neoplastic follicular cells; then it is released into the circulation. Hence serum Tg measurement can be used in clinical practice as a specific and sensitive tumour markers of differentiated thyroid cancer[26]. Tg concentration in serum of normal adults range from less than 1 to 25 ng/mL (<1.5 to 38 pmol/L), with the mean levels of 5 to 10 ng/ml.Values are slightly higher in females than in males. In neonatal period and during the third trimester of pregnancy, mean values are approximately two fold to fourfold higher. The gradual decline is seen from infancy to adolescence. Pituitary TSH regulates the secretion of Tg as the serum Tg is in positive correlation with TSH. Elevated serum Tg reflects increased secretory activity by stimulation of thyroid gland or damage to thyroid tissue, whereas values below or at the level of detectability indicate a paucity of thyroid tissue or suppressed activity. Serum Tg increases in early phase of subacute thyroiditis, TBG deficiency which are TSH mediated and in TSH nonmediated (Graves' disease & trophoblastic disease)[1,39]. A combined Tg-antithyroglobulin antibody is more valuable than measuring only Tg for recurrent and persistent diseases with differentiated thyroid disease[27]. Detectable levels of thyroglobulin are commonly observed in patients who had undergone incomplete thyroidectomies and $^{131}I$ remnant ablations. The baseline or stimulated serum Tg levels when greater then or equal to 2 ng/mL specifies the need for neck ultrasound and further scanning. In certain series of patients of differentiated thyroid cancer following thyroidectomy, about 21% of incidence of metastases showed serum Tg less then 1 ng/mL (while receiving thyroxine for TSH suppression), so stimulated serum Tg must be always used with neck ultrasound[11]. Patients with undetectable serum Tg levels without receiving levothyroxine therapy may be considered free of disease[28].

### Radio Iodine Uptake (RAIU) study

This assay is used to measure the ability of the thyroid gland to trap iodine. A number of radioisotopes are now available for investigative procedures and provision of more sophisticated and sensitive detection devices has substantially decreased dose and radiation exposure required for studies. $^{131}I$ an average dose given for scanning purposes is 50 μCi. Isotopes with slower physical decay, such as $^{125}I$ and $^{131}I$, are particularly suitable for long-term studies. Conversely, isotopes with faster decay, such as $^{123}I$ and $^{132}I$, usually deliver lower radiation dose and are advantageous for short term and repeated studies[29]. Normal values of RAIU uptake will depend on Iodine content in geographic region and also related to age (children having higher iodine uptake than adults)[30]. RAIU is the measure of the avidity of the thyroid gland for iodide and its rate of clearance relative to kidney, but the results of this test does not equate with the hormone production or release. Disease states like *hyperthyroidism* (Graves' disease, Plummer's disease, trophoblastic disease, resistance to thyroid hormone, TSH-producing pituitary adenoma), nontoxic goiter (endemic, inherited biosynthetic defects, generalized resistance to thyroid hormone, Hashimoto's thyroiditis) and excessive hormonal loss (nephritis, chronic diarrhea, hypolipidemic resins, diet high in soybean), decreased renal clearance of iodine (renal insufficiency, severe heart failure), recovery of suppressed thyroid (withdrawal of thyroid hormone and antithyroid

drug administration, subacute thyroiditis, iodine induced myxedema), Iodine insufficiency (endemic and sporadic dietary deficiency, excessive iodine losses in pregnancy or in dehalogenase defect), and TSH administration, the RAIU is elevated. But in case of *hypothyroidism* (primary or secondary), syndromes of TSH resistance, thyroid dysgenesis (hypoplasia, ectopy or agenesis), Na/I symporter defect, defect in iodide concentration (inherited trapping defect, early phase of subacute thyroiditis, transient hypothyroidism), suppressed thyroid gland caused by thyroid hormone (hormone replacement, thyrotoxicosis factitia, struma ovarii), and iodine excess (dietary, drugs) the RAIU is decreased[1].

### Iodide Uptake Study Using Radionucleides

The use of $^{99m}$Tc pertechnetate is ideal for studying the trapping of iodide by thyroid. It is available easily in all nuclear medicine laboratories and low of cost. It gives information regarding anion trapping. The administration amount ranges from 2 to 10 mCi (74 to 370 MBq) and imaging of thyroid is usually began 15 to 30 minutes after i.v. injection[31].This was followed by a single daily intake of 2 µg/kg of L-thyroxine, for 10 days. Thyroid imaging and uptake were then repeated. $^{99m}$Tc pertechnetate uptake after L-thyroxine suppression had a mean reduction of 58-87% in comparison to the baseline level. In trials all subjects were euthyroid by clinical and laboratory criteria and none complained of side-effects, despite significant suppression of TSH levels. This method was efficient for demonstration of autonomous thyroid tissue, since none of the patients showed significant reduction of thyroid uptake after L-thyroxine suppression compared with the control group. This test was effective as that of the original $T_3$ suppression test and more convenient to the patient with no side-effects, easy hormonal intake, low dosimetry and short stay in the nuclear medicine laboratory[32].

The thyroidal uptake of Thyroid suppression $^{99m}$Tc is approximately 10 times lower for $^{123}$I (0.4 to 4%). $^{123}$I images of gland may be obtained any time between 4 hours and 24 hours after administration[31].

### Calcitonin (CT)

Calcitonin is secreted by parafollicular C cells having low or barely detectable level of serum CT (sCT) in normal subjects. Increased sCT levels highly suggests of medullary thyroid carcinoma (MTC). Thyroid nodule is clinical manifestation of MTC that is either single or multinodular goiter, so the routine measurement of sCT is useful for evaluating thyroid nodule(s) which will facilitates the diagnosis of MTC. sCT measurement is far more sensitive than cytology in finding MTC. The major benefits is of this clinical practice is that it alerts surgeon for the need to perform total thyroidectomy and central compartment lymphadenectomy, being the minimal surgical treatment for MTC and outcome of MTC is favourably affected because it is usually identified at a less advanced stage. However, other non-MTC causes of hypercalcitoninaemia and false sCT positivity do exist and must be recognized[33]. If basal sCT exceeds 10 pg/ml, then it is analyzed by pentagastrin stimulation testing, after renal insufficiency and proton pump inhibitor medication have been ruled out. Thyroidectomy is advised in the individuals MTC which CT values >100 pg/ml. If stimulated CT exceeds 200 pg/ml, thyroidectomy and lymphadenectomy is strongly recommended. Pentagastrin-stimulated CT values <100 pg/ml are associated with a low risk of MTC, or very rarely, non-metastasizing micro-MTC (size <10 mm). Therefore, regular clinical and biochemical follow-up is the preferred treatment in such patients, unless thyroid malignancy is suspected otherwise[34]. Certain studies indicate the use of routine calcitonin screening for detection of medullary thyroid cancer (MTC) in patients with thyroid nodules that may improve patient outcomes. However, routine sCT screening in patients for thyroid nodules evaluation appears to be cost-effective, comparable to the measurement of thyroid stimulating hormone, colonoscopy, and mammography screening[35]. However, initial examination included thyroid examination, thyroid scans or ultrasonography, measurements of serum free triiodothyronine ($T_3$), free thyroxine ($T_4$), thyrotropin (TSH) levels, and antithyroid autoantibodies. Fine needle aspired cytology (FNAC) was performed in all patients with palpable or visible thyroid nodule in ultrasonography, and pentagastrin stimulation test[36].

### Fine Needle Aspiration Cytology (FNAC)

FNAC are viewed as 'gold standard' for diagnosis in most cases, as they play a vital role in selection of patients for surgery. The diagnostic accuracy is nearly 98%, with fewer than 2% false positives and false negatives. Miller et al[37] compared FNAC and cutting needle biopsy. FNAC

examination could detect majority of carcinomas, whereas additional procedure done by cutting needle biopsy especially for larger nodules (more then 2-3 cm) helped in diagnosis. FNAC is performed with guidance of ultrasound especially in smaller or partially cystic nodule and non palpable of 0.5 cm could be biopsied using this technique. FNAC is a minimally invasive, highly accurate and cost-effective procedure for the assessment of patients with thyroid lesions[38]. FNAC can also be performed both as therapeutic technique and as diagnostic tool if the nodule is cold and cystic, that will detect the percentage of cystic adenocarcinomas. If the nodule is cold and totally or partially solid, the therapeutic decision will depend on results of FNAC[39]. Molecular testing of thyroid nodules enhances the accuracy of FNAC cytology and is of particular value for thyroid nodules with indeterminate cytology. So far thirty-two mutations were found, including 18 *BRAF*, 8 *RAS*, 5 *RET/PTC*, and 1 *PAX8/PPAR*[40].

### Ultrasonography

The technique is noninvasive, involves less time, less expensive and more sensitive than scintiscanning. It was popularly known to be first line evaluation of thyroid nodules. It is the good technique which indicates cystic areas capsule around the nodule and lobe size[39].

### Magnetic Resonance Imaging (MRI)

MRI images are generated by computer – assisted analysis of the interaction of electromagnetic waves of a specific frequency and hydrogen atoms in patients body. The two properties of MRI are termed as T1 and T2. The hydrogen atoms of various tissues have specific T1 and T2 properties, differences in T1-weighted and T2-weighted images can be used to identify the thyroid gland, skeletal muscle, blood vessels or lymph nodes[41]. In general, normal thyroid is slightly more intense than muscle on a T1-weighted image whereas thyroid tumor appears still more intense or brighter[42]. The scintigraphy and ultrasonography are the primary imaging modalities for investigating thyroid disorders whereas MRI is used for specific indications, that evaluate the extent of substernal goiters, thyroid carcinomas, and localizing recurrent sites of thyroid neoplasia. MRI investigation is also done for congenital disorders of the thyroid gland, even evaluation of diffuse thyroid disease, such as Graves' disease, Hashimoto's thyroiditis, Riedel thyroiditis and hemochromatosis[43].

### Positron Emission Tomography (PET)

PET is a diagnostic technique which has become an important method in oncology. The basis for this test is the injection of a positron-emitting radionuclide that localizes in cancers and allows imaging. The most exclusively used is $^{18}$F –Fluorodeoxyglucose ($^{18}$FDG) and it is a radiolabeled analogue of glucose, actively concentrated in variety of malignant tumors including carcinoma[44]. PET plays an important role in the management of thyroid cancer patients. It may be involved in initial, sometimes inadvertent, diagnosis, in postoperative evaluation, in detection of occult metastases, in the evaluation of thyroid nodules, and also in prognosis of metastatic disease[45].

### Molecular Basis of Thyroid Cancer: Diagnostic and Therapeutic Implications

Thyroid cancer though uncommon it is estimated lifetime risk of 0.8% for women and 0.3% for men. The incidence appears to be increasing and now it is currently the eighth commonest cancer in women[46]. *Fagin*[47] reported MAP kinase-signaling cascade in thyroid cancer. He reported on studies using various inhibitors of thyroid oncogenes *RET, RAS, MAPK* and *BRAF* can be beneficial in subjects with radioiodine refractive papillary thyroid cancer and other inhibitors are mammalian target of rapomycin *(mTOR)* act as a distal effector of TSH and of signaling systems that involves the phosphoinositide cascade. Similarly *Williams*[47] reported on the genetics of thyroid tumors in the context of the occurrence of multiple tumours. He also mentioned that in 1965 about 10% of medullary thyroid cancer was thought to be genetically mediated while today this figure has increased to about 25-30%. Hence up to 70% of non-medullary thyroid cancer is thought to be genetically mediated. He emphasized the importance of studying gene involvement in tumors such as Cowden's or familial adenomatous polyposis (FAP) and also of the difficulty of determining the multiplicity of papillary thyroid cancers. The Author also commented that thyroid carcinoma can be resulted from the interaction germline mutations and environmental factors such as radiation or iodine deficiency with consequent TSH hyperstimulation. Recently *Dumont*[47] reported on the application of microarray technology to the study of gene expression in various thyroid regulatory pathways and even regulatory pathways common to all tumours, whereas *Fusco*[47] described altered expres-

sion of micro RNA (miRNA) in differentiated and undifferentiated thyroid cancer. MicroRNAs (miRNAs) is a recently identified class of small endogenous non coding RNAs that act as negative regulators of the protein-coding gene expression that may impact on cell differentiation, proliferation and survival, a fundamental cellular processes implicated in carcinogenesis. miRNA expression is deregulated in many types of human cancers, that can also include thyroid cancer. Hence the findings of miRNA in deregulation in thyroid tumors can show a potential role in thyroid cancer biology and molecular diagnostics[48]. *Haugen*[47] described the possible thyroidal side effects of new therapeutic agents such as tyrosine kinase inhibitor (TKI).

Follicular Variant of Papillary Thyroid Carcinoma (FVPTC) has become a diagnostic challenge for clinicians and pathologists. Molecular features of FVPTC may be similar in both pathologic features as well as in clinical behavior of follicular adenoma or carcinoma and histopathological diagnosis is difficult[49]. Elastography is a newly developed technique that uses ultrasound to provide an estimation of tissue stiffness, to differentiate malignant from benign lesions, to diagnosis of thyroid cancer, especially in indeterminate nodules on cytology with more accuracy[50].

Thyroid-stimulating hormone receptor messenger ribonucleic acid is a useful marker circulating in cancer that helps in diagnosis and management of differentiated cancer (DTS). When TSHR mRNA measured with fine needle aspiration (FNA) promotes preoperative detection of cancer in patients with thyroid nodules, reducing unnecessary surgeries, and immediate postoperative levels can predict residual/metastatic disease[51].

In human thyroid papillary carcinoma, follicular or classical variant it shows that level of the $P2X_7$ receptor ($P2X_7R$) much higher level than normal thyroid tissue. Hence it is a new potential marker of the disease[52].

### *Serological Tests for Specific Disorders*

Circulating antithyroid antibodies, specifically Antimicrosomal (AMA) and Antithyroglobulin (ATA) antibodies are usually present in patients with autoimmune thyroid disease. Anti-TPO was formerly known as Antimicrosomal antibodies. Anti-TPO autoantibodies are found in over 90% of patients with autoimmune hypothyroidism and Graves' disease. TgAb are found in less than 60% of patients with lymphocytic thyroiditis and 30% of Graves' disease patients. Autoimmune thyroid disease (AITD) is commonly seen in women between 30-50 yrs of age. It can cause several forms of thyroiditis ranging from hypothyroidism (Hashimoto's thyroiditis) to hyperthyroidism (Graves' disease). Graves' disease is one tenth as common as hypothyroidism and common in younger individual. Autoimmune thyroid disease is the result of a complex interaction between genetic and environmental factors. Genetic factors lies in HLA complex (HLA DR-3) and the T cell regulatory gene (CTLA 4) and environmental factors like viral infection, smoking, stress and iodine intake are associated with the disease progression. The main hallmarks of AITD are antibodies to thyroid peroxidase (TPO), thyroglobulin (Tg) and Thyroid stimulating hormone receptor (TSH R)[53]. In case of atrophic thyroiditis, the major antibody is the TSH-R blocking antibody. This results in diminished thyroid hormone output, atrophy of thyroid gland and the clinical state of hypothyroidism. Other antibody is $Na^+$/I-symporter (NIS) which is the fourth major thyroid autoantigen[54].

## References


1) Roy EW, Sharon YW, Samuel R. Diagnostic Tests of the Thyroid, In: DeGroot LJ, Leslie J, Jameson JL et al, eds. Endocrinology, volume 2, 5th edition. USA: Elsevier Saunders, 2006, pp 1899-1913.
2) Koutras DA. Disturbances of menstruation in thyroid disease. Ann NY Acad Sci 1997; 816: 280-284.
3) Carl AB, Edward RA, David EB. Reference information for Clinical Laboratory. Table 56-1 Reference intervals and values, In: Burtis, Carl A, Ashwood, Edward R et al, eds. Tietz textbook of Clinical Chemistry and Molecular diagnostics, 4th edition. USA: Elsevier Saunders, 2006, p 2298.
4) Chonchol M, Lippi G, Salvagno G, Zoppini G, Muggeo M, Targher G. Prevalence of subclinical hypothyroidism in patients with chronic kidney disease. Clin J Am Soc Nephrol 2008; 3: 1296-1300.
5) Welle S, O' Connell M, Danforth E Jr, Campbell R. Decreased free fraction of serum thyroid hormones during the carbohydrate overfeeding. Metabolism 1984; 33: 837-839.
6) Das BK, Baral N, Shyangwa PM, Toora BD, Lamsal M. Altered serum levels of thyroxine, triiodothyroinine and thyroid stimulating hormone in patients with depression. Kathmandu Univ Med J 2007; 5: 330-334.

7) Dumitrescu AM, Liao XH, Best TB, Brockmann K, Refetoff S. A novel syndrome combining thyroid and neurological abnormalities is associated with mutations in a monocarboxylate transporter gene. Am J Hum Genet 2004; 74: 168-175.

8) Dumitrescu AM, Liao XH, Weiss RE, Millen K, Refertoff S. Tissue-specific thyroid hormone deprivation and excess in monocarboxylate transporter (mct)8-deficient nice. Endocrinology 2006; 147: 4036-4043.

9) Peter AS. Clinical Approach to thyroid Function Testing. In: Stephen A. Falk eds Thyroid Disease: Endocrinology, surgery, nuclear medicine and radiotherapy, 2 edition. Philadelphia: Lippincott-Raven Publishers, 1997, pp 41-52.

10) Surks MI, Chopra IJ, Mariash CN, Nicoloff JT, Solomon DH. American Thyroid Association Guidelines for use of laboratory tests in thyroid disorders. JAMA 1990; 263: 1529-1532.

11) Fitzgerald PA. Endocrinology, Disease of Thyroid gland. In: Maxin AP, Stephen JMP, Lawrence M et al, eds. Current Medical diagnosis and treatment, 49th edition. USA: Mc Graw Hill publication; 2007, pp 1138-1142.

12) Carole S. Assay of thyroid hormones and related. Thyroid and its disease: last revised by February 6, 2004.

13) Ekins R. Measurement of free hormones in blood. Endocrine Rev 1990; 1: 5-6.

14) Szkudlinski MW, Kazlauskaite R, Weintraub BD. Thyroid-stimulation Hormone and Regulation of thyroid Axis, In: DeGroot LJ, Leslie J, Jameson JL et al, eds. Endocrinology, volume 2, 5th edition. USA: Elsevier Saunders, 2006, pp 1803-1860.

15) Gurnel M, PaoloBeck P, Chatterjee VK. Resistance to Thyroid Hormone, In: DeGroot LJ, Leslie J, Jameson JL et al, eds. Endocrinology, volume 2, 5th edition. USA: Elsevier Saunders, 2006, pp 2227-2234.

16) Delange F, Camus M, Ermans AM. Circulating thyroid hormones in endemic goiter. J Clin Endocrinol Metab 1972; 34: 891-895.

17) Sadow PM, Chassande O, Sadow, Koo EK, Gauthier K, Samarut J, Xu J, O'Malley BW, Weiss RE. Regulation of expression of thyroid hormone receptor isoforms and coactivators in liver and heart by thyroid hormone. Mol Cell Endocrinol 2003; 203(1-2): 65-75.

18) Koenig RJ. Ubiquitinated deiodinase: not dead yet. J Clin Invest 2003; 112: 145-147.

19) Nelson JC, Tomei RT. Direct determination of free thyroxin in undiluted serum by equilibrium dialysis/radioimmunoassay. Clin chem 1988; 34: 1737-1744.

20) Chopra IJ. A radioimmunoassay for measurement of 3,3',5'-triiodothyronine (reverse T3). J Clin Invest 1974; 54: 583-592.

21) Chopra IJ. A radioimmunoassay for measurement of 3, monoiodothyronine. J Clin Endocrinol Metabol 1980; 51: 117-123.

22) Wartofsky L, Dickey RA. Controversy in clinical endocrinology: the Evidence for a narrower thyrotropin reference range is compelling. J Clin Endocrin Metab 2005; 90: 5483-5488.

23) Kende M, Kandapu S. Evaluation of thyroid stimulating hormone (TSH) alone as a first-line thyroid function test (TFT) in Papua New Guinea. PNG Med J 2002; 45: 197-199.

24) Spencer CA. Clinical utility and cost-effectiveness of sensitive thyrotropin assays in ambulatory and hospitalized patients. Mayo Clin Proc 1988; 63: 1214-1222.

25) Joseph M, Rotman-Pikielny P, Michael S. Serum Thyrotropin Measurement in the community: Five year follow up in a large network of primary care physicians. Arch Intern Med 2007; 167: 1533-1538.

26) VanHerle AJ, Uller RP. Elevated serum Thyroglobulin-A marker of metastases in differentiated thyroid carcinomas. J Clin Invest 1975; 56: 272-277.

27) Aras G, Gultekin SS, Kucuk NO. The additive clinical value of combined thyroglobulin and antithyroglobulin antibody measurements to define persistent and recurrent disease in patients with differentiated thyroid cancer. Nucl Med Commun 2008; 29: 880-884.

28) Ozata M, Suzuki S, Miyamoto T, Liu RT, Fierro-Renoy F, DeGroot LJ. Serum Thyroglobulin in the follow-up of patients with treated differentiated thyroid cancer. J Clin Endocrinol Metab 1994; 79: 98-105.

29) MIRD/Dose Estimate report No 8: Summary of Current radiation dose estimates to humans from 99mTc as sodium pertechnetate. J Nucl Med 1976; 17: 74-77.

30) Modified from DeGroot LJ, ReedLarsen P, Hennemann G, et al. Thyroid and its diseases. New York: John Wiley and Sons, 1984.

31) Blum M. Thyroid imaging In: DeGroot LJ, Leslie J, Jameson JL et al, eds. Endocrinology, volume 2, 5th edition. USA: Elsevier Saunders, 2006, pp 1963-1977.

32) Ramos CD, Zantut-Wittmann DE. Thyroid suppression test with L-thyroxine and [99mTc] pertechnetate Clinical endocrinology 2000; 52: 471-477.

33) Elisei R. Routine serum calcitonin measurement in the evaluation of thyroid nodules. Best Pract Res Clin Endocrinol Metab 2008; 22: 941-953.

34) Karges W, Dralle H, Raue F, Mann K, Reiner C, Grussendorf M, Hüfner M, Niederle B, Brabant G, German Society for Endocrinology (DGE)–Thyroid Section. Calcitonin measurement to detect medullary thyroid carcinoma in nodular goiter: German evidence-based consensus recommendation. Exp Clin Endocrinol Diabetes 2004; 112: 52-58.

35) Cheung K, Roman SA, Wang TS, Walker HD, Sosa JA. Calcitonin measurement in the evaluation of thyroid nodules in the United States a cost-effectiveness and decision analysis. J Clin Endocrinol Metab 2008; 93: 2173-2180.

36) Hahm JR, Lee MS, Min YK, Lee MK, Kim KW, Nam SJ, Yang JH, Chung JH. Routine measurement of serum calcitonin is useful for early detection of medullary thyroid carcinoma in patients with nodular thyroid diseases. Thyroid 2001; 11: 73-80.

37) Miller JM, Hamburger JI, Kini S. Diagnosis of thyroid nodules.Use of fine needle aspiration and needle biopsy. JAMA 1979; 241: 481-484.

38) Uma H, Sukant G, Harsh M, et al. Role of fine needle aspiration cytology in diagnosis and ¬management of thyroid lesions: A study on 434 patients. J Cytology 2008; 25: 13-17.

39) Pacini F, DeGroot LJ. Thyroid Neoplasia In: DeGroot LJ, Leslie J, Jameson JL, et al, eds. Endocrinology, volume 2, 5th edition. USA: Elsevier Saunders, 2006, pp 2147-2180.

40) Yuri E. Nikiforov, David L. Steward, Toni M. Robinson-Smith, Bryan R. Haugen, Joshua P. Klopper, Zhaowen Zhu, James A. Fagin, Mercedes Falciglia, Katherine Weber, Marina N. Nikiforova. Molecular Testing for Mutations in Improving the Fine Needle Aspiration Diagnosis of Thyroid Nodules. J Clin Endocrinol Metab 2009; 10.1210/jc.0247. Published online on March 24, 2009.

41) Higgins CB, Auffermann W. MR imaging of thyroid and parathyroid glands-A review of current status. Am J Roentgenol 1988; 151: 1095-1106.

42) Tennvall J, Biorklund A, Moller T, et al. Studies of MRI relaxation times in malignant and normal tissues of the human thyroid gland. Prog Nucl Med 1984; 8: 142-148.

43) Gotway MB, Higgins CB. MR imaging of the thyroid and parathyroid glands. Magn Reson Imaging Clin N Am 2000; 8: 163-182.

44) MacDougall IR. Positron emission tomography in well differentiated thyroid cancer. In: Leonard W, Douglas VN. Thyroid cancer–A comprehensive guide to clinical management. 2nd edition. Place: Humana Press, 2006, pp. 319-328.

45) Robbins RJ, Larson SM. The value of positron emission tomography (PET) in the management of patients with thyroid cancer. Best Pract Res Clin Endocrinol Metab 2008; 22: 1047-1059.

46) Nix P, Nicolaides A, Coatesworth AP. Thyroid cancer review 1-presentation and investigation of thyroid cancer. Int J Clin Pract 2005; 59: 1340-1344.

47) Proceeding of the 32nd Annual meeting, European thyroid Association. Germany, 2007: September 1-5. Horm Res 2007; 68: 1-91.

48) Nikiforova MN, Chiosea SI, Nikiforov YE. MicroRNA expression profiles in thyroid tumors. Endocr Pathol 2009; 20: 85-91.

49) Salajegheh A, Petcu EB, Smith RA, Lam AK. Follicular variant of papillary thyroid carcinoma: a diagnostic challenge for clinicians and pathologists. Review article PG medical Journal–continuing professional development 2008; 84: 78-82.

50) Rago T, Santini F, Scutari M, Pinchera A, Vitti P. Elastography: new developments in ultrasound for predicting malignancy in thyroid nodules. J Clin Endocrinol Metabol 2007; 92: 2917-2922.

51) Chia S, Milas M, Reddy SK, Siperstein A, Skugor M, Brainard J, Gupta MK. Thyroid-stimulating hormone receptor messenger ribonucleic acid measurement in blood as a marker for circulating thyroid cancer cells and its role in the preoperative diagnosis of thyroid cancer. J Clin Endocrinol Metab 2007; 92: 468-475.

52) Solini A, Cuccato S, Ferrari D, Santini E, Gulinelli S, Callegari MG, Dardano A, Faviana P, Madec S, Di Virgilio F, Monzani. Increased P2X7 receptor expression and function in thyroid papillary cancer: a new potential marker of the disease? Endocrinology 2008; 149: 389-396.

53) Binoy K, Mohanty MS, Truptirekha S. Autoimmune thyroid failure is higher than usual among thyroid antibody-positive individuals on a more than adequate or excessive iodine diet. Indian J Clin Biochem 2005; 20: 9-17.

54) Li Y, Teng D, Shan Z, Teng X, Guan H, Yu X, Fan C, Chong W, Yang F, Dai H, Gu X, Yu Y, Mao J, Zhao D, Li J, Chen Y, Yang R, Li C, Teng W. Antithyroperoxidase and antithyroglobulin antibodies in a five-year follow-up survey of populations with different iodine intakes. J Clin Endocrinol Metab 2008; 93: 1751-1757.