Review Article

Interpretation of thyroid function tests

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Thyroid disorders are commonly encountered in clinical practice. Diagnosis and confirmation of thyroid disorders usually depends on accurate measurement and interpretation of thyroid function tests. The thyroid hormones (TH), T3 and T4 exert a negative feedback on TRH and TSH which helps maintain equilibrium. Modest changes in TH lead to major changes in TSH and hence TSH is often used as a screening test for thyroid hormone disorders. However, measurement of TH (Total T3 and T4 or Free T3 and T4) are often necessary for accurate interpretation and treatment of thyroid disorders. Raised T4 and T3 with suppressed TSH is suggestive of thyrotoxicosis and low T4 and T3 with elevated TSH is suggestive of hypothyroidism. Thyroid peroxidase antibodies and Thyroid stimulating hormone receptor autoantibodies (TRAbs) are useful for diagnosis of Hashimoto's thyroiditis and Graves' disease respectively. Serum Thyroglobulin is useful in follow-up of patients who are treated for thyroid cancer. Assay interference should be considered in cases where thyroid function test results do not correlate with the clinical status.

[*J Indian Med Assoc* 2018; **116:** 35-8 & 41]

Key words **: Thyroid hormones, thyroid function test, TRAbs.**

Thyroid disorders are commonly encountered endocrine problem in clinical practice. In recent studies in Indian school children and adults, goiter rate was 15.5% and 9.6%; hypothyroidism was present in 7.3% and 21% and hyperthyroidism in 0.3% and 0.6%, respectively. Prevalence of antithyroid peroxidase antibodies (TPOAb) was around 3.7% among children and adolescents and 13.3% among adults[1,2]. Patients with thyroid disorders often manifest symptoms and/or signs that are non-specific, and present to clinicians in many different specialties. A high clinical index of suspicion is required for diagnosis and confirmation of diagnosis usually depends on accurate measurement and interpretation of thyroid function tests (TFTs). In the majority of cases, the results of thyroid function tests (TFTs) are straightforward, and consistent with the clinical impression of thyroid status. However, in a small, but significant subgroup of patients, the interpretation of TFTs is more challenging, either because the results are not consistent with the clinical picture (eg, normal TSH in a patient with suspected thyrotoxicosis), or because different measurements appear to contradict each other (eg, raised thyroid hormones concentrations, but with non-suppressed TSH). Furthermore, recently laboratory values of thyroid function have gathered importance in view of rapidly changing cut-offs for the treatment of thyroid disorders during pregnancy[3]. Therefore, careful clinical reassessment of thyroid status, together with considering possible confounding factors [eg, pregnancy, intercurrent (non-thyroidal) illness, drug therapy] readily identifies the cause of apparently anomalous/discordant TFTs. 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]); and pathological assessment (fine needle aspiration).

- **Though TSH an ideal screening test for thyroid disorders, is often inadequate for accurate diagnosis.**
- **Total T3, T4 or free T3, T4 with TSH is necessary to start treatment.**
- **Anti-TPO and TRAbs are useful for diagnosis of Hashimoto's thyroiditis and Graves' disease respectively. Serum Tg is useful in follow-up of thyroid cancer.**
- **Assay interference should be considered where test results do not correlate clinicaly.**

Understanding Thyroid Physiology is Crucial for Interpretation of TFTs :

The hypothalamus-pituitary-thyroid (HPT) axis determines the set point of thyroid hormone (TH) production. Hypothalamic thyrotropin-releasing hormone (TRH) stimulates the synthesis and secretion of pituitary thyrotropin (thyroid-stimulating hormone, TSH), which acts at the thyroid to stimulate all steps of TH biosynthesis and secretion. The THs thyroxine (T4) and triiodothyronine (T3) control the secretion of TRH and TSH by negative feedback to maintain physiological levels of the main hormones of the HPT axis. Reduction of circulating TH levels due to primary thyroid failure results in increased TRH and TSH production, whereas the opposite occurs when circulating

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THs are in excess. Other neural, humoral, and local factors modulate the HPT axis and, in specific situations, determine alterations in the physiological function of the axis. The roles of THs are vital to nervous system development, linear growth, energetic metabolism, and thermogenesis. THs also regulate the hepatic metabolism of nutrients, fluid balance and the cardiovascular system. In cells, TH actions are mediated mainly by nuclear TH receptors, which modify gene expression. T3 is the preferred ligand of THR, whereas T4, the serum concentration of which is 100-fold higher than that of T3, undergoes extrathyroidal conversion to T3. This conversion is catalyzed by 5'-deiodinases (D1 and D2), which are TH-activating enzymes. T4 can also be inactivated by conversion to reverse T3, which has very low affinity for THR, by 5-deiodinase (D3). The regulation of deiodinases, particularly D2, and TH transporters at the cell membrane control T3 availability, which is fundamental for TH action.4,5

Changes in thyroid status are normally associated with concordant changes in TH and TSH concentrations (eg, raised T4 and T3 with suppressed TSH in thyrotoxicosis; low T4 and T3 with elevated TSH in hypothyroidism). However, the population reference ranges for TH are relatively broad – in contrast to the narrow individual variations of serum TH seen in normal subjects.6 As a result, changes in TH concentrations sufficient to render a subject hypo- or hyper-thyroid may not necessarily be associated with numerically abnormal T4 or T3 concentrations (as occurs in so-called 'subclinical' hypo- or hyperthyroidism). Accordingly, TSH has been recommended as a frontline screening test for thyroid dysfunction, as relatively modest changes in TH concentrations are associated with marked excursions in TSH. However, screening exclusively with TSH will result in misdiagnosis of some cases, whilst other conditions may be missed altogether (by virtue of returning a TSH result that falls within the reference range despite overt hypothalamic–pituitary–thyroid dysfunction). Conditions in which measurement of TSH alone may be misleading:

- Recent treatment for thyrotoxicosis (TSH may remain suppressed even when TH concentrations have normalised)
- Non-thyroidal illness
- TSH assay interference
- Central hypothyroidism (eg, hypothalamic/pituitary disorders)
- TSH-secreting pituitary adenoma (thyrotropinoma/TSHoma)
- Resistance to thyroid hormone (RTH)
- Disorders of thyroid hormone transport or metabolism

Laboratory Assessment of Thyroid Status :

It is also important to consider whether total (TT4 and TT3) or free (FT4 and FT3) TH levels are being measured. If the former, then changes in binding proteins can confound interpretation of results: T4 and T3 are heavily protein bound; thus, total, but not free, hormone measurements are affected by alterations in binding protein status (eg, exogenous oestrogen therapy and pregnancy increase TT4 levels through elevation of T4-binding globulin (TBG)).

Serum Thyroglobulin :

Major clinical value of measuring the level of serum Tg is in the management, but not in the diagnosis, of differentiated thyroid carcinoma[11,12]. Serum Tg concentrations are increased in patients with both benign and differentiated malignant follicular-cell derived tumors of the

Table 1 — *Comparison of Total and Free T3/T4*

Total T3	Total T4	Free T3 and T4	TSH and free T4
Routine measurement of serum T3 is not carried out, total T3 measurements performed in the following settings: • In patients suspected of having T3 thyrotoxicosis The combination of high T3, suppressed TSH and normal T4 is usually associated with toxic nodular goiter, whereas T3 and T4 are typically both elevated in Graves' disease (although T3 is usually more elevated than T4)[7] • In patients taking drugs that inhibit the peripheral conversion of T4 to T3 (such as dexamethasone, propranolol, PTU, amiodarone, and iodine-containing contrast media)[8]	Although total T4 does not give a true picture of the thyroid status of an individual, measurement of total serum T4 levels is highly sensitive in reflecting the hyperthyroid (85-95%) and the hypothyroid status (80-90%) of patients.[9]	Free T3 / T4 level estimation is preferred over total T3/T4 estimation because these hormones are extensively (>99%) bound to plasma proteins and only the unbound forms are active.[10]	Both serum TSH and FT4 are required principally for disorders where the pituitary thyroid axis is not intact or is unstable. • Optimizing thyroxine therapy in newly diagnosed patients with hypothyroidism • Diagnosing and monitoring thyroid disorders in pregnancy • Monitoring patients with hyperthyroidism in the early months after treatment • Diagnosis and monitoring treatment for central hypothyroidism • End-organ TH resistance • Sick euthyroid state • TSH-secreting pituitary adenomas • Possible subclinical hypothyroidism: If screening is performed, and a high serum TSH concentration is found, and the FT4 is normal, the measurement should be repeated 3–6 months later, along with measurement of serum FT4, after excluding nonthyroidal illness and drug interference • Overtly hypothyroid patients (who have serum TSH >10 mU/L and low FT4 concentrations) should be treated with thyroxine.[9]

thyroid and do not serve to distinguish between the two. After total thyroid ablation for papillary or follicular thyroid carcinoma, Tg should not be detectable, and its subsequent appearance signifies the presence of persistent or recurrent disease[13].

Thyroid Peroxidase Autoantibodies :

Thyroid peroxidase (TPO) is a key enzyme in the formation of thyroid hormones and a major autoantigen in autoimmune thyroid diseases. TPO antibodies (TPOAb) are frequently present in euthyroid subjects (prevalence 12-26%)[14]. Approximately 70-80% of patients with Graves' disease and virtually all patients with Hashimoto's, atrophic thyroiditis or postpartum thyroiditis have TPOAb detected.15 A euthyroid subject with detectable TPOAb is at increased risk of development of hypothyroidism. Detectable level of TPOAb typically precedes the development of an elevated TSH and is therefore a risk factor for hypothyroidism.

Thyroglobulin Autoantibodies :

The functional consequence of anti-Tg antibodies is not clear as they do not cause thyroid cell destruction. Circulating antibodies could be detected in about 10% of healthy young subjects and 15% of people >60 years of age. Among Hashimoto's thyroiditis patients, antibody prevalence was 60-80% and in 50-60% in Graves' disease patients. Another study identified anti-Tg antibodies in 70-80% of autoimmune thyroid disorders patients, 30-40% of Graves' disease patients, and 10-15% of patients with non-thyroid immune disorders[16]. Anti-Tg antibodies can cross the placenta barrier, but the effect on the neonate is unclear[17].

Thyroid Stimulating Hormone Receptor Autoantibodies (TRAbs) :

Thyroid stimulating hormone receptor (TSH-R) is the prime autoantigen in Graves' disease and atrophic thyroiditis. It is located on the basal surface of thyroid follicular cells[18]. In Graves' disease, thyroid stimulating antibodies (TSAbs) bind to the receptor and stimulate the thyroid cell to produce excessive amount of thyroid hormones resulting in hyperthyroidism. In patients with atrophic thyroiditis, the major antibody is the TSH to its receptor, thus preventing stimulation of thyroid cell. This results in diminished thyroid hormone output, atrophy of thyroid gland, and the clinical state of hypothyroidism[18.19]. Quantitation of TRAbs may be a useful indicator of the degree of disease activity in an individual patient and can confirm the clinical diagnosis of Graves' disease in a scientific manner. Demonstration of TRAbs may also be of diagnostic value in the euthyroid patient with exophthalmos, especially when it is unilateral. Graves patients usually test positive for TRAb, and they may have related ophthalmopathy, whereas patients with toxic nodular goiter are TRAb-negative and do not have Graves ophthalmopathy[20,21]. Unfortunately, in patients with low or negative titers, the test is much less helpful. Furthermore, the presence of iodine deficiency may also interrupt the development of hyperthyroidism despite the presence of TRAbs. TRAbs are also useful for differentiating Gestational thyrotoxicosis from Graves' disease during pregnancy. High TRAbs in a pregnant woman with Graves' disease increase the likelihood that neonatal thyrotoxicosis in the offspring[3].

Special Considerations Interpreting Thyroid Function Tests :

Non-thyroidal illness :

The effects of acute illness (and some chronic illnesses) on thyroid hormones and TSH, has been known since the 1970s and is currently referred to as the non-thyroidal illness syndrome (NTIS) and is defined by the absence of an intrinsic abnormality of HPT function – rather it is considered a secondary adaptive change[22]. Whether it is a beneficial response (eg, to reduce metabolic rate) or a maladaptive response (with potential benefit from TH replacement therapy) has been much debated, but compelling evidence for the use of T3 or T4 therapy in most patients with NTI is currently lacking[23].

Many mechanisms have been implicated in NTIS, and they may be multiple in any one individual[24]. Amongst the most likely are:

- abnormal TRH and TSH secretion;
- defective deiodinase activity;
- thyroid hormone binding protein (thyroglobulin, albumin and transthyretin) and transporter (eg, MCT8) defects; and
- altered nuclear thyroid hormone receptor activity.

Although the exact mechanisms causing these changes are unknown, cytokines such as IL-1, IL-6, and TNF-a may be responsible in some types of NTIS[25].

The biochemical changes of NTIS may begin within the first 24 hours of illness.26 The most common abnormality is a reduction of free T3 (and a rise in reverse T3 which is metabolically inactive but not measured clinically). Patients are often significantly ill with a poor prognosis when fT4 also becomes reduced. In a minority of acutely ill subjects T4 and fT3 are elevated.4 During recovery from intercurrent illness, TH and TSH concentrations return to normal, although in some patients TSH may become overtly elevated for a short period of time. This rise in TSH typically precedes the increase in T4 and T3 concentrations, suggesting that it is required for the restoration of Euthyroidism[27]. It is important to be aware of this transient phenomenon in order to avoid inappropriate diagnosis and treatment.

Assay Interference :

In any patient in whom anomalous or discordant TFTs are not readily explained, consideration must be given to whether one or other laboratory result could be erroneous. Genetic and acquired causes of interference in both TH and TSH assays are well recognised and can be screened for (eg cross-reacting heterophilic antibodies causing falsely low or elevated TSH are readily detected through TSH dilution studies, which return non-linear results in this context). Interfering antibodies are intrinsic antibodies that can cause unpredictable results on thyroid testing. They can be heterophile (nonspecific) antibodies, human anti-animal antibodies or autoantibodies to TSH, T4 or T3. Although assays are designed to minimise such interferences, problems still occur in between 0.03% and 3% of all samples[28,29].

It is most commonly first suspected by the physician who sees a gross discordance between the clinical presentation of the patient and the laboratory test result. When antibody interference is suspected, the first step should be to remeasure both TSH and FT4 using a different manufacturer's platform but at the same time it is important to note that simply sending a sample to another laboratory does not necessarily exclude assay interference (the same interference can affect different assay platforms) and specialist laboratory and/ or endocrine advice should be sought in suspected cases[30].

Biotin is a B vitamin that acts as a cofactor for carboxylase enzymes involved in gluconeogenesis and fatty acid synthesis. It is produced by gut bacteria and normal daily intake is 35-350 mcg daily. It is used in the treatment of biotinidase deficiency and proprionic acidemia and as a supplement for TPN. It is frequently used by individuals in doses of 5,000 to 10,000 mcg daily as a supplement to improve hair and nail growth and to treat hair and nail disorders

Many laboratory platforms for the measurement of fT4, fT3 TSH and thyroglobulin depend on the strong binding of biotin and streptavidin. If patients ingest biotin in doses of 5,000 to 10,000 mcg prior to blood being drawn for these analytes, measurements of fT4 and fT3 will be falsely high and thyroglobulin and TSH will be falsely low as biotin interferes in the assays[31]. The combination of a high fT4 and low TSH mimics hyperthyroidism[32,33]. These effects correspond to the blood level of biotin with a peak effect seen several hours after ingestion and potentially even lasting until the next day. Variable times between ingestion and blood measurements can results in confusing variations in these measurements not corresponding to patents clinical status. Confirmation of this effect can be made by measuring several hours after ingestion and after abstaining for 48 hours or by re-measuring in an assay not utilizing biotin. This effect is not limited to thyroid hormone measurements but have also been reported for PTH, DHEA-sulfate, estradiol and ferritin.

Conclusion :

Thyroid function tests are useful for diagnosis and treatment of patients with suspected thyroid hormone disorders. TSH, though and ideal screening test for thyroid disorders, is often inadequate for accurate diagnosis. This necessitates the measurement of Thyroid hormones, T3 and T4 before starting treatment. In most cases interpretation of thyroid function test is relatively straightforward. However, in case of confounding results, an Endocrinologist may be consulted for correct interpretation of thyroid function test and management of thyroid disorders.

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