

T3, T4, TSH Levels in Hypothyroidism

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Abstract:

Introduction: The Thyroid gland is a highly vascular organ, situated at the front and sides of the neck shaped like a butterfly. Its name is derived from Greek, which means 'shield gland'. The adult thyroid weights about 15-20gms, being slightly heavier in females and increasing in menstruation and pregnancy.

The hormones produced by the gland are found in the thyroid and the serum. They are two physiologically potent amino acids L-thyroxine (tetraiodothyronine) T4 L-triiodothyronine T3

PathoPhysiology: Hypothyroidism: Hypothyroidism may manifest as growth failure intellectual impairment, goiter, pubertal abnormalities (early or delayed), weight gain memorrhagia, and oilgomenorrhea. The diagnosis is usually established by a combination of TSH and free T4. Antithyroid antibodies (antithyroid peroxides and antithyroglobulin) antibodies usually establishment the etiology of autoimmune thyroiditis.

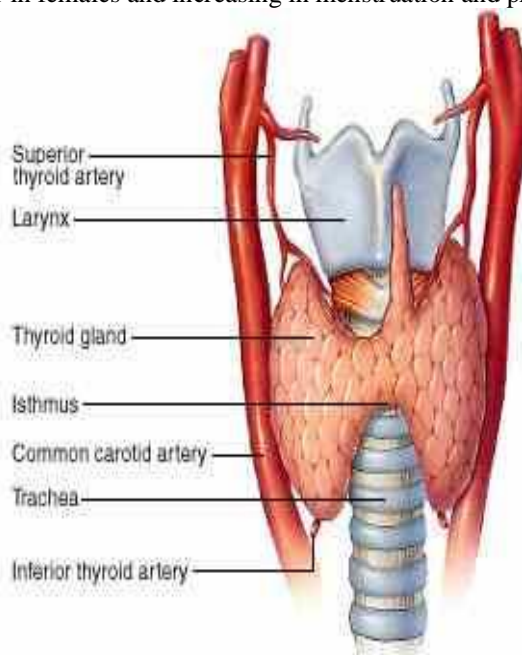
Materials and Method: In GGH, Kakinada, Bio Chemistry Lab 35 different cases of 13 years to 70 ages. Both males and females cases. Normal range is taken as control. TOSOH bioscience - AIA 360 Auto analyzer With TOSOH bioscience T3, T4, TSH Kits

Results: T3 P Value $P < 0.01$, T4 P Value $P < 0.02$, TSH P. Value $P < 0.001$.

Conclusion: T3 values lowered and it is significant in Hypothyroidism cases, T4 values are some what lowered and it is slightly significant, T3 is more significant and more lowered than T4, TSH values are raised so much and it is more significant, TSH is most significant and sensitive index in hypothyroidism cases which is studied in GGH Bio-Chemistry lab RMC Kakinada.

I. Introduction

The Thyroid gland is a highly vascular organ, situated at the front and sides of the neck shaped like a butterfly. Its name is derived from Greek, which means 'shield gland'. The adult thyroid weights about 15-20gms, being slightly heavier in females and increasing in menstruation and pregnancy.



The primary structural and functional unit of the organ is the follicle. It is roughly spherical with a diameter of 300μ . The cells lining the follicle are one cell thick. This is a mixture of thyroglobulin, iodoproteins and albumin. Thyroglobulin is a unique protein within the peptide sequence of which T4 and T3 are synthesized and stored.

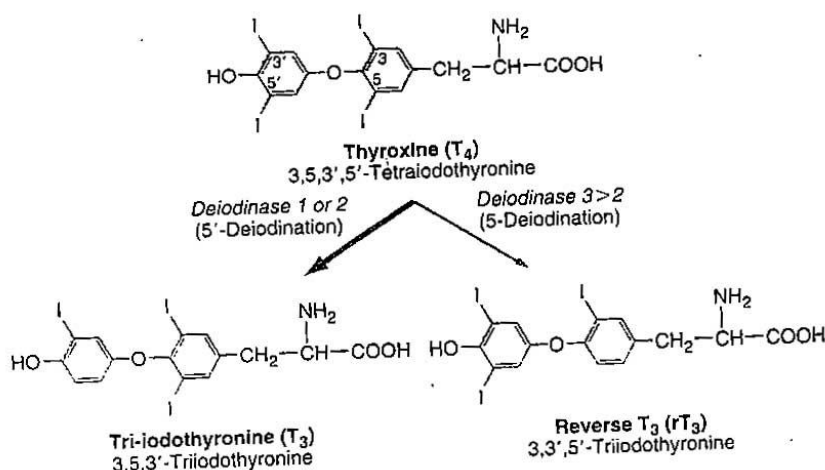


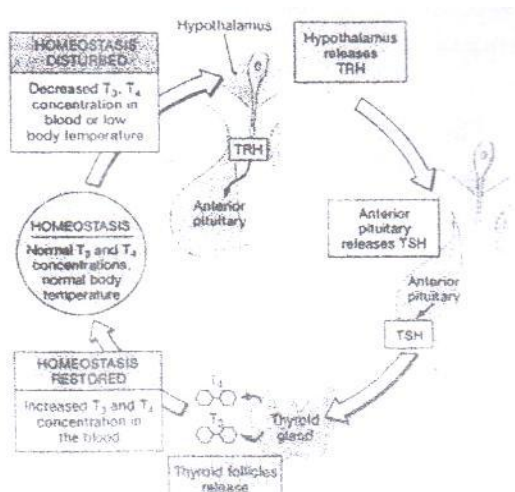
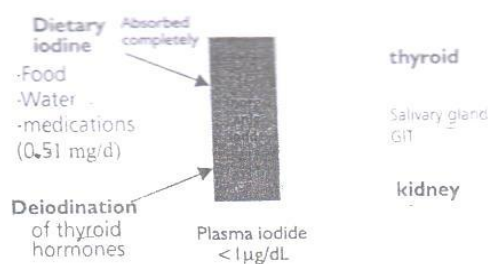
FIGURE 320-1 Structures of thyroid hormones. Thyroxine (T₄) contains four iodine atoms. Deiodination leads to production of the potent hormone, triiodothyronine (T₃), or the inactive hormone, reverse T₃.

II. Pathophysiology

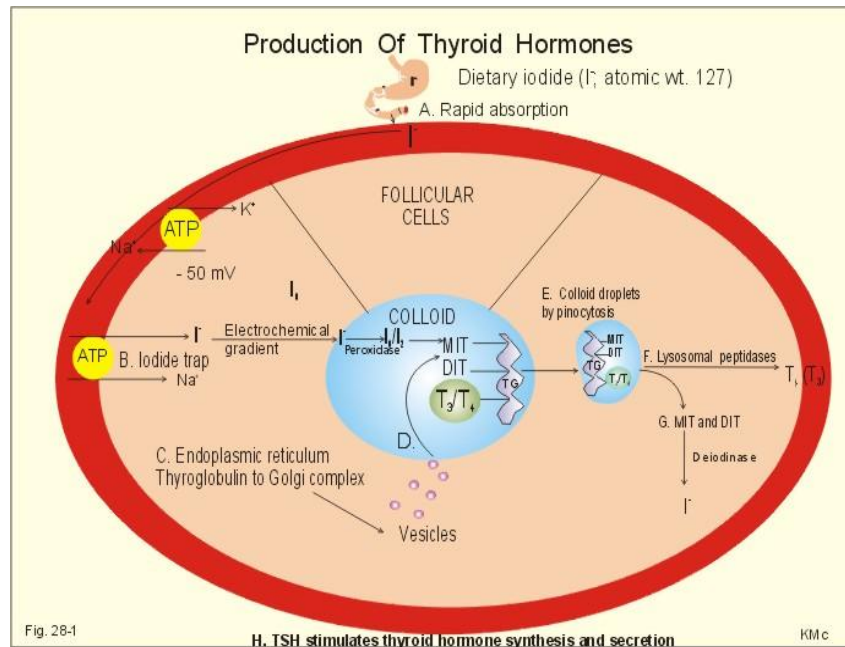
The hormones produced by the gland are found in the thyroid and the serum. They are two physiologically potent amino acids

1. L-thyroxine (tetraiodothyronine) T₄
2. L-triiodothyronine T₃

T₄ is metabolically active primarily after deiodination to T₃, but active when bound to receptors. T₃ is the active form of the hormone. Synthesis of the thyroid hormone depends upon absorption of adequate amounts of iodide by the thyroid. The iodide that enters the GI secretions is reabsorbed in effect the thyroid and kidneys compete for plasma iodide.



Thyroid Gland Function



The synthesis of the thyroid hormone can be divided into four steps.

1. Iodide trapping
 2. Oxidation of iodine and organic iodination.
 3. Coupling
 4. Release
- The thyroid is the only source of T4
 - Only 20% of T3 is produced in the thyroid; the rest is obtained from enzymatic removal in extraglandular tissues

Thyroid Hormones Transport

T4 and T3 are mostly protein bound. The plasma proteins are:

1. TBG (Thyroxin binding Globulin)
 - Increased affinity for T4
 - Major Determinant in binding
2. TTR (Transthyretin):
 - Binds only 15% of T4
 - Contribution to free hormone levels is comparable to TBG owing to higher dissociation constant
 - Does not bind much to T3
3. Albumin

Free Hormone Levels

Free or unbound form is the active form available to tissues. Only about 0.03% of T4 is Free. T3 is bound less firmly to TBG than T4 and not bound much to TTR. Hence the normal free proportion of T3 is 0.03% i.e ten times more than T4.

III. History

Change in total goiter prevalence between 1993 & 2003, by WHO region

WHO region	TGP General Population		% Change
	1993	2003	
Africa	15.6	28.3	+ 81.4
Americans	8.7	4.7	- 46.0
South-East Asia	13.0	15.4	+18.5
Europe	11.4	20.6	+80.7
Eastern Mediterranean	22.9	37.3	+62.9
Western Pacific	9.0	6.1	- 32.2
Total	12.0	15.8	+31.7

India

- The estimated population at risk for IDD in India was 270 million people. Assessment was that 70 million are already affected by IDD, an estimated 2.2 million by cretinism and 6.6 million with mild neurological disorders.
- The Himalayan goiter belt is the world's largest belt from Kashmir to Naga hills; covering J & K, Himachal Pradesh, Punjab, Harayana, Delhi, Uttar Pradesh, Bihar, West Bengal, Sikkim, Arunachal Pradesh, Assam, Nagaland, Mizoram, Meghalaya, Tripura and Manipur. This has an average goiter prevalence rate of 36%.
- Renewed surveys have detected areas outside this belt: parts of MP, Gujarat, Maharashtra, AP, Kerala, and Tamil Nadu.

IV. Hypothyroidism

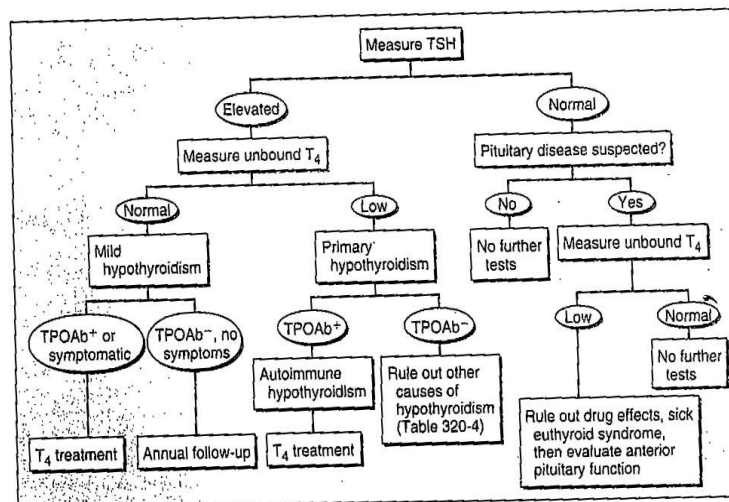
Hypothyroidism may manifest as growth failure intellectual impairment, goiter, pubertal abnormalities (early or delayed), weight gain memorrhagia, and oilgomenorrhea. The diagnosis is usually established by a combination of TSH and free T4. Antithyroid antibodies (antithyroid peroxides and antithyroglobulin) antibodies usually establishment the etiology of autoimmune thyroiditis.

- Iodine deficiency goiter
- Congenital Hypothyroidism
- Congenital Atrophic Hypothyroidism
- Infiltrative Disease
- Acquired Atrophic Hypothyroidism
- Transient Hypothyroidism
- Central Hypothyroidism
- Resistance to Thyroid Hormone

Autoimmune thyroid diseases are the commonest amongst autoimmune disorders of the endocrine glands. Humoral and cell-mediated immune mechanisms are implicated in causing cellular damage and inflammatory reactions in the thyroid gland.

Laboratory test commonly used for measuring thyroid antibodies are for thyroperoxidase antibodies (TPOAb), thyroglobulin antibodies (TgAb and thyroid receptor antibodies (TRAb), TPOAb and TgAb are often present in diseases of the gland like Hashimoto's thyroiditis.

Measurement of thyroid antibodies alone or in combination with other thyroid function tests like TSH and thyroxin (T4) have been used in screening populations for thyroid diseases



Evaluation of hypothyroidism. TPOAb⁺, thyroid peroxidase antibodies present; TPOAb⁻, thyroid peroxidase antibodies not present. TSH, thyroid-stimulating hormone.

V. Materials And Method

- In GGH, Kakinada, Bio Chemistry Lab 35 different cases of 13 years to 70 ages. Both males and females cases. Normal range is taken as control.
- TOSOH bioscience - AIA 360 Auto analyzer
- With TOSOH bioscience T3, T4, TSH Kits

T3:**SUMMARY AND EXPLANATION OF TEST**

Triiodothyronine (T3) and thyroxine (thyroxine; T4), regulate a variety of biochemical processes throughout the body⁽¹⁾. The majority of T3 in circulation is produced enzymatically by monodeiodination of T4 in the peripheral tissues, rather than from direct secretion from the thyroid gland⁽²⁾. Approximately one-third of all T4 secreted is deiodinated to yield T3⁽³⁾.

Serum T3 measurements can be a valuable component of a thyroid-function screening panel in diagnosing certain disorders of thyroid function in addition to conditions caused by iodide deficiency. Assays for T3 are valuable in early detection of hyperthyroidism and for monitoring the efficacy of treatment for thyroid disorders⁽⁴⁾. A normal T3 value in the presence of an elevated T4 and/or Free T4 (FT4) level may also help to rule out hyperthyroidism⁽⁵⁾.

SPECIFICITY

The following substances were tested for cross-reactivity. Cross-reactivity is expressed in terms of the percentage of the concentration of each substance which will be measured as T3.

Compound	Cross-reactivity
L-Triiodothyronine (L-T3)	100 %
D-Triiodothyronine (D-T3)	125
L-Thyroxine (L-T4)	0.15
D-Thyroxine (D-T4)	0.07
3, 3', 5'-Triiodothyronine	0.3
3, 5-Diiodo-L-tyrosine	< 0.001

SUMMARY AND EXPLANATION OF TEST

Evaluation of thyroid status is complex. The primary function of the thyroid gland is the secretion of thyroxine (T4) or triiodothyronine (T3). Abnormal secretion of T4 and/or T3 may lead either to hyper- or hypo-thyroidism. The synthesis and release of T4 and T3 are in response to a hypothalamic-pituitary signal, Thyroid Stimulating Hormone (TSH), which is released from the anterior pituitary and is the principal regulator of thyroid activity⁽¹⁾. The release of TSH is controlled by Thyrotropin Releasing Hormone (TRH) from the hypothalamus⁽²⁾. This combined system regulating the release of thyroid hormone is the hypothalamic-pituitary axis^(3,4). In the circulation, T4 is 99.97% protein bound (0.03% free) while T3 is 99.7% bound (0.3% free). Thyroxine Binding Globulin (TBG) is the primary binding protein. To a lesser extent, Thyroxine Binding Prealbumin (TBPA) and albumin can also bind T4^(5,6,7). Only unbound (free) forms exert the physiological actions.

T4 is largely converted to T3 in peripheral tissues by monodeiodination⁽⁸⁾. Total T4 rises and falls with the TBG level in euthyroid individuals. An erroneous interpretation of thyroid function may be obtained if a condition exists which changes the TBG concentrations. Certain drugs compete with T4 for binding to TBG, which results in decreased levels of total T4 through the negative feedback of thyroid hormone concentration on TSH secretion^(9,10).

PRINCIPLE OF THE ASSAY

The ST AIA-PACK TT3 is a competitive enzyme immunoassay, which is performed entirely within the AIA-PACK. Triiodothyronine, which is displaced from its binding proteins by ANS (8-anilino-1-naphthalene sulfonic acid), and free T3 present in the test sample compete with enzyme-labeled T3 for a limited number of binding sites on a T3 specific antibody immobilized on magnetic beads. The beads are washed to remove the unbound enzyme-labeled T3 and are then incubated with a fluorogenic substrate, 4-methylumbelliferyl phosphate (4-MUP). The amount of enzyme labeled T3 that binds to the beads is inversely proportional to the T3 concentration in the test sample. A standard curve using a range of known standard concentrations is prepared and unknown T3 concentrations are calculated using this curve.

Incubation time:	10 minutes
Specimen volume	25 µl
Specimen type:	Serum
Assay range:	up to 8.0 ng/ml
Calibration stability:	90 days
Sensitivity:	0.25 ng/ml

Reference range

0.79 - 1.58 ng/mL

T4:**PRINCIPLE OF THE ASSAY**

The ST AIA-PACK T4 is a competitive enzyme immunoassay, which is performed entirely within the AIA-PACK. Thyroxine, which is displaced from its binding proteins by ANS (8-anilino-1-naphthalene sulfonic acid), and free T4 present in the test sample compete with enzyme-labeled thyroxine for a limited number of binding sites on a thyroxine-specific antibody immobilized on magnetic beads. The beads are washed to remove the unbound enzyme labeled thyroxine and are then incubated with a fluorogenic substrate, 4-methylumbelliferyl phosphate (4MUP). The amount of enzyme labeled thyroxine that binds to the beads is inversely proportional to the thyroxine concentration in the test sample. A standard curve using a range of known standard concentrations is constructed and unknown thyroxine concentrations are calculated using this curve.

Incubation time:	10 minutes
Specimen volume	10 µl
Specimen type:	Serum
Assay range:	up to 24 µg/dl
Calibration stability:	90 days
Sensitivity:	0.5 µg/dl

Reference range

4.0 - 11.0 µg/dL

TSH:

SUMMARY AND EXPLANATION OF TEST

Thyroid Stimulating Hormone is a glycoprotein hormone secreted by the anterior pituitary gland. When feedback suppression of the pituitary is reduced by a reduced production of thyroid hormones (T4 and T3), TSH rises in an attempt to increase thyroid hormone production. This rise occurs while the patient is still asymptomatic and thus is an early and very sensitive indication of hypothyroidism⁽¹⁻⁵⁾. TSH is also controlled by the hypothalamic peptide, thyrotropin releasing hormone (TRH). Accurate determination of serum TSH is the most useful and sensitive test for primary hypothyroidism, where serum thyroid hormone concentrations are depressed and serum TSH concentrations are significantly elevated. Serum TSH determinations may also be used to differentiate between pituitary (secondary) and hypothalamic (tertiary) hypothyroidisms⁽⁶⁻⁹⁾. Through the use of monoclonal antibody technology, which provides the necessary specificity and sensitivity, the usefulness of TSH determination in the diagnosis of hyperthyroidism distinguished from euthyroidism has been well established.

PRINCIPLE OF THE ASSAY

The ST AIA-PACK TSH is a two-site immunoenzymometric assay, which is performed entirely in the AIA-PACK test cups. TSH present in the test sample is bound with monoclonal antibody immobilized on magnetic beads and monoclonal antibody conjugated with bovine alkaline phosphatase in the AIA-PACK test cups. The magnetic beads are washed to remove unbound enzyme-labeled monoclonal antibody and are then incubated with a fluorogenic substrate, 4-methylumbelliferyl phosphate (4MUP). The amount of enzyme conjugated with monoclonal antibody that binds to the beads is directly proportional to the TSH concentration in the test sample. A standard curve is constructed, and unknown sample concentrations are calculated using this curve

Incubation time:	10 minutes
Specimen volume	100 µl
Specimen type:	Serum
Assay range:	up to 100 µIU/ml
Calibration stability:	90 days
Sensitivity:	0.01 µIU/ml

SPECIFICITY

The following substances were tested for cross-reactivity. Cross-reactivity is expressed in terms of the percentage of the concentration of each substance, which will be measured as TSH.

Compound	Cross-reactivity(%)
TSH	100
HCG	< 0.01
FSH	< 0.01
LH	0.17
HGH	< 0.01

Reference range
0.39 – 3.55 µIU/ml

Table -1 Normal Range:

T3	0.79-1.58ng/ml
T4	4.0-11.0µg/dL
TSH	0.39-3.55µIU/ml

VI. Results

Table -2

Parameter	Mean	S.D	P Value
T3:			
Control (normal range)	1.08	0.28	P<0.01
Test (35 cases)	0.68	0.45	
T4:			
Control (normal range)	8.02	1.24	P<0.02
Test (35 cases)	4.76	3.80	
TSH:			
Control (normal range)	2.4	1.31	P<0.001
Test (35 cases)	20.97	15.8	

VII. Conclusion

T3 values lowered and it is significant in Hypothyroidism cases. T4 values are somewhat lowered and it is slightly significant. T3 is more significant and more lowered than T4 TSH values are raised so much and it is more significant.TSH is most significant and sensitive index in hypothyroidism cases which is studied in GGH Bio-Chemistry lab RMC Kakinada.

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