ABSTRACT
Thyroid hormones are secreted by thyroid gland. These hormones regulate many functions such as homeostasis, metabolic activities and normal growth and development in children. Differences either in the quantity or quality of these hormones from the normal levels lead to many abnormalities and have a negative effect on the function of one or more organ systems. Thyroid dysfunction progresses from early to more advanced forms. Moreover, today it is important to grade patients according to the results of thyroid function tests. Our study in different age and sex groups aims to characterise the abnormal condition based on the specific biochemical marker such as thyroid stimulating hormone TSH, triiodothyronine (T3) and tetraiodothyronine (T4).
KEYWORDS: Thyroid Stimulating Hormone TSH, Triiodothyronine (T3) and Tetraiodothyronine (T4).

INTRODUCTION
The thyroid gland is a part of the endocrine system, located in front of the lower neck, having a thin band of tissue called isthmus connecting 2 lobes, giving it a butterfly-like appearance. Iodine is selectively absorbed by the spherical follicles of the thyroid gland which is later utilized in the synthesis of iodine containing hormones, namely: thyroxine (T4) and triiodothyronine (T3). Another hormone called calcitonin is secreted by the parafollicular cells (C-cells) present in between the follicles. Homeostasis, metabolic activities and normal growth and development in children are dependent on the thyroid hormones. Reduction in the...
quantity or quality of these hormones leads to mental retardation in children and has a negative effect on the function of one or more organ systems.

**T3 and T4 production and action**

Thyroxine is synthesized by the follicular cells from the tyrosine residues of the protein called thyroglobulin (TG). Iodine, captured with the "iodine trap" is activated by the enzyme thyroid peroxidase (TPO) and linked to the 3' and 5' sites of the benzene ring of the tyrosine residues on TG. Upon stimulation by TSH, the follicular cells reabsorb TG and proteolytically cleave the iodinated tyrosine from TG, combining them into T4 and T3 (in T3, one iodine is absent compared to T4), and releasing them into the blood. In the blood, T4 and T3 are partially bound to thyroxine-binding globulin, transthyretin and albumin. Only the free fraction (not bound to these proteins) has hormonal activity.

Thyroid hormone receptors are present intracellularly, in nuclear chromatin or cytosol in the respective organs. When the hormone binds to the receptor, brings about gene expression and transcription producing mRNA which consequently is read for protein synthesis.

**T3 and T4 regulation**

**Regulation occurs in Three ways**

1. **Release of TRH from hypothalamus is inhibited by somatostatin and dopamine.** Apart from which secretion increases in cold; decreases in warmth and stress.

2. **Feedback action:** T₃ and T₄ act on the pituitary which releases TSH as well as on the hypothalamus which releases the TRH leading to inhibition of stimulation of thyroid gland. Increases secretion of T₃ and T₄ also leads to reduction in the TRH receptors on pituitary.

3. **Auto regulation:** the synthesis of the hormone depends on the iodine content in the blood. Increase in iodine content makes thyroid gland hyperactive while decrease makes it depressed.

**Calcitonin significance**

The synthesis of calcitonin is stimulated by hypercalcemic condition mainly exerting its effects on the bone. It plays an important role in calcium metabolism but the role is relatively small as there is very less fluctuation seen in calcium levels even after the removal of thyroid gland.\(^{[2]}\)
Iodine significance
It is essential for the production of 4 iodine containing hormone, thyroxine which is important for the metabolism and growth of animal kingdom. Lack of iodine in diet may cause enlargement of the gland as well as endemic goiter.

Thyroid disorders
The release of thyroid hormones is done by thyroid gland and is regulated by the pituitary which is in turn regulated by the hypothalamus. Over activity of any of these organs or glands leads to hyperthyroidism i.e., excessive thyroid hormone production and the under activity will lead to hypothyroidism i.e., deficiency of thyroid hormones. The pituitary gland senses the amount of thyroid hormone circulating in the blood and accordingly decreases or increases the stimulation of the thyroid gland affecting the production of the hormones either positively or negatively.

Causes for hypothyroidism
1. Hashimoto's thyroiditis (autoimmune thyroiditis)-most common cause wherein antibodies target the thyroid gland and destroy its ability to produce hormones.\(^7\)
2. Postpartum thyroiditis (inflammation of the thyroid gland after pregnancy)
3. Acute thyroiditis
4. Silent thyroiditis
5. Thyroid hormone resistance.

Causes for hyperthyroidism
1) Graves' disease
2) Toxic multinodular goiter
3) Toxic nodule ("hot" nodule)
4) Excessive intake of iodine
5) Hashitoxicosis
6) Medications (ingesting thyroid hormone).

The most common disorders that develop in the thyroid are:

1. **Hypothyroidism**
   Occurs due to the diminished activity of the thyroid gland, improperly developed at birth, surgically removed partially or wholly or becomes incapable of producing the hormone.
2. Hyperthyroidism
The most common cause is autoimmune disease called Graves’ disease, where the body ability to differentiate between its tissues and organs and foreign organisms, such as bacteria, is hampered.

3. Goiter
It is characterized by the enlargement of the thyroid gland due to hashimotos disease, Graves’ disease nutritional deficiencies etc.\cite{8}

4. Thyroid cancer
Small percentage of thyroid nodules is cancerous and it is more common in younger people mostly between the age groups of 22-50.

5. Thyroiditis\cite{9}
Swollen thyroid due to bacteria or virus is known as thyroiditis
(a) Subacute granulomatous thyroiditis: it is very painful around the neck, feeling of tenderness prevails.
(b) Acute infectious thyroiditis: often streptococcus or staphylococcus bacteria are responsible for this. It also comes under the painful type.
(c) Painless thyroiditis: It is thought that this type is responsible for 10\% of hyperthyroidism. Here thyroid does not become enlarged.

6. Hypothyroidism in pregnancy
It is rare as women with hypothyroidism do not ovulate or do not produce mature eggs. But it increases the chance of stillbirth or growth retardation of the fetus.

7. Hyperthyroidism in pregnancy
It occurs 1 in 2000 pregnancies, Graves’s disease bearing 95\% of the responsibility. Symptoms include some mimicking those of the normal pregnancy while the others include weight loss, vomiting, high BP, persistently fast heart rate.

Symptoms of thyroid diseases
Symptoms of hypothyroidism
In infants: constipation, poor feeding, poor growth, jaundice, excessive tiredness.
In children: excessive fatigue, Poor growth, and Poor school performance
In adults: Early symptoms like Easy fatigue, exhaustion, Poor tolerance to cold temperatures, Constipation, Carpal tunnel syndrome (pain at the wrists and numbness of the hands) and later symptoms like Poor appetite, Weight gain, Dry skin, Hair loss, Intellectual ability worsens, Deeper, hoarse voice, Puffiness around the eyes, Depression, Irregular menstrual periods or lack of menstrual periods.

Symptoms of hyperthyroidism
Insomnia, Hand tremors, Nervousness, Feeling excessively hot in normal or cold temperatures, Frequent bowel movements, Losing weight despite normal or increased appetite, Excessive sweating, Menstrual period becomes scant, or ceases altogether, Joint pains, Difficulty concentrating, Eyes seem to be enlarging.

Screening for thyroid diseases
Screening can be defined as “the application of a test to detect a potential disease or condition in a person who has no known signs or symptoms of that condition at the time the test is done.” Hence, screening with thyroid function tests may identify asymptomatic individuals who have mild, nonspecific symptoms such as cold intolerance or feeling “a little tired.”[2, 3] Subclinical thyroid dysfunction can be diagnosed before symptoms and complications occur, is viewed as a risk factor for developing hyperthyroidism and hypothyroidism complications. The aim of screening helps to identify and treat patients with subclinical thyroid dysfunction before they develop these complications.[1]

Thyroid dysfunction progresses from early to more advanced forms. Today, the most common approach is to classify patients according to the results of thyroid function tests. In this classification, “overt hypothyroidism” refers to patients who have an elevated thyrotropin (TSH) and a low thyroxine (T4) level. “Overt hyperthyroidism” refers to patients who have a low TSH and an elevated T4 or triiodothyronine (T3).[4, 5]

The primary rationale for screening is to diagnose and treat subclinical thyroid dysfunction. This rationale views subclinical thyroid dysfunction as a risk factor for the later development of complications and as a condition that may have symptoms that respond to treatment. The terms “subclinical hypothyroidism” and “mild thyroid failure” refer to patients who have an elevated TSH and a normal thyroxine level.[6]
Diagnosis
Diagnosis is done by blood tests, imaging tests, biopsies, and other tests. Basic physical check up is done prior to these tests which include:

Reflexes, heart rate, rhythm, blood pressure, weight and body temperature is tested along with examining the eyes and face, quality and quantity of hair, skin and nails.

Thyroid blood tests
Thyroid markers
1. TSH: This is the most common and most sensitive marker used in the thyroid function test. TSH level increase when T$_4$ level drops and vice versa.
   Laboratory reference range: 0.5-5.5
   Optimal reference range: 1.5-3.5
2. T$_4$(thyroxine): An excess of T$_4$ in the blood is indicative of overactive thyroid while low levels is indicative of under active thyroid.
   Normal range of total T$_4$: 4.8-10.4 μg/dl in adults
   Normal range of free T$_4$: 0.8-1.7 μg/dl.
3. T$_3$(triiodothyronine): increases levels of T$_3$ indicates hyperthyroidism and lower levels indicate hypothyroidism.

Blood tests
1. Thyroid stimulating hormone (TSH) test
This is the only test performed in the traditional health care model as a means to screen the patient for thyroid disorders; this is because they are only concerned for screening the thyroid for hormone replacement and not optimal physiological function.

The TSH level that is elevated, or above normal, is considered indicative of hypothyroidism, while TSH that is “suppressed” or below normal, is considered evidence of hyperthyroidism.\textsuperscript{[10, 11]}

The thyrotropin releasing hormone stimulates the pituitary to secrete thyroid stimulating hormone which then regulates the release of the hormones T$_3$ and T$_4$. A negative feedback which is sensitive to the circulating hormone level is monitored by the hypothalamus. This whole system is collectively called the hypothalamus-hypophyseal-thyroid axis. Any alteration in the function of this axis can influence the levels of the thyroid hormones. The
enzyme that is activated under the influence of the TSH is adenylate cyclase which triggers a downstream enzyme cascade which ultimately produces the hormone.

Radioimmunoassay is done for measuring hTSH level with the help of the antisera which was prepared by utiger et al.\textsuperscript{[14]} It is a sensitive method and the sensitivity can be enhanced by the use of chemiluminescent technology.

2. Total T\(_4\)/total thyroxine/serum thyroxine

This test measures the total amount of T\(_4\) circulating in the blood. A high value can indicate hyperthyroidism and a low value can indicate hypothyroidism. Once released into the circulation it is bound to carrier proteins. The greatest binding affinity for both hormones is to thyroxine-binding globulin (TBG) and, to a lesser extent, to prealbumin (TBPA). As a result, 99.97\% of circulating T4 and 99.7\% of circulating T3 bind leaving only small portions unbound.\textsuperscript{[15]}

Measurement of total T4 gives a reliable reflection of clinical thyroid status in the absence of binding abnormalities. However, changes in binding proteins can occur which affect the level of total T4 but leave the level of unbound hormone unchanged.\textsuperscript{[8]} The clinical importance of total T4 determination is in the diagnosis and confirmation of thyroid disorders.

3. Free T\(_4\)/free thyroxine

This test measures the amount of T\(_4\) that is unbound in the blood. This ensures no interference of the proteins and hence is a more useful test than total T\(_4\). Free T\(_4\) is elevated in hyperthyroidism and lowered in hypothyroidism. This test is thought to be more accurate as the hormone is in the active form.

More recently, radioimmunoassay and enzyme immunoassays have been developed for measuring free T4. These assays were performed with various combinations of analogue or non-analogue tracers and one-step or two-step incubation procedures.

Free T\(_4\) is required for the normal growth and development by maintaining body temperature and stimulating calorigenesis; affects all aspects of carbohydrate metabolism, foetal and neonatal development.
4. Total T₃/total triiodothyronine

T₃ is the active thyroid hormone also called triiodothyronine whose levels may fluctuate a bit in the blood but large fluctuations can be due to hyperthyroidism or hypothyroidism. The release of this hormone is monitored by the hypothalamus-hypophyseal-thyroid axis.

20% of the T₃ found in circulation is released directly from the thyroid gland while the rest of the T₃ found is derived by the enzymatic monodeiodination of T₄ to T₃.

5. Free T₃/free triiodothyronine

Free T₃ refers to the unbound level of triiodothyronine in the bloodstream. The levels are elevated in hyperthyroidism and diminished in hypothyroidism. About 0.2-0.4% of the circulatory T₃ is in the Free State. In most individuals the free fraction of these hormones correlates with the functional thyroid state. [12, 13]

Free T₃ is required for the normal growth and development by maintaining body temperature and stimulating calorigenesis; affects all aspects of carbohydrate metabolism, foetal and neonatal development.

MATERIALS AND METHODS

Sample collection

With aseptic precautions venous blood samples were collected in test tube. 2 hours later, blood sample was centrifuged at 3000 rpm for 5 minutes. Serum obtained were collected in polythene tube with stopper, which were then stored at -20°C until assayed.

For estimation of TSH separated serum or plasma should not remain in the stored condition more than 8 hours. If assays are not completed within 8 hours, serum or plasma should be stored at +2–8°C. If assays are not completed within 48 hours, the separated samples should be frozen at -15°C to -20°C. Frozen samples should be thawed only once to prevent analyte deterioration. A minimum of 0.3 mL serum is needed for the TSH. Sample volume for individual test is 55 µl.

Determination of T₃

Principle

In this test the sample is added along with a stripping reagent to release the T₃ bound to proteins, in a reaction vessel. T₃ in the sample competes with the T₃ analogue coupled to biotin to form anti-T₃ alkaline phosphatise conjugate. Of the resulting antigen: antibody
complexes, the T3 analogue: antibody complexes are bound to the streptavidin coated solid phase. These complexes are separated in a magnetic field and washing removes the sample T3: antibody complexes and other materials not bound to the solid phase. After these separation, the chemiluminescent substrate Lumi-Phos® 530 is added to the vessel and light generated by the reaction is measured with a luminometer. The light production is inversely proportional to the concentration of total T3 in the sample which is determined from a stored, multi-point calibration curve.

**Procedure**

Serum is pipette out into assigned wells and a working tracer reagent solution is added to it followed by swirling. To this biotinylated tT3 specific antibody conjugate solution is added. After swirling and incubation wash buffer is added and decanted several times following which working reagent solution is added to each well and incubated. The relative light in each well is read within 30 min of adding substrate solution.

**Determination of T4**

**Principle**

In the T4 EIA, a certain amount of anti-T4 antibody is coated on microtiter wells. To this a measured amount of serum sample, and a constant amount of T4 conjugated are added. During incubation, T4 and conjugated T4 compete for the limited binding sites on the anti-T4 antibody. After a 15 minutes incubation at 37-40℃, the wells are washed 5 times by wash buffer to remove unbound T4 conjugate. A solution of Substrate A/B mixed Reagent is added and incubated for 5 minutes. Read the light units a Chimiluminescence microplate reader.

**Procedure**

Standards, specimen and controls are pipetted out into appropriate wells, working conjugate reagent is added to it and mixed thoroughly and incubated. Rinse and flick the microtiter wells with working wash buffer solution and then add the working substrate solution to all the wells. After incubation read the light units are all wells with a chemiluminiscence microtiter. Results are read within 30 min after adding substrate.
Determination of TSH

Principle
Chemiluminescent immunoassay for the quantitative determination of thyrotropin, human thyroid-stimulating hormone (hTSH) levels in human serum and plasma. The Access Hypersensitive hTSH assay is a two-site immunoenzymatic assay. A sample along with goat anti-hTSH-alkaline phosphatase conjugate, paramagnetic particles coated with immobilized mouse monoclonal anti-hTSH antibody and buffered protein solution were added to a reaction vessel. The hTSH binds to the immobilized monoclonal anti-hTSH on the solid phase while the goat anti-hTSH-alkaline phosphatase conjugate reacts with a different antigenic site on the hTSH. After incubation, materials that are bound to the solid phase are held in a magnetic field the chemiluminescent substrate Lumi-Phos* 530 are added. The light generated is measured with a luminometer. The amount of light produced is proportionate to the total concentration of human thyroid-stimulating hormone in the sample.

RESULTS

T3 marker study on the different age and sex of subjects
Reference Range: 0.87 – 1.78 ng/ml

![Average T3 levels (ng/ml)](image)

Fig.1: Average T3 levels (ng/ml)
T4 marker study on the different age and sex of subjects

Reference Range: 6.09 – 12.23 ng/ml

![Average T4 levels (ng/ml)](image)

**Fig.2: Average T4 levels (ng/ml)**

TSH marker study on the different age and sex of subjects

Reference Range: 0.34 – 5.6 ng/ml

![Average TSH levels (ng/ml)](image)

**Fig.3: Average TSH levels (ng/ml)**

**DISCUSSION**

Serum T3 levels of the serum from the patients is tabulated in Table – 1. The results show that age group of 51 – 55 and 56 – 60 are found to be significantly higher level of T3 marker when compared to other age group.
Serum T4 levels of the serum from the patients is tabulated in Table – 2. The results show that age group of 51 – 55 and 56 – 60 are found to be significantly higher level of T3 marker when compared to other age group.

Serum TSH levels based on their age group is tabulated in Table – 3. Age group of 31 – 35 and 46 – 50 are found to be significantly higher level but the higher age groups like 51 – 60 showed a reduced TSH level. Serum TSH concentrations decrease in healthy elderly subjects due to an age – related decrease in TSH secretion by the pituitary. The mechanism in reduction in TSH secretion is still not clearly known.

From our study, there exist an age – dependent decline in serum TSH and T3. The decreased level of TSH secretion may be due to an increased sensitivity of the thyrotrophin negative feedback by T4, but other factors such as a reduced hypothalamic TRH secretion should also be taken for consideration (Shown in Purple). Though reduced TSH levels result in a reduced thyroidal T4 secretion in the elderly aged subject, total and free T4 (FT4) concentrations in serum remain unchanged. This is because, the degradation of T4 by outer ring deiodination decreases with age. This inactive metabolite rT3 seems to increase with age.

A clear age dependent decline in serum Thyroid stimulating Hormone (TSH) and free T3, whereas serum (free) T4 levels remain unchanged. The inactive metabolite rT3 seems to increase with age.

In addition to malnutrition and illness, use of medication may influence thyroid function tests in elderly aged groups. It is well known that there is a strong positive relation between age and medication use. 34 drugs can cause hypothyroidism (e.g. Lithium), hyperthyroidism (e.g. amiodarones) or abnormal thyroid function tests without a direct effect on thyroid function by affecting TBG status (e.g Heparin), by suppressing TSH secretion (e.g. Glucocorticoids, dopamine) or by suppressing T4 to T3 conversion ( e.g. Glucocorticoids, amiodaron, propanolol).

Therefore, such factors must be considered in interpreting altered thyroid function tests in the elderly aged groups. Also the parameters, such as genetic variation and psychological factors may influence thyroid function tests in the elderly aged groups.

In the last few years, evidence has emerged that genetic factors and thus genetic polymorphism in thyroid hormone pathway genes are major contributors to the variation in
thyroid function tests between different individuals. In elderly subjects polymorphisms in thyroid hormone pathway genes have been associated with serum T3 level, body composition and insulin.

REFERENCES

