A STUDY ON ALTERED TOTAL PLASMA HOMOCYSTEINE LEVEL AND LIPID PROFILE IN NEWLY DIAGNOSED HYPOTHYROID INDIVIDUALS

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BONAFIDE CERTIFICATE

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ABBREVIATIONS

NIS	Sodium-Iodide symporter
MIT	Monoiodo tyrosine
DIT	Diiodo tyrosine
T3	Triiodo thyronine
T4	Tetraiodo thyronine
Tg	Thyroglobulin
TBG	Thyroid binding globulin
TR	Thyroid receptors
TRH	Thyrotrophin releasing hormone
TSH	Thyroid stimulating hormone
CAMP	3’ – 5’ Cyclic Adenosine monophosphate
Ca2+	Calcium
BMR	Basal metabolic rate
TPO antibodies	Thyroid peroxidase antibodies
FMN	Flavin adenine mononucleotide
FAD	Flavin adenine dinucleotide
MTHFR	Methylene tetrahydrofolate reductase
PTH	Parathormone
ACTH	Adrenocorticotrophic hormone
THF	Tetrahydrofolate
NO	Nitric oxide
VLDL	Very low density lipoprotein

IDL	Intermediate density lipoprotein
LDL	Low density lipoprotein
HDL	High density lipoprotein
Hcy	Homocysteine
HMG coA	Hydroxymethyl glutaryl coA
CETP	Cholesteryl ester transfer protein
ASK 1	Apoptosis – regulating signal kinase 1
MMP	Matrix metallo proteinase
MAPK	Mitogen activated protein kinase
SERCA	Sarcoplasmic endoplasmic reticulum calcium ATPase
SAH	S-Adenosyl homocysteine
DHAP	Dihydroxy acetone phosphate
4-AAP	4-Aminoanti pyrine
PVS	Polyvinyl sulfonic acid
PEGME	Polyethylene glycol methyl ether
BMI	Body mass index
MCT	Monocarboxylate transporter
OATP 1C1	Organic anion transporting polypeptide 1C1

ABSTRACT

One of the major complication of hypothyroidism is atherosclerosis and cardiovascular disease. Hyperhomocysteinemia is an important and independent risk factor for atherosclerosis. Hypothyroidism decreases hepatic levels of enzymes which converts homocysteine to methionine that leads to increase in homocysteine level in the circulation of hypothyroid individuals. The aim of the study was to assess fasting total plasma homocysteine level in recently diagnosed hypothyroidism. The study included thirty recently diagnosed hypothyroid individuals, thirty treated hypothyroidism and thirty apparently healthy subjects with age and sex matched. The study group was selected after obtaining ethical committee clearance and consent from subjects attending outpatient department of Endocrinology, Madras Medical College, Chennai. Thyroid profile, homocysteine and lipid profile were measured in fasting blood samples. Total plasma homocysteine levels was significantly more in recently diagnosed hypothyroidism compared to controls and treated hypothyroidism with p value 0.006. By this study we confirmed hyperhomocysteinemia in hypothyroidism which may lead to atherosclerosis. Hypothyroidism is one of the treatable cause for hyperhomocysteinemia. Lipid profile was altered in recently diagnosed hypothyroidism. Total cholesterol, triglycerides, LDL cholesterol and HDL cholesterol were high in recently diagnosed hypothyroidism compared to controls and treated hypothyroidism. Lipid profile was highly significant between the study groups with the p value <0.001 except HDL cholesterol. During treatment with thyroxine hypothyroid patients should be monitored for lipid profile, total plasma homocysteine levels. In hypothyroidism estimation of total plasma homocysteine level is may be used as screening test to identify and monitor cardiovascular risk.

Keywords : Hypothyroidism, Homocysteine, Lipid profile

INTRODUCTION

Thyroid diseases are common worldwide. In India too, thyroid diseases are more prevalent. Thyroid disorders are the most common among all the endocrine diseases[1]. In pregnancy thyroid diseases are the second most common endocrine disease and its prevalence is 0.3-0.5 %[2].

Approximately 42 million people are affected by thyroid diseases. In India 11 % of the population are affected from hypothyroidism. Among these 12 % of adults have palpable goiter, 16.7 % of adults have anti-thyroid peroxidase and 12.1 % have anti-thyroglobulin antibodies. The older population were affected more than the younger population. Women were three times more prone for hypothyroidism than men[3].

Hypothyroidism [myxoedema] was first described by Thomas Curling in 1850. Thyroidectomy was first performed in 1880. Anti-thyroid drugs and radio-iodine therapy were developed in early 1940's [4].

Homocysteine is a sulphur containing amino acid which is formed as an intermediate in the methionine metabolism and methionine is the only source of homocysteine in the human body.

Homocysteine undergoes auto-oxidation and produces hydrogen peroxide which is a free radical. Glutathione peroxidase prevents oxidative degradation of nitric oxide[5]. Among thiol containing

aminoacids homocysteine has unique property to inhibit glutathione peroxidase [5]. Hyperhomocysteinemia destroys glutathione peroxidase, decreases its intracellular concentration and increases oxidative degradation of nitric oxide, thereby decreasing its bioavailability. Damage to endothelial cell disrupts the normal function of endothelium resulting in leukocyte and platelet adhesion, thrombosis, smooth muscle proliferation, lipid accumulation and finally atheroma [6,7,8].

Hyperhomocysteinemia is defined as the levels of homocysteine > 10 μmol/L. There are various causes for hyperhomocysteinemia. Hypothyroidism is one of the treatable cause for hyperhomocysteinemia. For each 1 μmol/L increase in homocysteine concentration there is a 1 % increase in risk to develop cardiovascular events or death [9].

Hyperhomocysteinemia is an important and independent risk factor for atherosclerosis. 60% of the patients affected by cardiovascular disease have hyperhomocysteinemia[10]. Many studies have reported mild hyperhomocysteinemia as an independent risk factor for venous and arterial occlusive disease [11,12,13].

AIM OF THE STUDY

AIM OF THE STUDY

Hypothyroidism is the most common endocrine problem in recent years, more prevalent in India and about 11 % of population[3] were affected. Almost 1 in every 10 person are affected with hypothyroidism. Overt hypothyroidism has been found to be associated with cardiovascular disease. 60% of the patients affected by cardiovascular disease have hyperhomocysteinemia[10]. Thyroid dysfunction has adverse role in nearly all major metabolic pathways like carbohydrate, protein and lipids.

In hypothyroidism common metabolic abnormalities are dyslipidemia, hyperhomocysteinemia and Insulin resistance. Hyperhomocysteinemia causes oxidative stress to the cell, increase production of reactive oxygen species and leads to complications. Homocysteine has been identified as an independent risk factor for atherosclerosis. Patients with unexplained hyperhomocysteinemia should be screened for thyroid status[14]. Hypothyroidism is one of the treatable cause for hyperhomocysteinemia.

Objectives:

1. Estimation of Total Plasma Homocysteine level in recently diagnosed hypothyroid individuals and to compare them with the lipid profile in the same group.

2. To compare the levels of homocysteine and lipid profile between recently diagnosed hypothyroid cases and apparently normal individuals.

3. To compare the homocysteine levels and lipid profile between recently diagnosed hypothyroid cases and treated hypothyroid patients.

4. To compare the homocysteine levels and lipid profile between treated hypothyroid patients with apparently normal individuals.

5. To correlate TSH level with lipid profile and homocysteine levels in all the above groups.

REVIEW OF LITERATURE

REVIEW OF LITERATURE

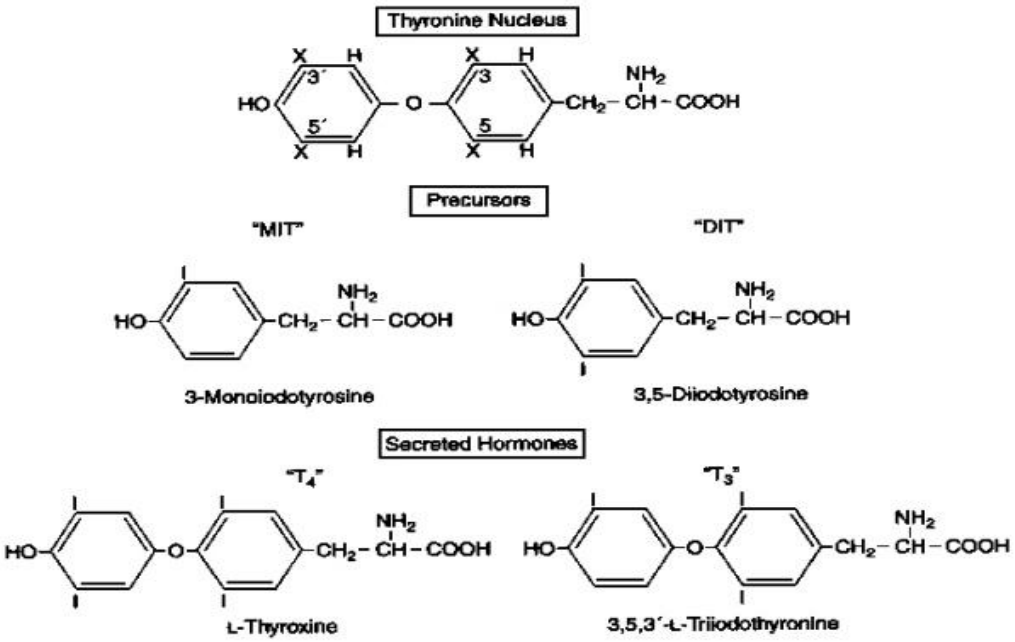
Thyroid gland Anatomy and Embryology :

The word Thyroid is derived from the Greek word [thyreos-shield], situated anterior to the trachea between cricoid cartilage and suprasternal notch[15]. It has two lobes joined by an isthmus. It is composed of many spherical follicles made up of follicular cells which synthesize thyroglobulin. In the center of the follicle, is the lacuna filled with secreted colloid proteineous fluid containing predominantly thyroglobulin. The follicular cells becomes columnar when it is active and cuboidal when it is inactive[12].

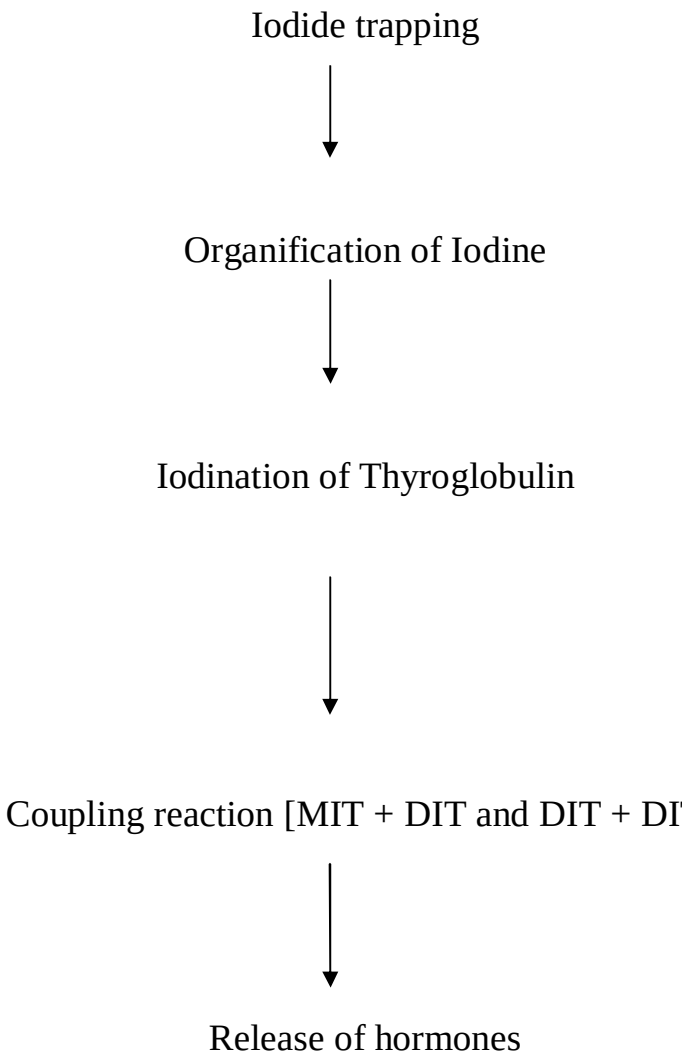
Thyroid gland is an important endocrine gland which develops at the 24th day of intrauterine life from an anterior out pouching of the foregut, and then it descends along with thyroglossal duct to reach its location by 7 weeks of gestation. At about 20 weeks the fetus starts producing thyroid hormones due to maturation of hypothalamic-pituitary-thyroid axis[18].The thyroid medullary C cells which produces calcitonin – a calcium lowering hormone develops from ultimobranchial body, a derivative of neural crest[15].

Thyroid hormone synthesis:

Thyroglobulin is the precursor of thyroid hormones, which is synthesized from the rough endoplasmic reticulum of the follicular cell. Thyroglobulin is a glycoprotein homodimer of 660 kDa. It consists of 2769 amino acids, including 134 tyrosine residues . Among these only 25 – 30 residues are iodinated. Principal stimulator of thyroglobulin synthesis is TSH[18].



Thyroid hormone synthesis :

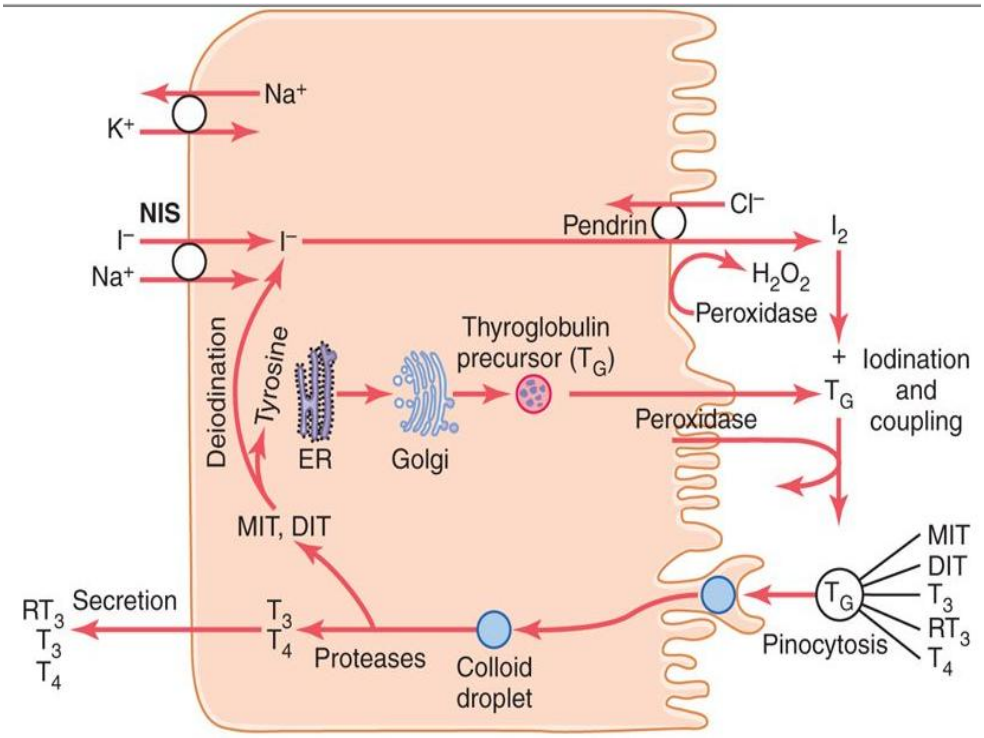


Iodide trapping:

Recommended dietary allowance of iodine is 150 microgram / day[11].

In thyroid hormone synthesis, iodide uptake is the first critical step which is mainly derived from the diet. Ingested iodine is absorbed and bound to albumin in the circulation. Unbound iodine is excreted through

FIGURE 1 : THYROID HORMONE SYNTHESIS



Courtesy : Guyton and Hall. Textbook of Medical Physiology [12 th edition]

Calibrators :

CALIBRATORS	CONCENTRATION
Calibrator 1	**2 µmol/L**
Calibrator 2	**4 µmol/L**
Calibrator 3	**8 µmol/L**
Calibrator 4	**15 µmol/L**
Calibrator 5	**30 µmol/L**
Calibrator 6	**50 µmol/L**

HOMOCYSTEINE STANDARD CONCENTRATION	ABSORBANCE
50 µmol/L	**0.2443**
30 µmol/L	**0.3447**
15 µmol/L	**0.4887**
8 µmol/L	**0.8004**
4 µmol/L	**1.1251**
2 µmol/L	**1.3059**

kidney. Iodide is transported to the follicular cells by active transport via sodium-iodide symporter [NIS]. Sodium-Iodide symporter is encoded by the gene SLC5A5. It is a glycoprotein which has 643 aminoacids [12]. The iodine concentration in the follicular cells is 30-40 times greater than the circulation[18].

Na+/I- symporter is present in the basolateral surface of the follicular cells, highly expressed in the thyroid gland and low in salivary glands, placenta, lactating mammary gland[15].The iodide transport mechanism is highly regulated by dietary supply of iodine. Low iodine intake increases the expression of NIS and stimulates the uptake. Whereas high intake suppress NIS [15]. Iodide is transported in to the lacuna by another transporter pendrin situated on apical surface of the follicular cell. It is a glycoprotein encoded by the gene SLC26A4[12]. Pendrin[Chloride / iodide antiporter] is a passive iodide transporting glycoprotein also present in kidney and inner ear[12,16].

Organification of Iodine:

Iodide after entering in to the lacuna gets oxidized to iodine by an enzyme thyroid peroxidase which is a heme containing protein. In this reaction hydrogen peroxide acts as an electron acceptor, and generates

reactive iodine. Hydrogen peroxide is produced at the apical membrane by the action of DUOX 1 and 2 [Dual oxidase][12].

Iodination of Thyroglobulin :

The reactive iodine atom is added to the selected tyrosine residues in the thyroglobulin to produce either monoiodotyrosine or diiodotyrosine. This reaction is catalyzed by thyroperoxidase.

Coupling reaction:

The coupling reaction is catalyzed by thyroperoxidase enzyme to produce either triiodo / tetraiodothyronine. T_3 is formed by transfer of one monoiodinated phenolic group from MIT to one DIT residue. T_4 is formed from two DIT residues with the transfer of one di-iodinated phenolic group to another DIT residue[18]. These T_3 and T_4 are bound to thyroglobulin and acts as a reservoir of thyroid hormones in the colloid.

Release of Thyroid hormones:

The coupled thyroglobulin is taken back in to the follicular cell by pinocytosis. This phagosome is endocytosed by primary lysosome and fused to form secondary lysosome, in which Tg gets digested and T_3 ,T_4, DIT ,MIT and amino acids are released. This lipophilic T_3 and T_4

molecules exit from lysosome and by diffusion across the follicular cell plasma membrane it enters into the thyroid capillaries[18].

In the follicular cell cytoplasm uncoupled MIT and DIT are deiodinated by an enzyme dehalogenase and free iodine are recycled for hormone synthesis[18].

Transport of thyroid hormones:

The major hormone secreted from thyroid gland is T_4 which is 20 times more than that of T_3. Both are bound to thyroxine-binding globulin, transthyretin, and albumin. These plasma-binding proteins increase the level of circulating hormones, alter the hormone delivery to the target tissues, and delays the hormone clearance. Free thyroid hormone in plasma is physiologically active. Only 0.03 % of total T_4 and 0.3 % of total T_3 is in the free form [12].

- **Thyroid binding globulin:**
  - Thyroid hormones are bound to this protein which is present in low concentration in the circulation and has a high affinity for T3.
  - Each TBG molecule has one iodothyronine binding site.

TBG level is increased in [11]:

Pregnancy

Drugs: Estrogen pills

Clofibrate

TBG level is decreased in :

Liver disease

Drugs : Glucocorticoids

Androgens

Phenytoin [17,20]

Salicylates [19,20]

Cancer chemotherapeutic agents – eg. Mitotane

- **Transthyretin :**
  - Also called as Thyroxine-binding prealbumin
  - Affinity for thyroid hormones is low.

- **Albumin :**
  - Even though it has a low affinity for thyroid hormones because of its high plasma concentration, albumin is a major carrier protein for thyroid hormones[15].
  - Binds about 10% of the thyroid hormones.

Table - 1: Effect of Alterations in the Concentrations of Thyroid Hormone-Binding Proteins in the Plasma on Various Parameters of Thyroid Function after Equilibrium Has Been Reached.

Condition	Concentrations of Binding Proteins	Total Plasma T_3, T_4, RT_3	Free Plasma T_3, T_4, RT_3	Plasma TSH	Clinical State
Hyperthyroidism	Normal	High	High	Low	Hyperthyroid
Hypothyroidism	Normal	Low	Low	High	Hypothyroid
Estrogens, methadone, heroin, major tranquilizers, clofibrate	High	High	Normal	Normal	Euthyroid
Glucocorticoids, androgens, danazol, asparaginase	Low	Low	Normal	Normal	Euthyroid

Courtesy : Ganong 12 th Edition

Table – 1 shows that the concentration of thyroid hormone binding protein is unaffected by hypo/hyperthyroidism.

Steroids and drugs like Clofibrate, danazol affect this binding protein concentration. Inspite of this, individuals may be in euthyroid state because free thyroid hormone concentration is normal.

Characteristics of thyroid hormones are as follows :

Table 2 : Characteristics of Circulating T_4 and T_3

Hormone Property	T_4	T_3
Serum concentrations		
Total hormone	8 g/dL	0.14 g/dL
Fraction of total hormone in the free form	0.02%	0.3%
Serum half-life	7 days	0.75 day
Fraction directly from the thyroid	100%	20%
Production rate, including peripheral conversion	90 g/d	32 g/d
Intracellular hormone fraction	20%	70%
Relative metabolic potency	0.3	1
Receptor binding	10^{-10}M	10^{-11}M

Courtesy : Harrison 18 th Edition

Table 3 : Effects of some drugs on thyroid function

Cause	Drug	Effect
Inhibit TSH secretion	Dopamine L-dopa Glucocorticoids Somatostatin	↓ T_4; ↓ T_3; ↓ TSH
Inhibit thyroid hormone synthesis or release	Iodine Lithium	↓ T_4; ↓ T_3; ↑ TSH
Inhibit conversion of T_4 to T_3	Amiodarone Glucocorticoids Propranolol Propylthiouracil Radiographic contrast agents	↓ T_3; ↑ rT_3; ↓, ↔, ↑ T_4 and FT_4; ↔, ↑ TSH
Inhibit binding of T_4/ T_3 to serum proteins	Salicylates Phenytoin Carbamazepine Furosemide Nonsteroidal antiinflammatory agents Heparin (in vitro effect)	↓ T_4; ↓ T_3; ↓ FT_4E, ↔, ↑ FT_4; ↔ TSH
Stimulate metabolism of iodothyronines	Phenobarbital Phenytoin Carbamazepine Rifampicin	↓ T_4; ↓ FT_4; ↔ TSH
Inhibit absorption of ingested T_4	Aluminum hydroxide Ferrous sulfate Cholestyramine Colestipol Iron sucralfate Soybean preparations Kayexalate	↓ T_4; ↓ FT_4; ↑ TSH
Increase in concentration of T_4 binding proteins	Estrogen Clofibrate Opiates (heroin, methadone) 5-Fluorouracil Perphenazine	↑ T_4; ↑ T_3; ↔ FT_4; ↔ TSH
Decrease in concentration of T_4 binding proteins	Androgens Glucocorticoids	↓ T_4; ↓ T_3; ↔ FT_4; ↔ TSH

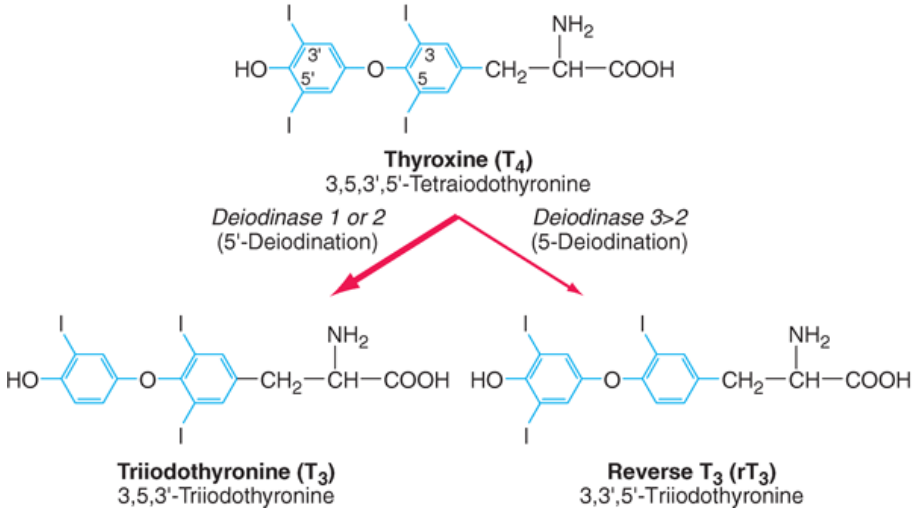
Courtesy: Tietz Textbook of clinical chemistry and molecular diagnostics fourth edition.

Metabolism of thyroid hormones:

T_4 and T_3 are metabolized by the enzyme deiodinases. There are three different deiodinases which are active in homodimeric forms. This enzyme has selenocysteine in its active site which is the cause for its catalytic activity[11].

- **Type 1 [D1] -** present in thyroid, liver, kidney and anterior pituitary gland.
  - D1 – converts T_4 to T_3 [5' deiodination] or rT_3 [5 deiodination]
  - Thyroid hormone stimulates D1 gene transcription and its activity
  - Km for D1-T4 complex is 1000 times greater than D2 or D3
- **Type 2 [D2]** present in thyroid, brown adipose tissue, CNS, anterior pituitary, heart, skeletal muscle and placenta.
  - D2- converts T4 to T3
  - Thyroid hormone suppress D2 gene expression
- **Type 3 [D3]** found in liver, CNS, endometrium and placenta.
  - D3- converts T4 to rT3 [18]

13 % of circulating T3 is secreted by thyroid gland, while 87 % is formed by deiodination of T4[11].



- Some of the T3, T4 is further metabolized to deiodotyrosines and conjugated in the liver to form glucuronides and sulfates. They are excreted through bile[11].

Mechanism of action of thyroid hormone:

- Circulating thyroid hormones enter into the cells through passive diffusion and certain transporters like MCT 8, MCT 10 and OATP 1C1. MCT 8 and 10 are expressed in many tissues. OATP 1C1 is expressed mainly in the brain [20].

- Crosses the nuclear membrane and binds to the intranuclear thyroid receptors.

- Thyroid receptors are members of the steroid hormone supergene family.

- T3 has 10-15 times more affinity than T4 for the receptor binding. There are two forms of thyroid receptor - α and β. Their expression differs in various tissues.

- The aporeceptors bind to corepressors that inhibit gene transcription.

- After binding of thyroid hormones to the receptor, conformational change occurs that dissociates corepressors and recruits co-activators. TR has carboxy terminal ligand binding domain and central DNA binding domain. This DNA binding domain binds to thyroid response element, a specific DNA sequence in the promoter region of the target genes.

- Usually receptors bind most commonly as heterodimers with Retinoid X receptor or as homodimers.

- Depending upon the nature of the regulatory elements in the target gene the activated receptor can either induce or inhibit gene transcription.

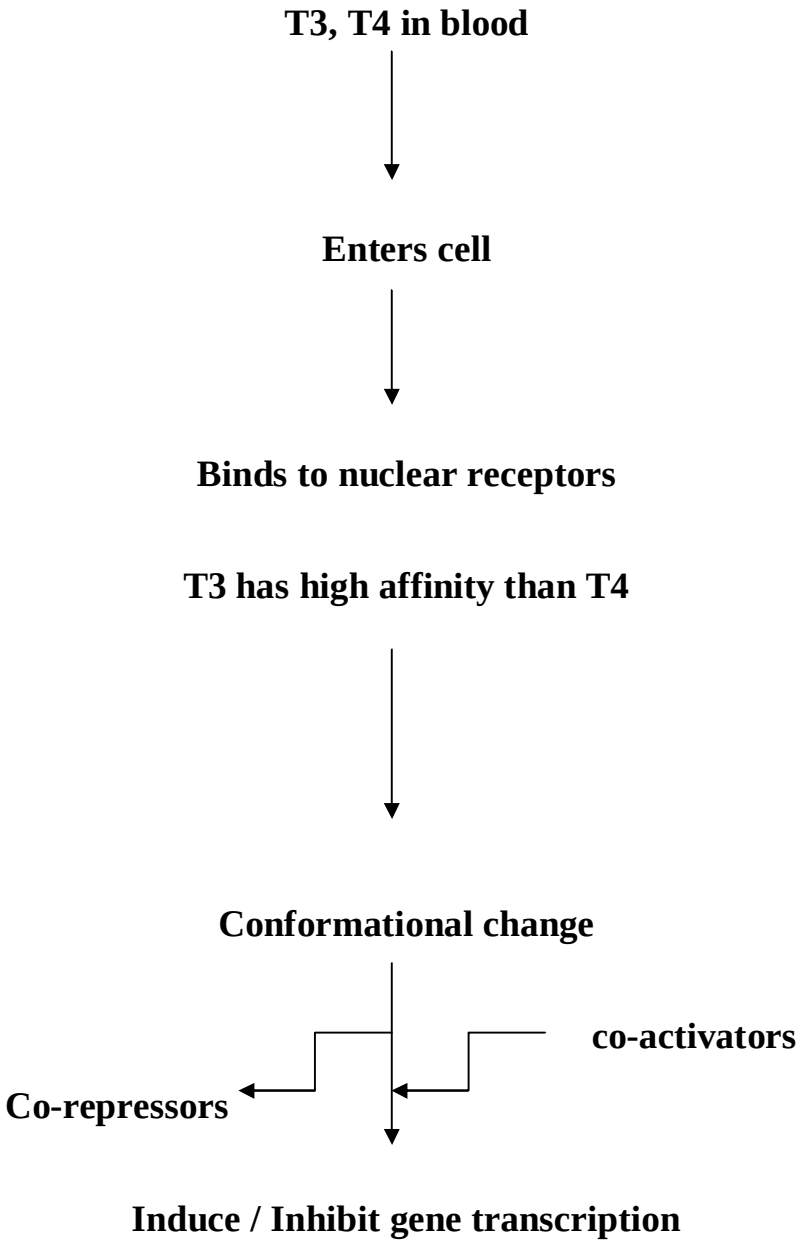
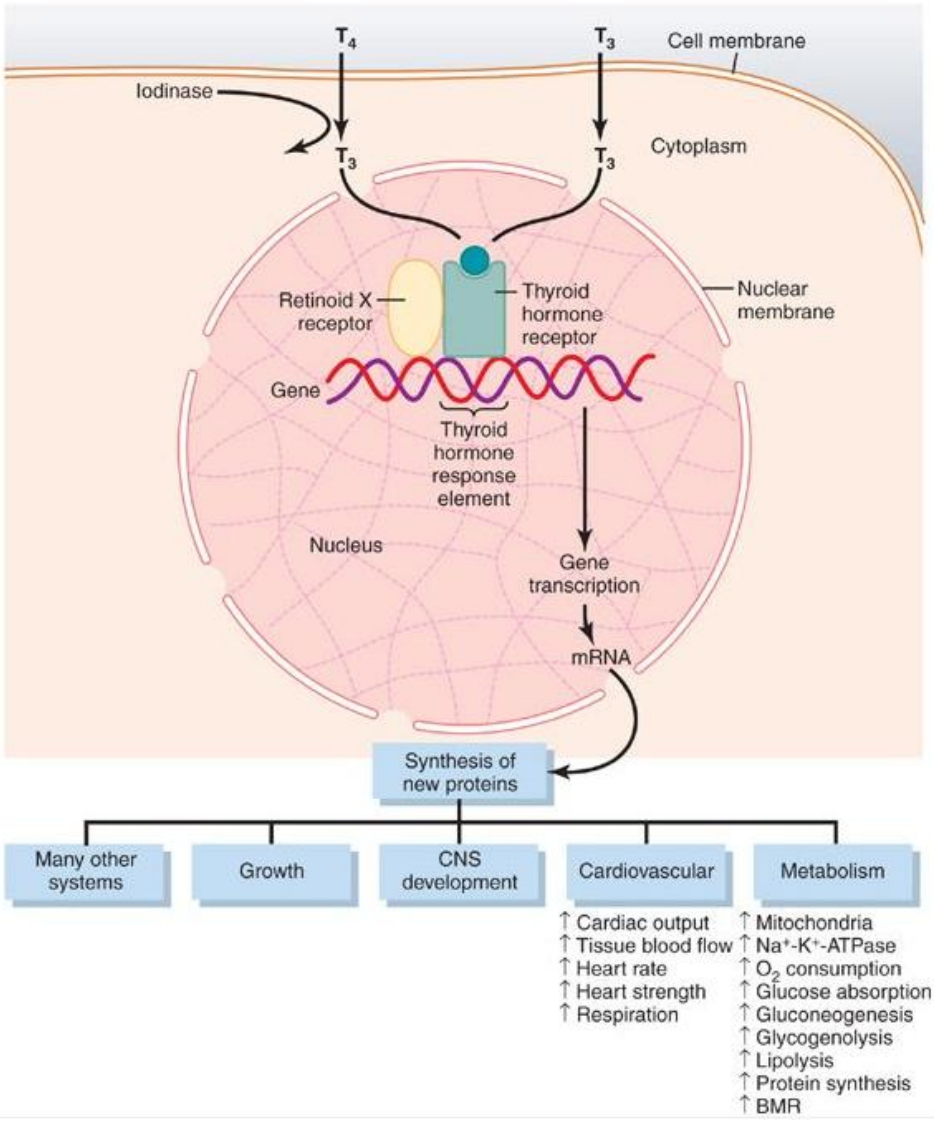

T3, T4 in blood
Enters cell
Binds to nuclear receptors
T3 has high affinity than T4
Conformational change
co-activators
Co-repressors
Induce / Inhibit gene transcription

FIGURE 2 : MECHANISM OF THYROID HORMONE



Courtesy : Guyton and Hall. Textbook of Medical Physiology [12 th edition]

Regulation of thyroid hormone :

Hypothalamic – Pituitary –Thyroid axis is a classical example for feedback control. This axis senses variation in the thyroid hormone concentration in the circulation and regulates them.

Thyrotropin Releasing Hormone regulates TSH secretion :

Hypothalamus secretes thyrotropin releasing hormone[TRH] which is a tripeptide – polyglutamyl-histidyl-proline-amide. It is secreted from the median eminence of hypothalamus and reaches the pituitary via hypothalamic-pituitary portal blood.

TRH bind to its receptor in thyrotroph membrane of the pituitary and activates phospholipase C that converts phosphatidylinositol diphosphate into inositol triphosphate and diacylglycerol which acts as a second messenger [21]. Inositol triphosphate increases intracellular calcium and stimulates Ca^{2+} -calmodulin dependent kinases. Diacylglycerol stimulates protein kinase C. Both kinases phosphorylate proteins and releases thyrotrophin from the pituitary thyrotroph cells. T_3 in the hypothalamus inhibits pre-pro-TRH mRNA[21,22] and also blocks ability of TRH to stimulate TSH release.

Neuropeptides like leptin and α melanocyte stimulating hormone regulates pre-pro TRH synthesis by stimulating TRH promoter through leptin receptor and melanocortin 4 receptor respectively. Fasting decreases leptin secretion which may decrease TRH synthesis and amplitude of pulsatile TSH release [21,23].

Thyrotropin/TSH regulates thyroid hormone secretion :

Thyroid stimulating hormone otherwise called thyrotropin, is a glycoprotein secreted by thyrotrophs from the anteromedial portion of adenohypophysis. It consists of α and β subunits. Among these α subunits are identical to other glycoprotein hormones like Leutinizing hormone, Follicle stimulating hormone and Human chorionic gonadotrophin. Beta subunit is unique for TSH.TRH increases transcription of both subunits and post-translational modification of TSH.

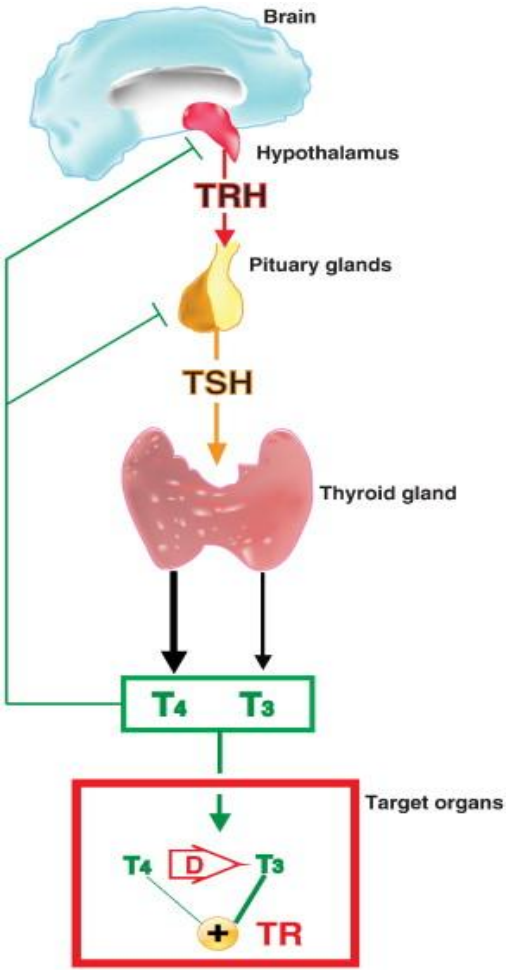
TSH undergoes two types of variations. Such variations are pulsatile and circadian variation. TSH is secreted in 1 – 2 hours interval in pulsatile manner. This is decreased during fasting, after surgery and during illness. TSH levels start to rise at about 9 pm, peaks at midnight and declines during the day. Half-life of TSH is 60 minutes. Most part is degraded by kidney and to a small extent by the liver.

TSH binds to its receptor in the thyroid gland. TSH receptor is a G protein coupled receptor with seven transmembrane domains spanning the membrane. After binding of TSH to its receptor it activates adenyl cyclase through G stimulatory and increases the intracellular cAMP concentration and activates protein kinase that phosphorylates many proteins.

TSH increases thyroid hormone secretion by :

- Increasing proteolysis of thyroglobulin stored in the colloid to increase T_3 and T_4 concentration
- Increasing the activity of iodide pump and iodide trapping
- Increasing the iodination of tyrosine residues
- Increasing the secretory activity of follicular cells
- Increasing the number of cells and converting inactive cuboidal to active columnar cells.

FIGURE 3 : REGULATION OF THYROID HORMONE



Courtesy : Camilla Pramfalle, Matteo Pedrelli, Paolo Parini. Role of thyroid receptors β in Lipid metabolism: BBA Molecular Basis of Disease. Volume 1812, issue 8, August 2011: 929-937.

Thyroid hormone receptors :

- Thyroid hormone receptors are encoded by two genes – α and β. By different splicing of mRNA various isoforms produced. There are four thyroid hormone receptors namely α_1, α_2, β_1 and β_2.
- Each receptors has 3 domains: [21]
  - ❖ Transactivation domain at amino terminus , to interact with transcription factors.
  - ❖ DNA binding domain binds to promoter region of DNA known as Hormone Response Element.
  - ❖ Ligand binding domain and dimerization domain at carboxy terminus.
- DNA binding domain of all four receptors are very similar.
- α_2 has unique carboxy terminus and does not bind to T3.
- Isoforms differ in their tissue expression. All tissues express α_1, α_2, β_1 isoforms. β_2 is present in developing ear, hypothalamus and anterior pituitary.
- Thyroid receptor α is the major receptor in the heart. It regulates the contraction and relaxation of the heart and the heart rate.

- β_1 is the major receptor in liver, plays an important role in lipid metabolism [24].
- In the absence of thyroid hormone, receptors binds to DNA and usually leading to transcriptional repression.
- After binding of the thyroid hormone to its receptor it induces conformational change in the receptor and acts as a transcriptional activator.

Thyroid hormones which acts through TRβ_2 regulates TSH and TRH production by feedback loop mechanism[15].

Decrease in thyroid hormone directly increases TSH production from the pituitary or by the action of TRH from hypothalamus . Thyroid hormones are the major regulator of TSH secretion. The feedback effect of thyroid hormones are mainly on the pituitary.

Functions of thyroid hormones:

Generally thyroid hormone activates transcription of large number of genes in almost all cells in the body. This increases the synthesis of enzymes, transport proteins, structural proteins, etc. In overall it increases the functional capacity of the cell. Also thyroid hormones have non-genomic cellular actions that are independent of gene transcription

effects. These effects are explained in several tissues including pituitary, heart and adipose tissue [25].

Thyroid hormone upregulates the expression of:

- Growth hormone
- Myelin basic protein
- Alpha-myosin heavy chain
- Sarcoplasmic reticulum calcium ATPase

Thyroid hormone downregulates the expression of :

- TSH beta chain
- Beta myosin heavy chain

The non-genomic thyroid hormone action is on Plasma membrane, cytoplasm and some organelles . For example in mitochondria it has effect on oxidative phosphorylation, regulation of ion channels, activation of intracellular second messengers eg. cAMP, Protein kinase signaling cascade[25].

Increase of cellular metabolic activity :

Basal metabolic rate- Defined as energy required by an awake individual during physical, emotional and digestive rest. It is the

minimum energy required to maintain life or sustain vital functions like respiration, circulation, heart and brain function.

- Basal metabolic rate is regulated by thyroid hormones. When this hormone secretion is increased , BMR increases upto 60-100 % above the normal level [25].

- Thyroid hormones increase oxygen consumption by metabolically active tissues in the body except brain, anterior pituitary, spleen, lymph nodes, uterus and testis. This is known as calorigenic action of thyroid hormones[11].

- Thyroid hormones increase active transport through cell membrane. One of the ion channels that gets activated is Na+/K+ ATPase. This uses energy and produces heat. This is one of the mechanisms leading to increased metabolic rate.

Effects on growth:

- Effects are mainly seen in growing children.
- Enhances growth hormone action on tissues.
- In hypothyroidism rate of growth is retarded.
- In hyperthyroidism rate of growth is increased and epiphysis close at an early age.
- Promotes growth and development of the brain during fetal life.

Effects on carbohydrate metabolism :

- Increases absorption of glucose from gastrointestinal tract.
- Rapid uptake of glucose by the cells
- Stimulate gluconeogenesis
- In hyperthyroidism plasma glucose level suddenly rises after a carbohydrate meal and falls rapidly

Effects on Fat metabolism:

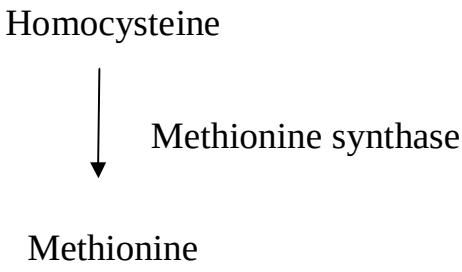
- Thyroid hormone increases lipolysis
- Stimulates Fatty acid oxidation
- Lowers plasma cholesterol and triglycerides level
    - ❖ By increasing LDL receptors in the liver , resulting in excess removal by hepatic tissue.
    - ❖ By increasing cholesterol secretion in the bile and loss in feces.

Effects on protein metabolism :

- At physiological concentration thyroid hormone increases protein synthesis and also its degradation to some extent.

- Thyroid hormone provides gluconeogenic substrate by protein degradation mainly from the muscle which is exaggerated in hyperthyroidism.

- Thyroid hormone increases hepatic levels of methionine synthase or methyltetrahydrofolate-homocysteine methyl transferase enzyme that methylates homocysteine to methionine which maintains homocysteine concentration in the normal range.



Effects on Cardio Vascular System :

- Thyroxine has chronotropic and ionotropic effect on heart muscle.
- Increases blood volume by increasing cellular metabolism and vasodilatation that leads to activation of Renin-Angiotensin-Aldosterone system
- Increases cardiac output
- Increases heart rate
- It sensitizes heart to adrenaline

Effects on Respiratory System :

Increases respiratory rate and depth of respiration due to increased utilization of O2 and formation of CO2.

Effects on Gastro Intestinal Tract :

Increases appetite and gastrointestinal motility

Effects on Central Nervous System :

- Increases the activity of brain
- Thyroid hormone is necessary for brain development.
- From the circulation T3 enters into brain

Effects on Muscles :

- Increase in thyroid hormones weaken the muscle due to excess protein catabolism.
- Lack of hormones lead to sluggish and delayed relaxation of muscles after contraction.

Effects on sleep:

Thyroid hormone excess produce increased reactivity of the neuronal synapses leading to insomnia. In hypothyroidism somnolence is a characteristic feature.

Effects on other Endocrine glands [25]:

- Increases insulin secretion from pancreas and has secondary effects on glucose metabolism
- Increases PTH – which increases many metabolic activities related to bone formation
- Thyroid hormone increases ACTH levels by increasing the metabolism of adrenal glucocorticoids in the liver.

Effects on gonadal function :

It has direct metabolic effects on the gonads and indirectly through anterior pituitary hormones that regulate gonadal function.

Other functions:

- Conversion of beta carotene to vitamin A
- Metabolism of glycosaminoglycans

Table 4: Physiologic Effects of Thyroid Hormones

Target Tissue	Effect	Mechanism
Heart	Chronotropic Inotropic	Increased number of adrenergic receptors
		Enhanced responses to circulating catecholamines
		Increased proportion of myosin heavy chain (with higher ATPase activity)
Adipose tissue	Catabolic	Stimulated lipolysis
Muscle	Catabolic	Increased protein breakdown
Bone	Developmental	Promote normal growth and skeletal development
Nervous system	Developmental	Promote normal brain development
Gut	Metabolic	Increased rate of carbohydrate absorption
Lipoprotein	Metabolic	Formation of LDL receptors
Other	Calorigenic	Stimulated oxygen consumption by metabolically active tissues (exceptions: testes, uterus, lymph nodes, spleen, anterior pituitary)
		Increased metabolic rate

Courtesy : Ganong's Review of Medical physiology 23 rd edition.

Hypothyroidism :

Hypothyroidism is due to deficiency of thyroid hormone secretion and action. Women are more affected than men and the incidence increases with age. Myxoedema is the adult form of hypothyroidism characterised by accumulation of mucopolysaccharides in the tissues and skin which leads to swelling and thickening of the skin. Cretinism is the severe form of hypothyroidism that occurs in infants.

Primary hypothyroidism:

It is caused by structural or functional abnormalities of thyroid gland. Diseases of the gland or any treatment that destroys thyroid tissue interferes with hormone synthesis and action.

Secondary hypothyroidism:

Diseases of the pituitary and hypothalamus cause decreased TSH secretion and leads to hypothyroidism.

Symptoms:

- Tiredness, weakness, Fatigue
- Dry skin
- Feeling cold
- Hair loss

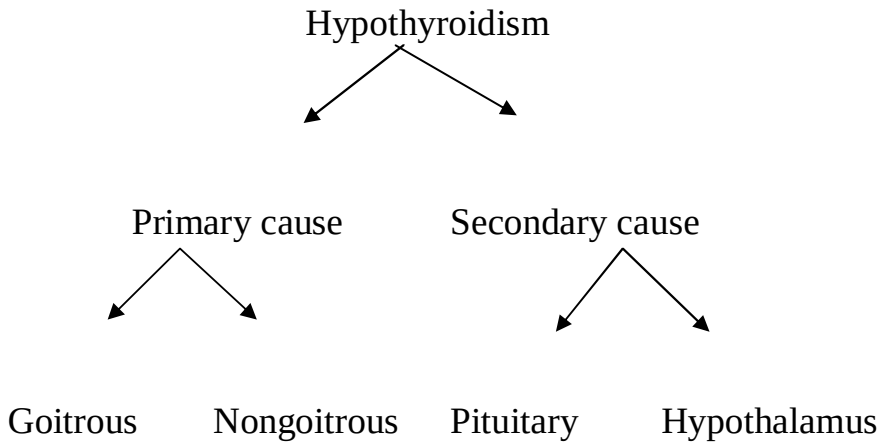
- Poor memory and difficulty in concentrating
- Constipation
- Weight gain with poor appetite
- Dyspnea
- Hoarseness of voice
- Menorrhagia
- oligomenorrhea or amenorrhea
- Paresthesia
- Impaired hearing

Signs

- Dry coarse skin
- cool peripheral extremities
- Puffy face, hands, and feet (myxoedema)
- Diffuse alopecia
- Bradycardia
- Peripheral edema
- Delayed tendon reflex relaxation
- Carpal tunnel syndrome
- Serous cavity effusions

Causes :

There are various causes for hypothyroidism. It is divided into primary causes and secondary causes. Iodine deficiency is the most common cause for hypothyroidism. In iodine sufficient areas autoimmune disease is the most common cause. Approximately 99 % of cases are due to primary hypothyroidism [12].



Primary causes of Hypothyroidism:

- Nongoitrous :[loss of thyroid tissue]
  - Surgical removal of thyroid gland
  - Radioablation for toxic goiter or cancer
  - Autoimmune thyroiditis
  - Thyroid gland dysgenesis
  - Thyroid gland hypoplasia

- Goitrous : [decrease in thyroid hormone synthesis]
  - Dietary deficiency of iodine
  - Intake of goitrogens
  - Drugs like :
    - Lithium
    - Iodine [wolff chaikoff's effect][7]
    - Antithyroid agents
    - Sodium or Pottasium perchlorate
    - Paraamino salicylic acid
    - Phenylbutazone
    - Aminoglutethimide

Secondary causes :

- Pituitary :[deficient TSH production]
  - Trauma
  - Injury
  - Infarct
  - Infection
  - Tumors
- Hypothalamus :[deficient TRH production]
  - Trauma
  - Injury
  - Infection

Iodine requirement and deficiency :

- Recommended Dietary Allowances for iodine
- Adults – 150 microgram/day
- Pregnancy – 220 microgram/day

Iodine deficiency is most common in the mountainous areas because of low iodine content in soil and water. Ingested iodine is converted to iodide and absorbed from gut . 80 % of ingested iodine is excreted through kidneys. Only 20 % of iodine is utilized for thyroid hormone synthesis by the gland .

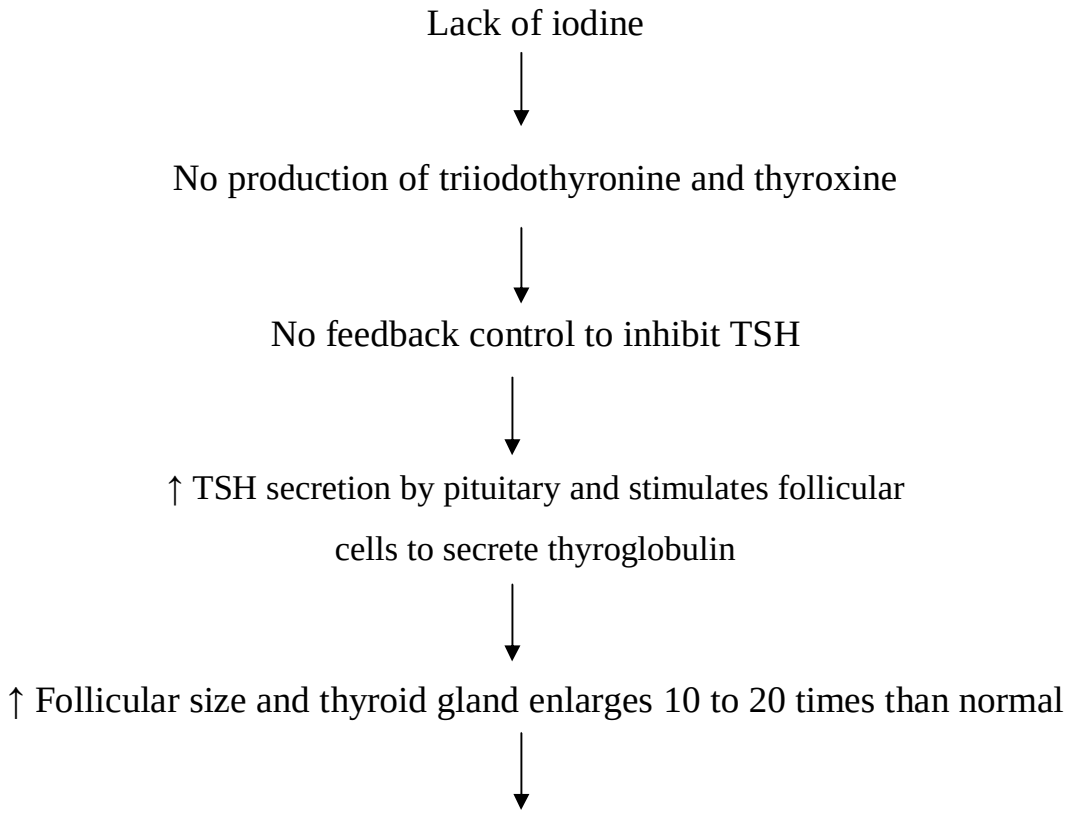
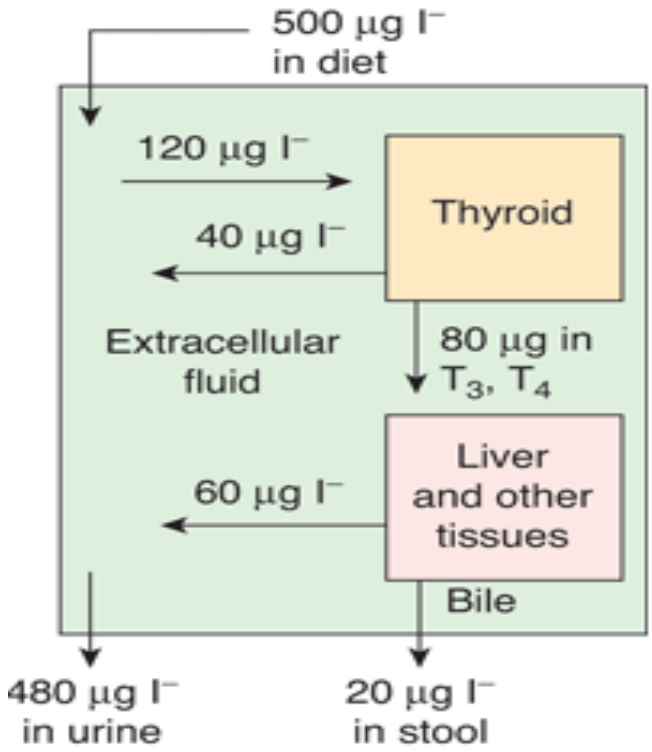


FIGURE 4 : IODINE METABOLISM



Courtesy : Ganong 23 rd edition.

Endemic goiter is usually due to Iodine deficiency. Enlargement of the thyroid gland is called goiter .

Grading of Goiter :

Grade 0 : No visible or palpable swelling

Grade 1 : No visible but palpable swelling when the neck is in normal position.

Grade 2 : swelling is visible and palpable when the neck is in normal position

Grade 3 : the struma is very large and extends into retrosternal region

Table salt is iodised one part of Sodium Iodide to every 1,00,000 parts of sodium chloride to prevent iodine deficiency. [30].

Goitrogenic Foods :

Ingredients present in naturally occurring foods which prevents utilization of Iodine in the diet are called goitrogens. Generally goitrogenic foods are divided into two categories: soybean-related foods and cruciferous vegetables. Also strawberries, millet, peaches contain goitrogens which is not included in this category.

Goitrogenic foods	Goitrogens	Mechanism
Millet, soybeans	Flavonoids	Inhibits thyroperoxidase
Cruciferous Vegetables : Cabbage, Cauliflower, Broccoli, turnips	Thiocyanates	Inhibits Iodine uptake of thyroid
Cassava meal	cyanogenic glycoside converted to thiocyanates	Inhibits Iodine uptake of thyroid

Autoimmune thyroiditis :

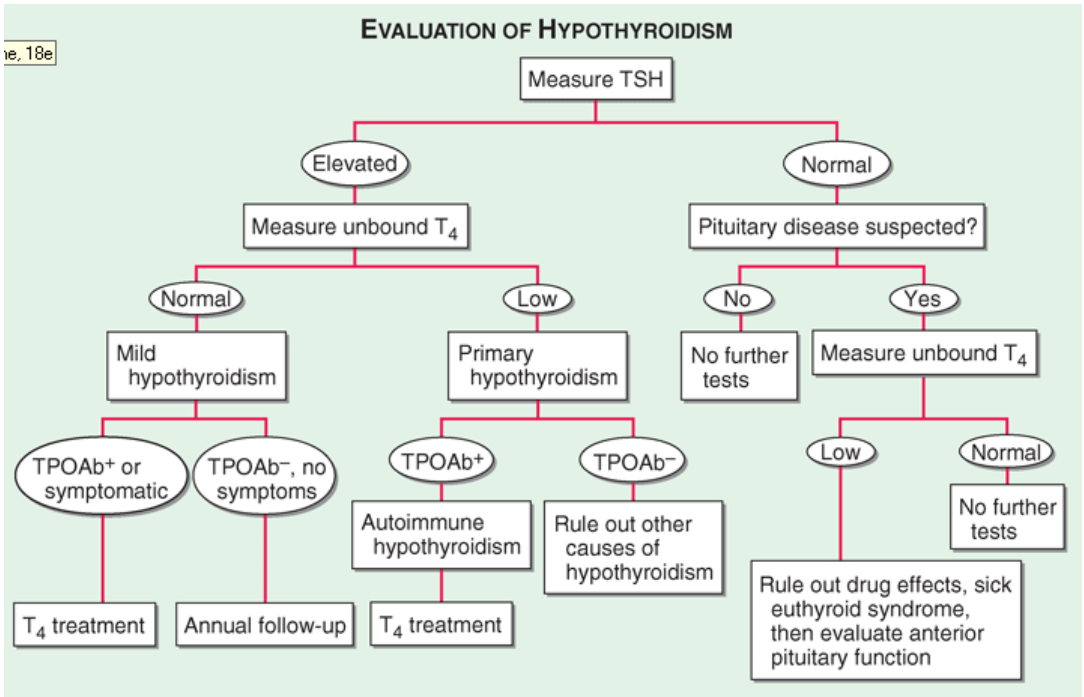
Among autoimmune thyroiditis hashimoto's is the most common. Its prevalence is 4 per 1000 women and 1 per 1000 men [33]. It is more common in females than males and increases with age. Presence of autoantibodies to either thyroid peroxidase, thyroglobulin or TSH receptors. In this condition lymphocytic infiltration occurs in the thyroid gland. Lymphocytic infiltration is composed of CD4, CD8, T cells and B cells. T cells produces cytokines which includes tumor necrosis factor, interleukins, interferon gamma. This stimulates follicular cells to undergo apoptosis. Thyroid cell destruction is mainly caused by CD8- cytotoxic T cells either by apoptosis or perforin induced cell necrosis[33]. This causes atrophy of thyroid follicles and leads to fibrosis. So there is impaired

secretion of thyroid hormones. This condition is diagnosed by elevated TPO antibodies and confirmed by thyroid biopsy.

Risk factors : [33]

- Genetic susceptibility – HLA DR polymorphism individuals
- Age
- Female sex – due to sex steroid effects on immune response
- Infection
- Pregnancy - post partum thyroiditis
- Drugs - Iodine , Amiodarone

EVALUATION OF HYPOTHYROIDISM



Courtesy : Harrison's 18 th edition

Complications of Hypothyroidism :

Infertility :

Hypothyroidism affects ovulation and impairs fertility.

Birth defects :

Hypothyroidism during pregnancy affects brain and physical development of the fetus. Hypothyroidism should be diagnosed by newborn screening as early as in the neonatal period to prevent developmental complications.

Heart problems :

Hypothyroidism increases LDL cholesterol in the plasma and can lead to atherosclerosis and myocardial infarction. Hypothyroidism can also lead to cardiac enlargement, cardiac failure and pericardial effusion.

Mental health :

Hypothyroidism gradually decreases mental functioning and leads to depression.

Peripheral neuropathy :

Peripheral nerves may get damaged if long term uncontrolled hypothyroidism occurs. Symptoms are pain, numbness and tingling sensation over the affected area. It also leads to muscle weakness.

Myxoedema :

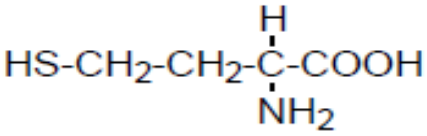
It is a rare, emergency and life threatening condition. Symptoms and signs include extreme fatigue, intense cold intolerance, drowsiness and unconsciousness.

Homocysteine :

Homocysteine is the sulphur containing aminoacid which is formed as an intermediate in the methionine metabolism. Methionine is an essential aminoacid and is the only source of homocysteine in the human body. Only small amount of homocysteine is present in free form, approximately 80 % are protein bound. Animal protein contains twice the amount of methionine than in plant protein.

Homocysteine Structure :

It is an aliphatic mono amino mono carboxylic aminoacid. It is a derived aminoacid.



Homocysteine Metabolism :

Homocysteine metabolism involves one of the two pathways : [26]

- Transmethylation
- Transsulfuration

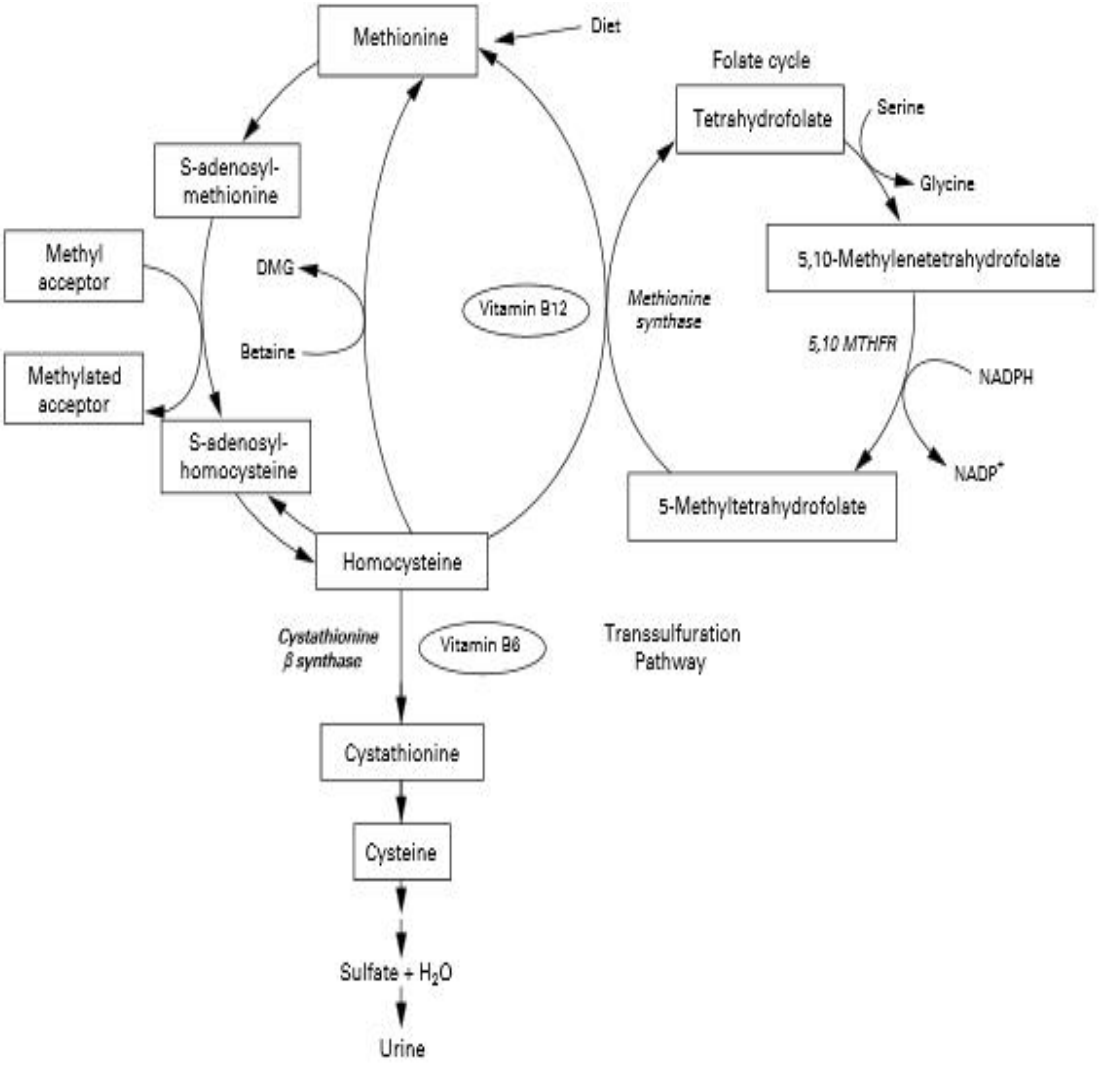
Transmethylation pathway :

Homocysteine is converted to methionine by the enzyme methyltetrahydrofolate - homocysteine methyl transferase and betaine – homocysteine methyl transferase which is present only in liver [27,28]. In this reaction methyl tetrahydrofolate and vitamin B12 acts as a cofactor. This is a reversible reaction . Methyl transferase enzyme has Km of < 0.1 mmol/L and has high affinity for homocysteine even in low concentration[27,28]. Methionine is converted to its active form S-adenosyl methionine by methionine adenosyl transferase . High concentration of S-adenosyl methionine inhibits methyl transferase and is the methyl donor for transmethylation reaction.

Transsulfuration pathway :

Homocysteine is metabolised to cysteine with the help of two vitamin B6 dependent enzymes. The Km of cystathionine β synthase is > 1mmol/L and has low affinity for homocysteine. It is converted to cysteine only in high concentration[27,28].This cysteine is further metabolised and excreted in the urine. Transsulfuration pathway is irreversible and this pathway is present only in liver, kidney, pancreas and small intestine [29].

FIGURE 5 : HOMOCYSTEINE METABOLISM

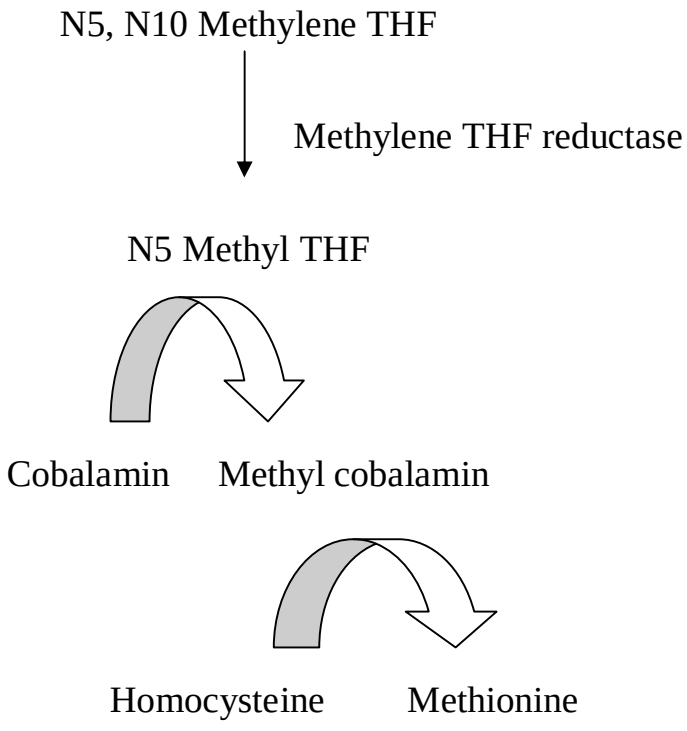


Courtesy: KILLIAN ROBINSON. Homocysteine, B vitamins, and risk of cardiovascular disease.

Hypothyroidism and Homocysteine :

Plasma total homocysteine level is increased in hypothyroidism and decreased in hyperthyroidism[31,32]. Hypothyroidism decreases the enzyme involved in remethylation pathway of homocysteine and thus leads to hyperhomocysteinemia[34]. Thyroid hormones stimulate flavokinase involved in the synthesis of flavin adenine mononucleotide and flavin adenine dinucleotide[35,36,37].

In hypoyhroidism there is defective conversion of riboflavin to its FAD co-enzyme[38]. Methylene tetrahydrofolate reductase is the flavoprotein enzyme that converts methylene THF to methyl THF. The methyl THF is necessary for methylation of vitamin B12 and further conversion of homocysteine to methionine. MTHFR activity is decreased in hypothyroid individuals which leads to hyperhomocysteinemia[39,40].



Thyroid hormone replacement therapy in hypothyroidism decreases homocysteine level [41,42]. Normalization of homocysteine usually occurs after 3-9 months of therapy[43].

Homocysteine on hypothyroidism :

Total plasma homocysteine levels [micromoles/L] :

- 10 μmol/L - Normal
- 10-12 μmol/L - Mild hyperhomocysteinemia
- 12-30 μmol/L - Moderate hyperhomocysteinemia
- 30-100 μmol/L - Intermediate hyperhomocysteinemia
- >100 μmol/L – Severe hyperhomocysteinemia [10]

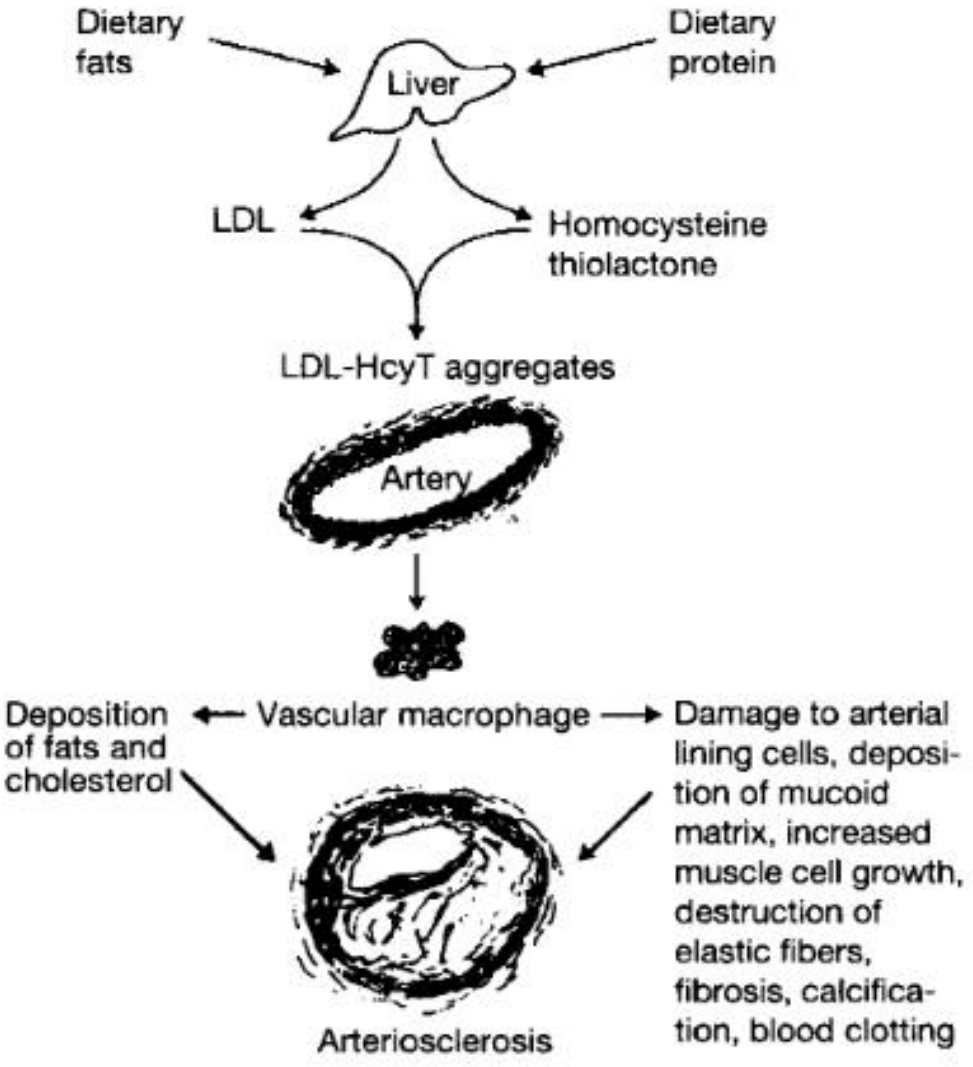
The reference range of total plasma homocysteine level has changed from 5-15 μmol/L to 5-10 μmol/L in recent times. Hyperhomocysteinemia is defined as > 10 μmol/L[44]. Mccully was the first person to report an association between hyperhomocysteinemia and atherosclerosis[45]. For atherothrombotic and atherosclerotic vascular diseases mild hyperhomocysteinemia is an independent risk factor [46,47].

There is a dose – response relationship between homocysteine and atherosclerosis. For each 5 μmol/L increase in homocysteine there is a 33 % risk of developing atherosclerosis [48,49].

In protein synthesis by fautly mechanism methionine may be replaced by homocysteine because of structural similarity. This will be recognised by one of the site of Methionyl tRNA synthetase and forms thioester bond during error-editing reaction and produces homocysteine thiolactone which is removed by Methionyl tRNA synthetase [50].

Homocysteine thiolactone is a highly reactive molecule which causes LDL cholesterol to aggregate and then phagocytosed by vascular macrophages present in early atherosclerotic plaques to form foam cells. Then homocysteine thiolactone is released from foam cells and produce free radicals [51]. Homocysteine thiolactone induces endothelial cell apoptosis independent of caspase pathway [52]. Endothelial cells are very sensitive even to mild increase in homocysteine concentration because of absence of cystathionine β synthase activity. Homocysteine thiolactone is a irreversible inhibitor of lysyl oxidase and impairs extracellular matrix maturation [53].

FIGURE 6 : HOMOCYSTEINE THEORY OF ARTERIOSCLEROSIS



Courtesy : Andrew U. Chai M.D., Jonathan Abrams M.D. Preventive Cardiology Homocysteine: A new cardiac risk factor ?

Homocysteine induced atherogenesis include [54]:

- ❖ Endothelial damage
- ❖ Platelet activation
- ❖ Smooth muscle proliferation
- ❖ Endothelial – leukocyte interactions

Endothelial damage:

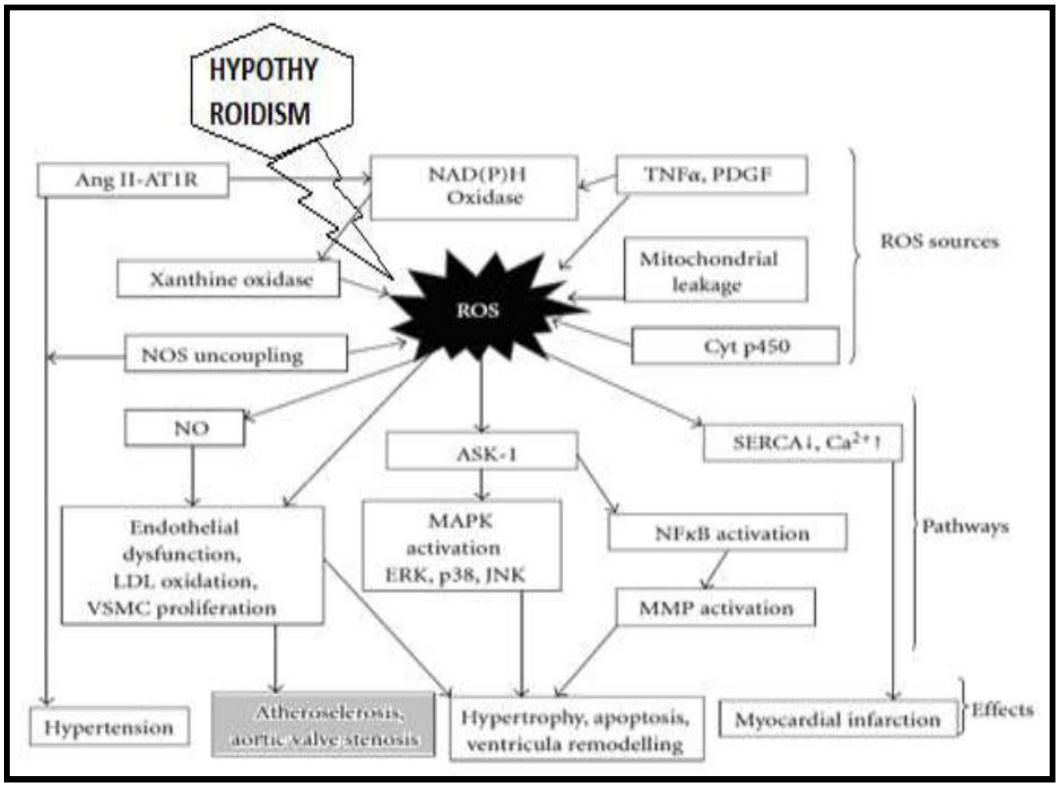
The endothelium maintains vascular homeostasis by several mechanisms. Any injury or damage to endothelial cell disrupts the normal function of endothelium resulting in leukocyte and platelet adhesion, thrombosis, smooth muscle proliferation, lipid accumulation and finally atheroma[6,7,8].

Homocysteine undergoes auto-oxidation catalysed by copper which results in formation of hydrogen peroxide which is a free radical.

Homocysteine + NO	= nitrohomocysteine
Homocysteine + LDL oxidized	= free radicals of O2
Superoxide radical o- + NO	= peroxynitrites
Homocysteine + Iron + copper	= Superoxide ion + H2O2

Homocysteine destroys glutathione peroxidase which liberates H2O2 and decreases the intracellular glutathione causes the imbalance between oxidized glutathione and reduced one [55].This increases oxidative stress of endothelial cells and causes damage [56].

FIGURE 7 : FREE RADICAL PRODUCTION IN HYPOTHYROIDISM



Generation of Reactive oxygen species :

Generation of free radicals in the vessel wall by vascular smooth muscle and endothelial cells is mainly due to TNF α which activates enzymes like NADPH oxidase and xanthine oxidase. [vinoth 36-43].This

stimulates the pathways like ASK 1, SERCA. ASK 1 activates MAPK and NFkB which in turn activates MMP and leads to hypertrophy, apoptosis and atherosclerosis[99,100].

Effects of endothelial dysfunction :

Nitric oxide:

Endothelial cell

↓

NO

↓

Vasodilatation

↓

Inhibits platelet activation and adhesion

↓

Limits smooth muscle proliferation

↓

Regulates endothelial leukocyte interactions

Initially homocysteine increases synthesis of nitric oxide [57] and reacts to form S-nitrosohomocysteine[58]. It has some properties of nitric oxide which contributes to its protective mechanism against atherosclerosis. But prolonged exposure to high homocysteine [59] creates

a self-perpetuating cycle, because the levels of homocysteine exceeds the endothelial cell capacity to produce S-nitrosohomocysteine and oxidatively degrades nitric oxide activity[60]. Antioxidants and folic acid prevents this homocysteine induced toxicity by maintaining cellular oxidative metabolism and also folic acid stimulates nitric oxide synthesis. [13,61]

Glutathione peroxidase :

Glutathione peroxidase catalyses hydrogen peroxide and lipid peroxides to form their respective alcohol[62,63] and prevents oxidative stress. Also prevents oxidative degradation of nitric oxide[5]. Among thiol containing aminoacids homocysteine has an unique property to inhibit glutathione peroxidase [5].

Haemostasis :

Endothelium synthesizes both anticoagulant and pro-coagulant. Balance between these two mechanisms is important for maintaining vascular homeostasis. Normally endothelial surface has glycosaminoglycans – antithrombin III and thrombomodulin-protein C complex[64,65]. Also by production of nitric oxide and prostacyclin, endothelium inhibits coagulation pathway[66].

In hyperhomocysteinemia, homocysteine stimulates protease endothelial cell activator of factor V and directly activates coagulation in the absence of thrombin[67].

Moderate and severe hyperhomocysteinemia reduces endothelial cell expression of glycosaminoglycans[68]. Homocysteine reduces protein C concentration because it competes with thrombin for thrombomodulin binding - component of protein C pathway[69].

Mild hyperhomocysteinemia favours binding of lipoprotein[a] to fibrin thus reduces plasminogen activation and inhibits fibrinolysis[70].

By all these above mechanisms endothelial cell produces imbalance and leads to activation of coagulation pathway.

Platelet activation :

There is indirect effect of homocysteine on platelets. Homocysteine decreases vasodilator nitric oxide concentration by inhibiting its synthesis and degradation[71] and relieves inhibition of platelet aggregation. In very severe hyperhomocysteinemia there is increased synthesis of thromboxane from platelets which is a vasoconstrictor and platelet aggregator[72].

Smooth muscle cell proliferation :

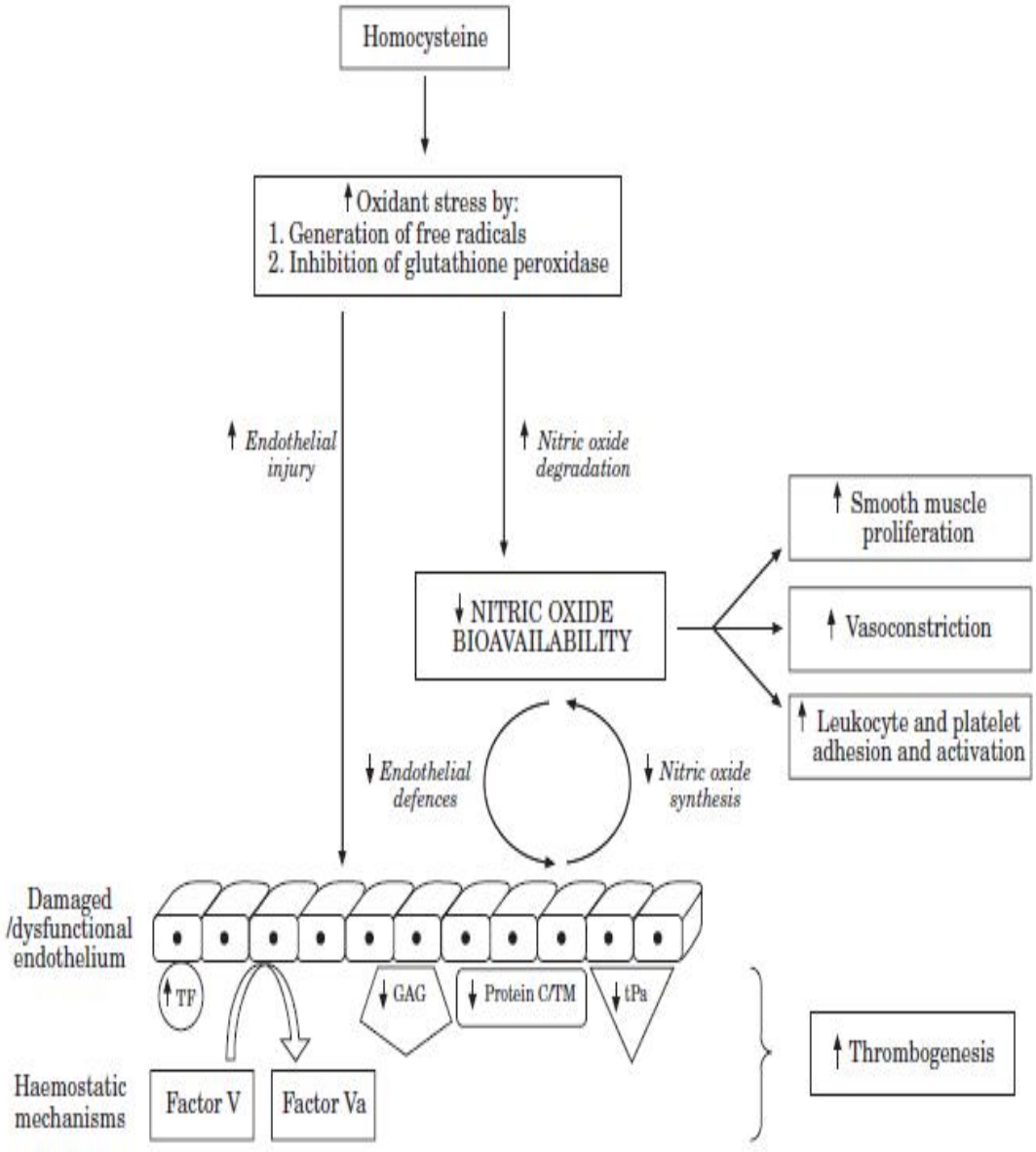
Smooth muscle cells are less susceptible to damage by homocysteine than endothelial cells. Smooth muscle cell proliferation is stimulated directly by homocysteine or homocysteine-induced platelet derived mitogenic factors. Mild hyperhomocysteinemia increases collagen expression in vascular smooth muscle cells[73].

Homocysteine increases synthesis of smooth muscle cells by involving cyclins and cyclin dependent kinases. Cyclin stimulates quinescent cells to re-enter the cell cycle and cyclin dependent kinases which is required for DNA replication[74,75] .

Endothelial-leukocyte interaction :

Homocysteine stimulates neutrophil docking protein complex CD 116 / CD 18 [76] and increase expression of monocyte chemoattractant protein-1[77].

FIGURE 8 : POTENTIAL ADVERSE EFFECTS OF HOMOCYSTEINE



Courtesy: J. Thambyrajah and J. N. Townend. Homocysteine and atherothrombosis — mechanisms for Injury

GAG, Glycosaminoglycan-Antithrombin III anticoagulant pathway; Protein C/TM, Protein C-Thrombomodulin anticoagulant pathway; TF, Tissue factor; tPa, tissue plasminogen activator.

An overview of Lipoprotein Metabolism : [78]

Lipids are transported in the plasma as lipoproteins. Lipoproteins are composed of nonpolar lipid core which consists of cholesteryl ester and triacylglycerol and encircled by single surface layer of amphipathic phospholipids and cholesterol. There are four major classes of lipids carried by lipoproteins – cholesteryl ester (36%), phospholipids (30%), triacylglycerols (16%) , cholesterol (14%) and much less fraction of free fatty acids.

Four major classes of Lipoproteins :

- **Chylomicrons** – transports exogenous triglycerides to liver and extrahepatic tissues.
- **Very low density lipoprotein** – transports endogenous triglycerides from liver to extrahepatic tissues.
- **Low density lipoprotein** – transports cholesterol from liver to extrahepatic tissues.
- **High density lipoprotein –** transports cholesterol from peripheral tissues to liver

Metabolism of Chylomicrons:

Chylomicron is responsible for transport of all dietary lipids. They are formed in the endoplasmic reticulum of intestinal epithelial cells

which is mainly composed of triacylglycerols. This is exported to the lymphatic vessels of the intestine and then to the systemic circulation. Nascent chylomicrons has apo B48 and accepts apo E and C from HDL in the circulation.

Chylomicrons are catabolised by lipoprotein lipase which is present in the blood capillaries, anchored to endothelium by heparan sulphate. It is present in heart, lung, spleen, adipose tissue, aorta, renal medulla, diaphragm and lactating mammary gland. This enzyme requires phospholipids and apo cII as cofactors and triacylglycerol is hydrolysed to free fatty acids and glycerol. Fatty acids are mainly delivered to heart, skeletal muscle, adipose tissue (80%) and 20% goes to the liver. Lipoprotein lipase in heart has high affinity for triglycerides than adipose tissue.

Insulin stimulates lipoprotein lipase synthesis in adipose tissue and its translocation to capillary endothelium . The resultant chylomicron remnant consists of apo E, cholesteryl ester, cholesterol and 10-30% of triglycerides. This is taken up by receptor mediated endocytosis in the liver and gets hydrolysed.

Very low density lipoprotein Metabolism :

Very low density lipoproteins are synthesized in the liver and mainly carries endogenous triglycerides to the extrahepatic tissues.

Formation of VLDL is similar to the formation of Chylomicrons except that it is released into the Space of Disse and then to the sinusoids. Nascent VLDL has apo B100 and accepts apo C and E from HDL in the circulation.

Triglycerides in the VLDL is metabolised by lipoprotein lipase to yield fatty acids , glycerol and VLDL remnants – also known as intermediate density lipoproteins. IDL is either taken up by the liver through LDL receptor (apo B100, E) and LDL receptor-related protein or it is converted to LDL. In humans, most of the IDL is converted to LDL.

Metabolism of Low density lipoproteins :

LDL is mainly composed of cholesterol and apo B100. LDL receptors are expressed in liver and many extrahepatic tissues. The receptors recognize apo B100 in the LDL. Approximately 70% of the LDL is taken up by the liver and nearly 30% by the extrahepatic tissues. Alternatively LDL is removed by phagocytes via non specific endocytosis or scavenger receptors – class A (SRA) and class B, type 1(SRB1) which recognizes altered LDL. This is responsible for cholesterol deposition in the vessel walls [79].

Metabolism of High density lipoproteins :

HDL is synthesised from liver and intestine. Intestinal HDL does not contain apo C and E. It is transferred from HDL synthesised from liver. The major function of HDL are reverse cholesterol transport and storage of apo C and E required for chylomicron and VLDL metabolism.

Nascent HDL consists of phospholipids, apo A and free cholesterol. LCAT and apo AI- activator of LCAT binds to the HDL disk. LCAT converts cholesterol and phospholipids into cholesteryl esters and lysolecithin. This cholesteryl ester moves into the hydrophobic core of the bilayer and lysolecithin released into the plasma. This reaction continues until spherical HDL is formed. HDL3 is smaller and more denser. HDL3 accepts further free cholesterol from peripheral tissues and forms HDL2 which is larger and less denser. Concentration of HDL2 is inversely related to atherosclerosis. Hepatic lipase hydrolyses triglycerides and phospholipids on the HDL2 and converts to HDL3 after releasing cholesteryl ester.

Lipid profile in Hypothyroidism :

Thyroid hormone enhances adipose tissue metabolism and increases production of adipokines.

- Thyroid hormone stimulates hydroxymethylglutaryl CoA [HMG CoA] reductase the rate limiting step in cholesterol synthesis and increases intracellular cholesterol concentration [96].

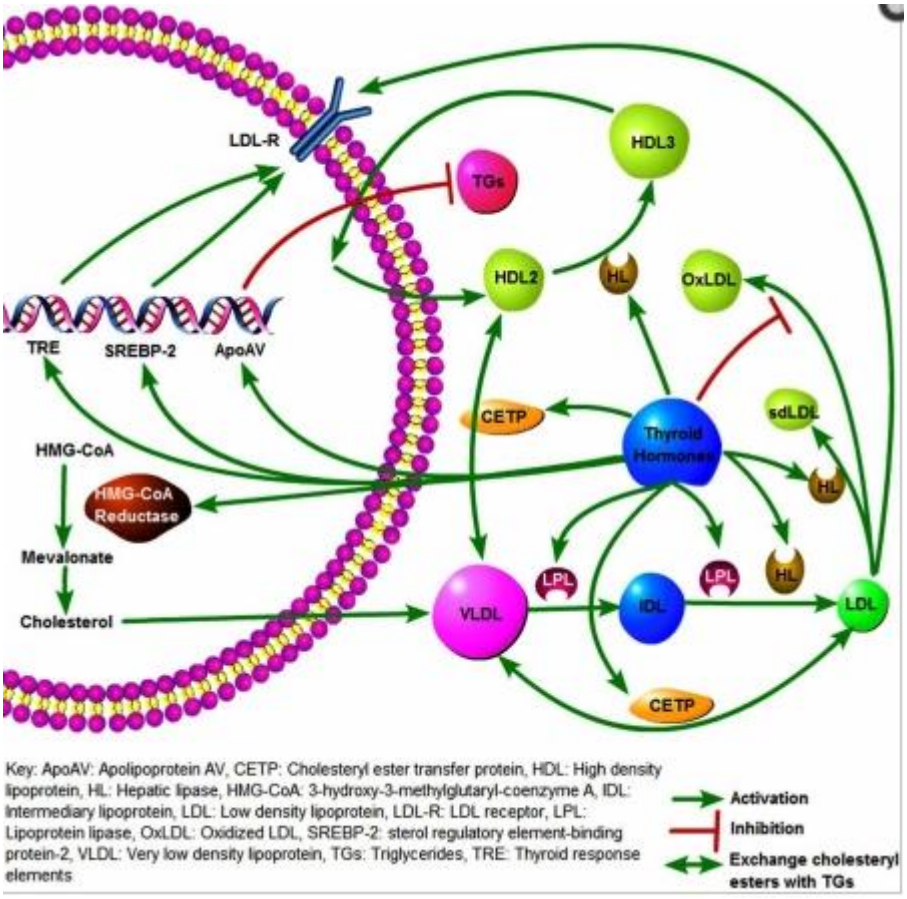
- It increases lipoprotein lipase activity which metabolises triglyceride containing lipoproteins VLDL, IDL and Chylomicrons into fattyacids and glycerol.

- It affects HDL metabolism by stimulating cholesteryl ester transfer protein which exchanges cholesteryl ester from HDL2 to VLDL , IDL and triglycerides in the opposite direction. Also it increases hepatic lipase activity which metabolizes HDL2 to HDL3 [80,81].

- Thyroid hormone increases LDL receptor gene expression by stimulating promoter region of LDL receptor gene which contains thyroid response element and regulates LDL receptor at mRNA level [82].

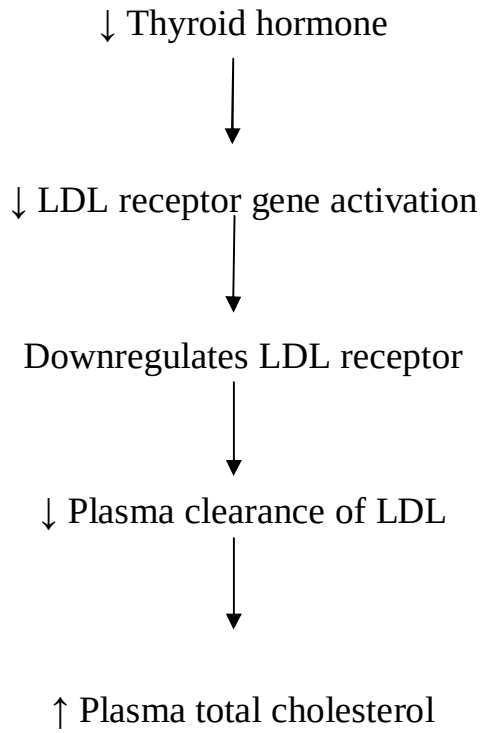
- Increases cholesterol convertion to bile acids and its excretion through bile.

FIGURE 9 : EFFECTS OF THYROID HORMONE IN LIPOPROTEIN METABOLISM



Courtesy: Shah Kruti N, Gohil Priyanshee V. Hypothyroidism and Atherosclerosis: From Etiology to Pathophysiology

In hypothyroidism lipid metabolism is altered and leads to elevated total cholesterol inspite of decreased activity of HMG CoA reductase. It is due to decreased LDL receptor activity and LDL clearance from plasma[97]..



Hypothyroidism decreases the activity of lipoprotein lipase which is present in the capillaries anchored to the endothelium by heparan sulphate. Due to its decreased activity metabolism of triglyceride containing VLDL and chylomicrons are decreased and leads to hypertriglyceridemia.

Hypothyroidism

↓

↓ Lipoprotein lipase activity

↓

↓catabolism of VLDL & chylomicrons

↓

↑ plasma triglycerides

Hypothyroidism individuals often present with normal or elevated High density lipoprotein due to decreased activity of hepatic lipase which catabolizes HDL_2 to HDL_3 and CETP which exchanges cholesteryl ester and triglycerides. Normally hepatic lipase activity is inhibited in premenopausal females by oestrogen and activated by androgens in males.

Hypothyroidism

↓

↓ hepatic lipase activity

↓

↓ catabolism of HDL2 to HDL3

↓

↑ plasma HDL cholesterol [83]

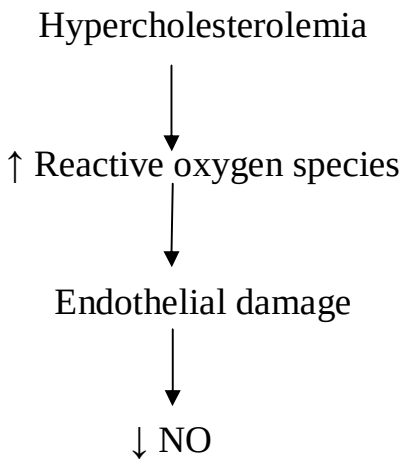
Effects of altered Lipid profile on Hypothyroidism :

Hypothyroidism increases low density lipoprotein and promotes LDL oxidation [84] . 3 % of T4 is bound to plasma lipoproteins. Among that 0.8 % binds to VLDL, 6.7 % to LDL, 92 % to HDL [85,86]. This T4 bound LDL is protected from oxidation. In hypothyroidism decrease in T4 level may induce LDL oxidation [87].

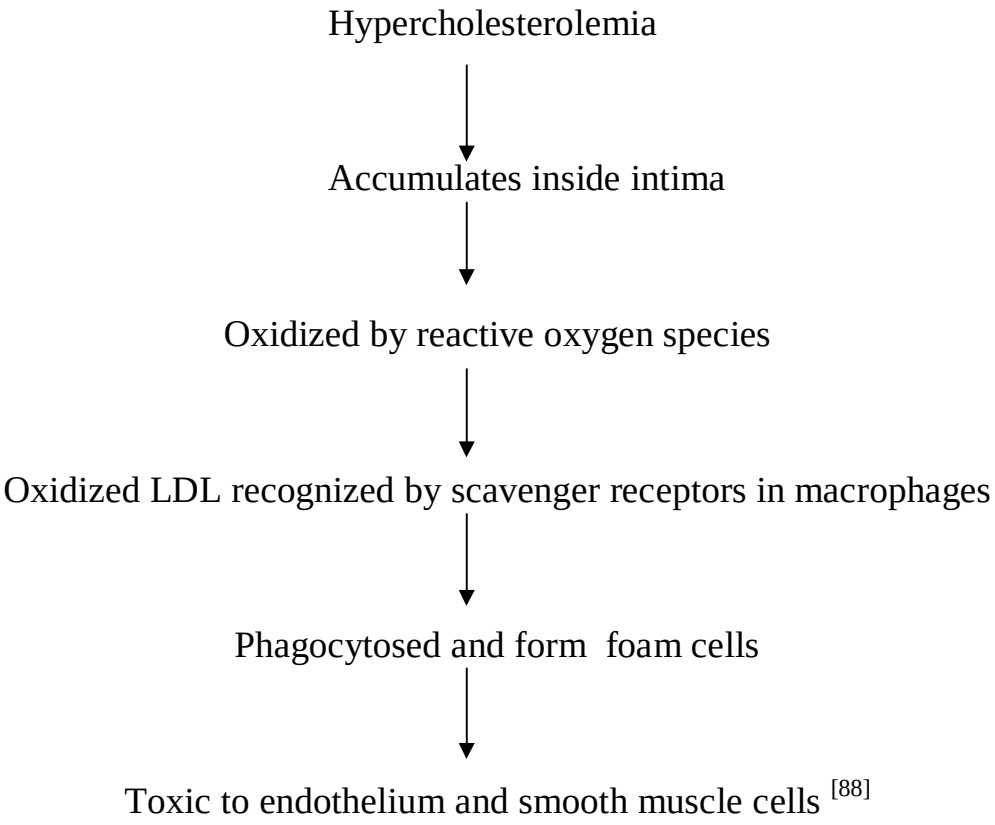
Low density lipoprotein is formed from Very low density lipoprotein after catabolism of triglycerides by lipoprotein lipase and transfer of apo A1 and apoE to High density lipoprotein. LDL is mainly composed of cholesterol and apolipoprotein B100. LDL receptors in the liver and extrahepatic tissues recognise apo B100. LDL receptor is present in the plasma membrane which is coated with clathrin. After binding of LDL to the receptor it is internalised into the cell and forms endosomes which gets fused with primary lysosome to form secondary lysosome. Protons enter in to lysosome thus decreases pH and separates LDL from its receptor and the receptor is recycled to the plasma membrane. The contents of the LDL gets metabolised by enzymes present in the lysosomes.

The normal function of LDL is to transport cholesterol from liver to extrahepatic tissues which is necessary for many functions like membrane lipids, steroid synthesis , vitamin D synthesis etc.

In hypothyroidism hypercholesterolemia leads to production of reactive oxygen species that oxidises LDL. This damages endothelial cells and decreases nitric oxide production that leads to loss of vasodilatation property. Oxidized LDL favours formation of atheromatous plaque through number of mechanisms like its cytotoxicity, chemotactic effects and thrombogenic effects. Oxidized LDL loses its normal function and gets deposited on arterial walls. This stimulates immune response and leukocytes migrate to that site and cause inflammation[88]. Oxidized LDL stimulates formation of matrix metalloproteinase in vascular endothelium, fibroblast and upregulates the expression of receptors in endothelial cells which is responsible for foam cell formation [[102]



The oxidised LDL cannot perform its function and get deposited in the walls of the arteries. This chemoattracts leukocytes which causes inflammation and are phagocytosed by macrophages and become foam cells. Early atheromatous plaque is formed in the arterial walls.



Treatment with thyroxine decreases cholesterol level by upregulation of LDL receptors and increases its excretion through bile. Thyroxine treatment stimulates reverse cholesterol transport due to increased activity of CETP and increases HDL2 metabolism by hepatic lipase, so it decreases HDL levels. Also it inhibits LDL oxidation [89].

In worldwide, atherosclerosis is one of the leading cause of death and disability. Arteriosclerosis is a syndrome characterized by the deposit and infiltration of lipid substances in the medium and large arterial walls. Is the most common form of arteriosclerosis. Causes an inflammatory reaction and multiplication and smooth muscle cells migration of the wall producing ranging narrowing of the arterial lumen and forms atheroma plaques[88].

Coronary artery atherosclerosis leads to angina pectoris and myocardial infarction. Cerebral artery atherosclerosis leads to stroke and cerebral ischemia. Peripheral artery atherosclerosis causes deep venous thrombosis[90]. The relationship between hypothyroidism and atherosclerosis was first raised by E.Theodor Kocher in 1883[101].

Hyperhomocysteinemia and dyslipidemia in hypothyroidism leads to increased free radical production, endothelial dysfunction, decreased nitric oxide bioavailability, LDL oxidation, deposition of cholesterol in vessel walls, leukocyte migration, platelet activation and adhesion leading to local inflammation, formation of foam cells and results in atheromatous plaque formation.

MATERIALS AND METHODS

METHODOLOGY

This cross sectional study was conducted after obtaining Ethical Committee clearance. All the participants and their relatives were informed about the study. The study group was selected from Endocrinology Out-patient Deparment, at Madras medical College and Rajiv Gandhi Government General Hospital.

Study population:

Cases:

This study population was divided into three groups.

Inclusion criteria:

Group 1:

It included 30 newly diagnosed hypothyroid individuals of age around 15-45 years including 26 females and 4 males. This study population was selected as diagnosed by clinician and confirmed by thyroid profile [TSH-High,T3,T4-low].

Group 2:

It included 30 hypothyroid patients between > 3 months to < 1 year duration of treatment. Age around 15-45 years including 29 females and 1

male. This study population was selected from history of decrease / absent symptoms of hypothyroidism with normal thyroid profile.

Group 3:

It included 30 controls with age, gender and other confounding factors like smoking, alcoholism were matched with 26 females and 4 males. They had no symptoms of hypothyroidism with normal thyroid profile.

Exclusion criteria for both cases and controls:

1. Chronic smokers
2. Pregnancy & lactation
3. Diabetes mellitus
4. Renal disease
5. Liver disease
6. Megaloblastic anemia by peripheral smear and
7. Patients on other medication for a long time were excluded.

Sample collection and storage:

After an overnight fast of about 10-12 hours, 2 ml of blood was collected from study groups from antecubital vein under an aseptic precaution. The blood was transferred to EDTA – anticoagulant

containing test tube upto the mark. Gently shaken the test tube upside down for uniform mixing.

Samples tubes are labeled and kept in ice box during transportation to the Biochemistry laboratory. EDTA tubes were centrifuged at 3000 g for 15 minutes . Plasma was separated from each tube, aliquoted into Eppendorf tubes within one hour from the collection time. stored at -20 $^{\circ}$ C until analysis of Homocysteine and Lipid profile.

Estimation of Plasma Total Homocysteine :

Method:

Competitive Enzyme linked Immunosorbent Assay[84,91]

Kit used:

Axis-shield Homocysteine EIA. The intra-assay precision is 7 %. The kit was stored in cold storage room at 4 $^{\circ}$ C .

Principle :

- Mixed disulfide and protein-bound homocysteine in the sample are reduced to free Homocysteine with the use of dithiothreitol.

- Protein-ss-Hcy /Hcy-ss-Hcy $\xrightarrow{\text{DTT}}$ Hcy

- Homocysteine in the sample is converted to S-adenosyl-L-homocysteine by the reagent containing SAH hydrolase and excess of adenosine.

- Hcy + Adenosine $\xrightarrow{\text{SAH hydrolase}}$ SAH + H2O

- An immobilized SAH was already bound to the walls of the microtitre [ELISA] plate.

- Pre-treated standards and samples were added to the wells.

- Then Monoclonal anti-s-adenosyl –L-Homocysteine antibody was added to the each well. This antibody competes for SAH in the sample and immobilized SAH.

- Then Bound SAH interacts with enzyme conjugate [Horse Radish Peroxidase] and substrate.Thus develops the colour.

- The intensity of the colour is inversely proportional to the concentration of SAH in the standard and the sample.

Reagents:

1. SAH coated microtitre plate 12 * 8 wells
2. SAH standards [2,4,8,15,30,50 micromoles/L] each vial 1.5 ml
3. Assay buffer-54 ml

4. Adenosine, dithiothretiol reagent -3.5 ml
5. Recombinant SAH hydrolase -3.5 ml
6. SAH hydrolase inhibitor -55 ml
7. Monoclonal mouse SAH antibody -25 ml
8. Enzyme conjugate -15 ml
9. Substrate solution -15 ml
10. Stop solution -20 ml
11. Wash buffer -60 ml [20x]

Procedure:

All the reagents and microtitre strips are equilibrated to the room temperature before use.

Sample pre treatment procedure:

Sample pretreatment solution was prepared not more than one hour prior to the start of the assay.

In the first step, assay buffer, adenosine/dithiothretiol, SAH hydrolase were calculated for 100 samples as Reagent A- 45 ml, Reagent B-2.5ML, Reagent C-2.5 ml were pipette into the clean beaker and mixed well.

Each calibrators / samples were diluted in plastic tubes by adding 25 µL of calibrators / samples with 500 µL of sample pre-treated solution , mixed and incubated for 30 minutes at 37° C. Then followed by the addition of 500 µL of enzyme inhibitor to each tubes, mixed and incubated for 15 minutes at 18-25° C. After the incubation time it was followed with addition of 500 µL of Adenosine deaminase, phenol red dye containing reagent , mixed and incubated for 5 minutes at 18-25° C. This results in pink color. At the end of this step there was formation of SAH by the enzyme dithiothreitol and SAH hydrolase.

Microtitre plate procedure:

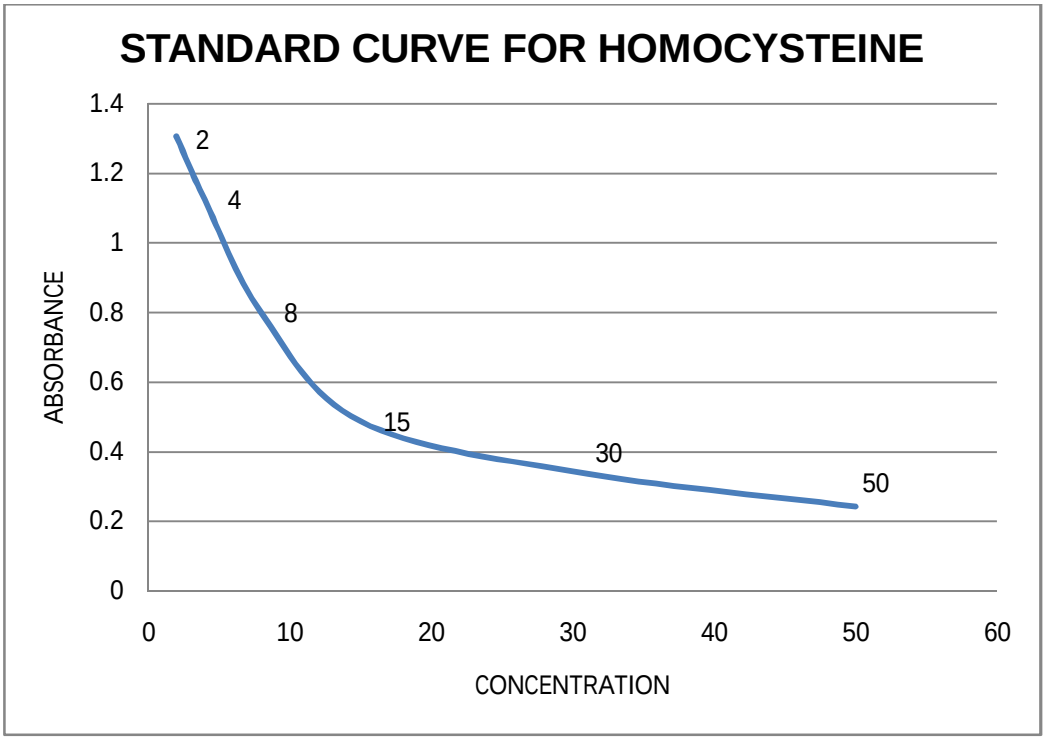
25 µL of calibrator / sample from the pre-treatment solution were pipette into the plate wells, which was coated with SAH. Then 200 µL of SAH antibody was pipette into the each well and allowed for incubation for 30 minutes at 18-25° C. During the incubation period sample / calibrator SAH and coated well SAH are competes for SAH - antibody.

Wash buffer solution were prepared during the incubation period by diluting with distilled water. 60 ml of wash buffer was added to clean wash buffer container and followed by 420 ml distilled water [1:9 ratio] to obtain 500 ml of wash buffer.

Following the incubation period, ELISA plate was washed with diluted wash buffer by using 400 μL for three times using an automated plate washer. At each wash , complete removal of solution was ensured. After washing , the wells are blotted on dry tissue paper. This was followed by the addition of 100 μL of enzyme conjugate to each well and incubated for 20 minutes at 18-25° C. This conjugate bound to antigen-antibody complex. The excess conjugate were removed by washing, using automated ELISA washer. After washing the wells are carefully blotted on dry tissue paper.

This step was followed by addition of 100 μL of substrate solution to each of the well and allowed for incubation for 10 minutes to react at 18-25° C. After the incubation period the reaction was stopped by the addition 100 μL of stop solution that contains 0.8M sulphuric acid and read the absorbance at 450nm immediately.

STANDARD CURVE FOR HOMOCYSTEINE



Graph shows homocysteine standard curve with concentration in X axis against the absorbance in Y axis.

Because of competitive immunoassay, the intensity of the colour is inversely proportional to the concentration of homocysteine. So the absorbance is obtained in the decreasing order.

Results :

Optical density and concentration of the standards were read from ELISA reader. After checking the calibration curve , samples optical density and concentration were noted. The concentration of homocysteine is expressed in micromoles /L.

Limit of detection:

- 1 micromoles /L

Measuring range:

- 2- 50 micromoles /L

Reference range :

- < 10 micromoles /L

Estimation of TSH :

Method :

Non-competitive Sandwich ELISA

Kit used :

ERBA- Thyro kit

Principle :

Sample was added to the each well which was coated with streptavidin. Anti-TSH-horseradish peroxidase / biotin conjugate was added. TSH in the samples forms sandwich between two specific TSH antibodies. By washing unbound conjugate was removed. By the addition of substate, developed colour intensity is directly proportional to the concentration of TSH. Measured photometrically at 450 nm.

Reagents :

- Streptavidin coated plate – 96 wells
- TSH enzyme reagent
- Wash buffer
- Substrate solution A & B
- Stop solution
- Calibrators – 0, 0.5, 2.5, 5.0, 10, 20, 40 μIU / L

Reagent preparation :

Wash buffer :

20 ml of wash concentrate is diluted to 1 litre with the distilled water.

Working substate solution :

Substrate solution A is poured into solution B.

Procedure :

- 50 μl of calibrators and samples are pipette into the corresponding well.
- Addition of 100 μl of TSH enzyme reagent to the each well.
- Mix the microplate gently for 20-30 seconds and incubated at room temperature for one hour.

- By using an automatic ELISA plate washer, the plate was washed for 3 times using 350 µl of wash buffer.
- Gently blotted on dry tissue paper.
- 100 µl of working substrate solution was added to the each well and incubated for 15 minutes at room temperature.
- Then 50 µl of stop solution was added to the each well and mix gently for 15-20 seconds.
- Measured photometrically at 450 nm.

Calculation of results :

For standards Optical density was noted. After observing the standard curve, optical density and concentration of the samples were noted. The concentration of TSH is expressed in micro international unit / L.

Reference range :

0.4 – 4.8 µIU / L.

Estimation of Total T3 :

Method :

Competitive Enzyme Linked Immunosorbent Assay

Kit :

ERBA Thyrokit

Principle :

The sample was added to the each well which has been coated with streptavidin. T3- horseradish peroxidase conjugate and anti-T3 biotin - conjugate was added. This antibody conjugate binds to the streptavidin . During incubation, sample T3 competes with Enzyme T3 conjugate for anti T3 binding. This complex reacts with substrate to produce colour and measured photometrically at 450 nm.

The intensity of the colour is proportional to bound HRP-T3 conjugate and inversely proportional to concentration of the T3 in the sample.

Reagents :

- Steptavidin coated microwell plate
- Total T3 biotin conjugate
- Total T3 anti horse radish peroxidase conjugate
- Substrate solution
- Stop solution
- Wash solution
- Standards -0, 0.5, 1.0, 2.0, 4, 8 ng/ml

Procedure :

- Pipette 50 µl of calibrators and samples into the assigned well.
- 50 µl of T3 enzyme conjugate was added to the each well.
- Then 50 µl of anti-T3- biotin conjugate was pipette into the each well.
- Gently shake the plate for 10 seconds and incubate at room temperature for one hour.
- Then washed the plate by using 350 µl of wash buffer for 3 times in automatic washer.
- Gently tapped the plate on dry tissue paper.
- 100 µl of substate solution was added and incubated for 15 minutes at room temperature.
- Stopped the reaction by addition of 100 µl of stop solution to the each well.
- Measured photometrically at 450 nm.

Results :

Optical density and calibration curve of calibrators were observed. Then optical density and concentration of samples were noted. The concentration of total T3 is expressed in nanograms / ml.

Reference range :

0.74 – 1.79 ng/ml.

Estimation of Total T4 :

Competitive Enzyme Linked Immunosorbent Assay

Kit :

ERBA Thyrokit

Principle :

The sample was added to the each well which was coated with streptavidin. Then Biotin-T4 conjugate and anti-T4-HRP conjugate were added to the well. During the incubation period sample T4 competes with Biotin-T4 conjugate for the binding site of anti-T4 which binds to streptavidin in the wells. This anti-T4-HRP-Biotin T4 conjugate reacts with substrate to produce colour and measured at 450 nm.

The intensity of the colour is inversely proportional to the concentration of T3 in the sample.

Reagents :

- Streptavidin microwell plates
- Total T4 biotin conjugate
- Total T4 anti HRP conjugate

- Substrate solution
- Stop solution
- Standards -0, 20, 40, 80, 150, 300 ng/ml

Procedure :

- Pipette 50 µl of standards and samples into the corresponding wells.
- Then pipette 50 µl of T4 – biotin conjugate and anti-T4-HRP conjugate into all the wells.
- Gently shake the plate for 10 seconds and incubated for one hour at room temperature.
- At the end of the incubation period the plate was washed for 3 times using wash buffer.
- After blotting on the tissue paper 100 µl of substrate solution was added.
- After incubation at room temperature for 15 minutes 100 µl of stop solution was added.
- Measured photometrically at 450 nm by ELISA reader.

Results :

After observing the calibration curve optical density and the concentration of the samples were noted. The concentration of the Total T4 measured in nanograms/ml.

Reference values :

47 – 120 ng /ml.

Estimation of other Biochemical parameters:

- Total cholesterol
- Triglycerides
- Low density Lipoprotein -Cholesterol
- High density Lipoprotein –Cholesterol
- Urea
- Creatinine
- Random blood sugar
- Total protein
- Albumin
- Alanine transaminase
- Peripheral smear

All these parameters were estimated in EM 360 Auto analyzer using Transasia kit except peripheral smear, it was done by Pathology Laboratory.

Calibration :

EM 360 auto analyzer was calibrated by using XL multical calibrator and controls such as Erba norm and Erba path.

Estimation of Plasma Total Cholesterol:

Method :

Cholesterol Esterase-Oxidase-Peroxidase

Principle:

Cholesterol Ester is hydrolysed by Cholesterol Esterase to form Cholesterol and then oxidized by cholesterol oxidase to form cholestenone and hydrogen peroxide. This hydrogen peroxide react with phenol and aminoantipyrine to produce pink colored complex and quantitated at 505 nm.

$$\text{Cholesterol Esters} \xrightarrow{\text{Cholesterol Esterase}} \text{Cholesterol + fatty acids}$$

$$\text{Cholesterol + O2} \xrightarrow{\text{Cholesterol Oxidase}} \text{Cholest-4-en-3-one + H2O2}$$

$$2H_2O_2 + \text{Phenol} + 4\ \text{AAP} \xrightarrow{\text{Peroxidase}} \text{Quinoneimine Dye} + 4H_2O$$

Reagent composition:

- Cholesterol Esterase ≥ 200 U/L
- Cholesterol Oxidase ≥ 50 U/L
- Peroxidase ≥ 3000 U/L
- Phenol 5 mmol/L
- 4-Aminoantipyrine 0.3 mmol/L
- Goods Buffer 50 mmol/L

Expected values :

- Desirable - < 200 mg/dl
- Border line - 200- 239 mg/dl
- High - > 239 mg/dl

Linearity :

Upto 695 mg/dl

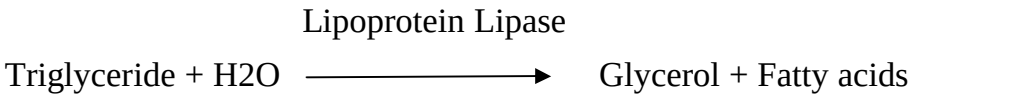
Estimation of Plasma Triglycerides :

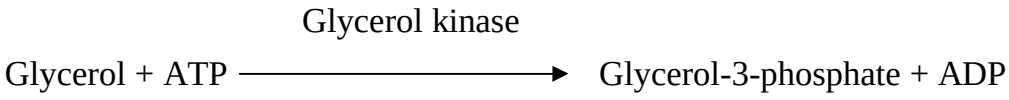
Method :

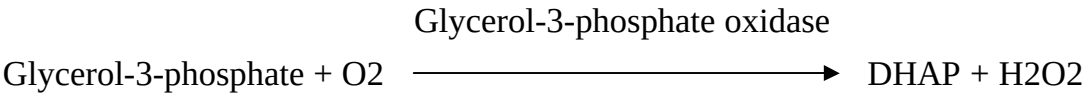
Glycerol phosphate oxidase method

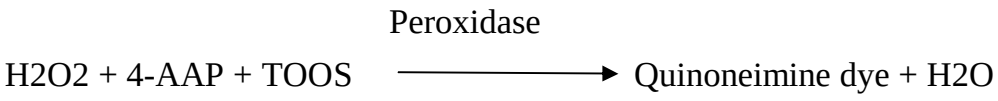
Priniciple :

Triglycerides are hydrolysed by lipoprotein lipase to form glycerol. Then acted upon by glycerol kinase to produce glycerol-3-phosphate, which on action by oxidase produces hydrogen peroxide and DHAP. Hydrogen peroxide reacts with 4-aminoantipyrine to produce pink colored complex and measured at 546 nm. The intensity of the color is directly proportional to the concentration of triglycerides in the sample.









Reagent composition :

R1

- Glycerol kinase ≥ 1.5 U/ml
- Glycerol-3-phosphate oxidase ≥ 6 U/ml
- PIPES buffer ≥ 50 mmol/L
- ATP 2.85 mmol/L
- Mg 60 mmol/L
- TOOS 0.48 mmol/L

R2

- Peroxidase ≥ 15 U/L
- Lipoprotein Lipase ≥ 25 U/L
- 4-Aminoantipyrine 1.5 mmol/L

Expected values :

- Male : 40-160 mg/dl
- Female : 35-135 mg/dl

Linearity :

Upto 1000 mg/dl

Estimation of plasma HDL Cholesterol :

Method :

Direct method

Principle :

The assay is based on use of polyvinyl sulfonic acid and polyethylene glycol methyl ether coupled classic precipitation method. LDL, VLDL, Chylomicrons in the sample react with PVS and PEGME and makes inaccessible for cholesterol esterase and oxidase . So the enzyme selectively reacts with HDL to produce cholesterol and hydrogen peroxide respectively. Hydrogen peroxide react with 4-AAP to give color complex and measured at 600 nm. The intensity of the color is directly proportional to the HDL concentration in the sample.

$$\text{HDL + VLDL + LDL + CM} \xrightarrow{\text{PVS,PEGME}} \text{HDL +}$$

[LDL+VLDL + CM] .PVS / PEGME

$$\text{HDL} \xrightarrow{\text{Cholesterol Esterase}} \text{Cholesterol + Fatty acid}$$

$$\text{Cholesterol + O2} \xrightarrow{\text{Cholesterol Oxidase}} \text{Cholest-4-en-3-one + H2O2}$$

$$\text{H2O2 + 4-AAP + TODB} \xrightarrow{\text{Peroxidase}} \text{Quinoneimine + 5 H2O}$$

Reagent composition :

R1

- Polyvinyl sulfonic acid 50 mg/L
- Polyethylene-glycol-methyl ester 30 ml/L
- Mgcl2 2 mmol/L
- MES buffer [pH 6.5] 6.5 mmol/L

R2

- Cholesterol Esterase 5000 U/L
- Cholesterol Oxidase 20 kU/L
- Peroxidase 5 kU/L
- 4-Aminoantipyrine 0.9 g/L
- MES buffer [pH 6.5] 6.5 mmol/L
- Detergent 0.5 %

Expected values :

- Male : 35-80 mg/dl
- Female : 42-88 mg/dl

Linearity :

Upto 190 mg/dl

Calculation of LDL cholesterol :

LDL cholesterol was calculated by using Friedwald's formula.

LDL cholesterol (mg/dl) = Total cholesterol - [Triglycerides / 5 + HDL cholesterol]

[Triglycerides < 400 mg/dl]

Body mass index Calculation :

We measured Height and Weight of the each individual in the study groups to calculate BMI.

$$\text{BMI } kg/m^2 = \frac{body\ weight\ of\ the\ subject\ (kg)}{(height\ of\ the\ subject)^2\ (m^2)\ .}$$

Urea estimation :

Method :

Urease – Glutamate Dehydrogenase

Principle :

Urea is hydrolysed by urease enzyme into ammonia and carbon dioxide. Then ammonia combines with alpha ketoglutarate in the presence of reduced nicotinamide adenine dinucleotide and glutamate

dehydrogenase to produce L-glutamate. The rate of decrease in absorbance at 340 nm as NADH is converted to NAD is measured.

$$\text{Urea} + H_2O \xrightarrow{\text{Urease}} 2NH_3 + CO_2$$

$$NH_3 + \alpha\text{ Ketoglutarate} + \text{NADH} \xrightarrow[\text{Dehydrogenase}]{\text{Glutamate}} \text{L-Glutamate} + \text{NAD}$$

Reference values :

15 – 40 mg/dl

Linearity :

Upto 300 mg/dl

Creatinine estimation :

Method :

Modified Jaffe's method

Principle :

Creatinine reacts with alkaline picrate to produce red colour and measured at 505 nm by using Kinetic method.

Reference values :

- Males : 0.7 – 1.3 mg/dl
- Females : 0.6 – 1.1 mg/dl

Linearity :

Upto 18 mg/dl

Estimation of blood sugar :

Method :

Glucose oxidase – Peroxidase

Principle :

Alpha D-glucose is converted to beta D- glucose by the enzyme mutarotase. In the presence of glucose oxidase beta D- glucose converted to glucoronic acid and hydrogen peroxide. By the enzyme peroxidase hydrogen peroxide combines with phenol and 4-aminoantipyrine to produce red colour complex and measured at primary wavelength – 505 nm and secondary wavelength at 660 nm.

$$\alpha \text{ D-Glucose} \xrightarrow{\text{Mutarotase}} \beta \text{ D-Glucose}$$

$$\beta \text{ D-Glucose} + H_2O + O_2 \xrightarrow{\text{Glucose Oxidase}} \text{Glucoronic acid} + H_2O_2$$

$$H_2O_2 + \text{phenol} + \text{4- Aminoantipyrine} \xrightarrow{\text{Peroxidase}} \text{Red dye} + 2H_2O$$

Reference values :

< 140 mg/dl

Linearity :

Upto 500 mg/dl

Estimation of Total serum protein :

Method :

Biuret method

Principle :

Protein reacts with copper salts in the alkaline medium to produce violet-blue coloured complex. The intensity of the colour is directly proportional to the protein concentration and it is measured at 540 nm.

Reference range :

6 – 8 mg/dl

Linearity :

Linear up to 10 g/dl

Estimation of serum Albumin :

Method :

Bromo cresol green method

Principle :

Albumin in a slightly acidic pH in the presence of bromocresol green produces colour change from yellow to green-blue and measured at 620 nm. The intensity of the colour is directly proportional to the albumin concentration in the sample.

Reference range :

3.5 – 5.2 mg/dl

Linearity :

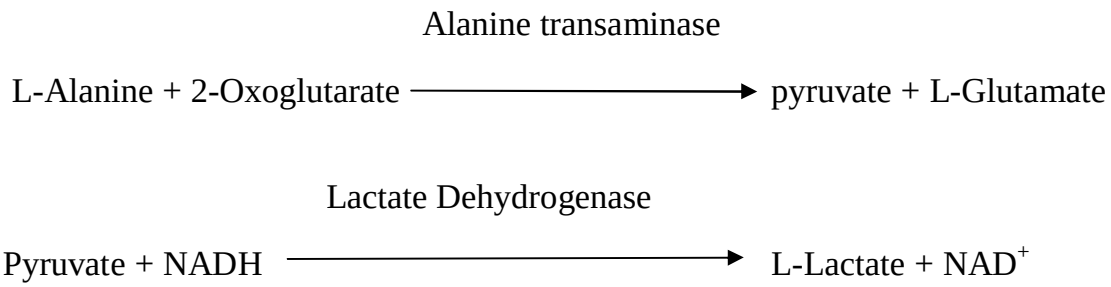
Upto 8 g/dl

Estimation of Alanine Transaminase :

IFCC, without pyridoxal phosphate

Principle :

The amino group transferred from Alanine to 2-Oxoglutarate to produce pyruvate and L-Glutamate. In the presence of lactate dehydrogenase and reduced nicotinamide adenine dinucleotide, pyruvate converted to lactate and NAD. The rate of decrease in absorbance due to oxidation of NADH is measured at 340 nm.



Reference range :

< 45 U/L

Linearity :

Linear upto 1600 U/L.

Peripheral smear[92]:

Blood films are made on clean glass slides which measures 75* 25 mm and 1 mm thick. After placing a small drop of EDTA blood about 1 cm from one end of the slide in the center line, by using spreader at 30 ° in front of the drop, moved to make contact with drop and spread the drop of blood along the slide. The blood film is about 3 cm in length. The glass slides are allowed to dry in the air. Addition of 8 drops of staining solution which is the mixture of ethanol and Leishman's stain and wait for 5-7 minutes. Then add 16 drops of buffered water and kept for 2 minutes. Then the slide was washed in thin running water and set in upright to dry. After drying the slide was placed in the microscope under 100x oil emersion field to ruled out features of Megaloblastic anemia.

STATISTICAL ANALYSIS

STATISTICAL ANALYSIS

- Data was analysed using SPSS 16 statistical software and considered as statistically significant if the p value is < 0.05.
- ANOVA was used to compare Age, Body mass index, Fasting lipid profile, TSH, Homocysteine levels between three groups.
- Post HOC test was used to compare Age, Body mass index, Fasting lipid profile, TSH, Homocysteine levels between two groups.
- Correlation of TSH with Homocysteine and other parameters was done by using Pearson Correlation Coefficient.

RESULTS

MASTER CHART

Characteristics and Biochemical parameters of Newly Diagnosed Hypothyroidism

S No	Age	Sex	BMI	TSH (µIU/ml)	T3 (ng/ml)	T4 (ng/ml)	Hcy (µMol/l)	Chol (mg/dl)	TGL (mg/dl)	HDL (mg/dl)	LDL (mg/dl)	Total protein (g/dl)	Alb (g/dl)	SGPT (IU/L)	Urea (mg/dl)	creat (mg/dl)	sugar (mg/dl)	HB (g/dl)	Peripheral smear
1	24	F	32.2	98.5	0.21	31.6	13	182	182	62	83.6	6.5	3.6	40	18	0.7	82	9.6	N
2	23	F	41.5	35.1	0.6	38.3	17	164	111	63	78.8	6.2	3.5	15	24	0.8	95	10.2	N
3	32	F	32.5	25.9	0.4	36	11.5	170	69	66	90.2	6.1	3.4	18	20	0.6	76	10.5	N
4	18	M	28.7	8.6	0.67	38	12	139	139	50	61.2	7.3	3.6	23	23	0.8	88	10	N
5	41	F	31.2	17.2	0.5	40	8	240	318	78	98.4	7.1	3.8	28	26	1	112	10.6	N
6	35	M	27.7	17.4	0.43	32	13	143	209	55	46.2	6.3	3.6	23	28	0.8	101	13.9	N
7	29	F	26.7	42.9	0.62	42.5	11.5	158	149	62	66.2	6.2	3.9	36	20	0.9	96	10.2	N
8	38	F	29.5	16.2	0.62	40	10.9	152	70	63	75	6	3.4	34	24	0.8	104	10.4	N
9	28	F	27.1	8.5	0.6	42	19.5	145	99	61	64.2	7.4	4.1	14	30	0.7	100	9.8	N
10	27	F	31.2	14.6	0.5	34	15	178	92	74	85.6	6.8	3.7	19	35	0.9	93	9.6	N
11	35	F	33.2	10.2	0.65	31	10	221	156	88	101.8	6.3	4	20	38	1	60	9.5	N
12	45	F	21	51	0.31	31	9.5	212	300	76	76	6	3.5	37	27	0.72	92	9.5	N
13	30	F	33.2	48.8	0.35	37	10.2	125	139	53	44.2	6.3	3.8	28	18	0.6	105	9.1	N
14	30	F	17.3	18.6	0.57	37	7.4	237	145	99	109	6.5	3.7	27	22	0.8	98	10.3	N
15	27	F	32	6.7	0.68	42	9.2	181	201	69	71.8	7.2	3.8	31	31	0.9	78	11.1	N
16	19	F	16.6	10.5	0.3	31	9.7	134	52	66	57.6	7	3.9	38	16	0.7	86	11.3	N
17	36	F	17.4	16.8	0.2	27	8	183	100	89	74	6.9	4	32	21	0.74	90	11.5	N
18	28	F	21.2	150	0.7	30	8.3	156	188	62	56.4	6.2	3.7	19	26	0.61	96	10.6	N
19	40	F	23.7	56.9	0.4	36.3	9.4	139	292	49	31.6	6.8	3.6	18	33	0.76	87	9.7	N
20	32	F	24.8	200	0.2	34	12.4	174	130	68	80	6.1	3.6	26	30	0.81	106	9.9	N
21	24	F	18.1	150	0.16	36	7	167	64	69	85.2	6.4	3.7	27	28	0.85	110	10.4	N
22	27	M	23.9	88	0.2	32	9	143	199	53	50.2	7.4	4.1	29	32	1.2	108	11.2	N
23	29	F	29.3	9.5	0.68	42	10.2	171	169	56	81.2	6.5	3.6	32	19	0.6	96	10.2	N
24	43	F	24.8	17.5	0.6	35	10.2	111	332	37	7.6	6.6	3.7	35	27	0.71	89	10.4	N
25	25	M	22	100	0.55	27	23.6	117	79	47	54.2	6	3.4	24	24	0.6	80	12.8	N
26	29	F	31.1	7.3	0.5	42	9.3	189	199	44	105.2	7.1	3.5	26	25	0.63	84	11.4	N
27	45	F	30	13.4	0.6	43	11.5	207	169	54	119.2	6.8	3.7	34	19	0.7	97	11.5	N
28	30	F	29.9	100	0.34	20	12	226	142	52	145.6	6.2	3.8	33	22	0.65	95	9.2	N
29	18	F	21.1	7.9	0.3	31	14	144	113	62	59.4	6.5	3.8	25	26	0.84	102	9.5	N
30	30	F	24	15	0.2	37	17	220	170	36	150	6.4	3.6	19	24	0.81	100	10.2	N

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Characteristics and Biochemical parameters of treated Hypothyroidism

S No	Age	Sex	BMI	TSH (µIU/ml)	T3 (ng/ml)	T4 (ng/ml)	Hcy (µMol/l)	Chol (mg/dl)	TGL (mg/dl)	HDL (mg/dl)	LDL (mg/dl)	Total protein (g/dl)	Alb (g/dl)	SGPT (IU/L)	Urea (mg/dl)	creat (mg/dl)	sugar (mg/dl)	HB (g/dl)	Peripheral smear
1	29	F	20	2.3	0.7	102	7	130	66	54	62.8	6.3	3.8	24	20	0.8	95	12.4	N
2.	32	F	37	3.3	1.14	90	8.2	189	165	56	100	6.1	3.7	31	28	0.7	85	11.5	N
3	33	F	35.5	4.6	0.8	87	11	197	140	48	121	6.7	3.6	30	16	0.7	90	11.8	N
4	35	F	26.6	0.7	0.9	80	8.7	204	99	64	120.2	6.6	3.9	26	15	0.56	75	11.9	N
5	19	F	25.3	3	1.18	109	10.2	121	114	52	46.2	6.8	4	28	18	0.67	123	9.7	N
6	37	F	28.9	4.2	1.25	94	11.5	187	259	63	72.2	6.4	4	25	21	0.81	119	10.5	N
7	20	F	25.7	0.45	0.82	106	11.2	183	136	66	89.8	6.2	3.9	18	20	0.9	132	9.8	N
8	33	F	28.1	1.4	0.78	104	9	239	203	61	137.4	6.3	3.8	30	21	0.7	128	8.6	N
9	25	F	35.9	1.9	0.98	89	4.1	214	185	51	126	6.8	4.3	32	28	1.2	90	9.3	N
10	32	F	23.9	2.7	1.43	105	10	178	155	59	88	6.3	4	26	32	0.6	94	10.8	N
11	38	M	21.8	0.8	1.02	82	11.6	291	89	54	219.2	7.2	4.1	28	30	0.8	82	10.3	N
12	23	F	26.1	3.9	0.96	67	8.4	144	184	57	50.2	7.5	4.4	34	21	0.76	89	12.1	N
13	23	F	24	0.9	0.8	108	11.8	172	72	66	91.6	7.2	3.9	32	22	0.69	98	10.8	N
14	30	F	21.7	2.7	0.6	59	7.6	197	330	67	64	6.9	3.7	19	29	0.84	93	10.3	N
15	30	F	24.7	1.3	0.5	77	8	198	222	47	106.6	7	3.8	17	26	0.91	106	9.6	N
16	25	F	29.2	4.1	0.9	92	7	203	167	60	109.6	7.1	3.9	21	34	1	78	9.8	N
17	45	F	27.2	3.2	0.9	62	10.5	222	136	53	141.8	6.7	3.8	25	30	1.1	86	12.3	N
18	33	F	24.4	4	1.03	109	9	142	111	57	62.8	7.1	4	29	20	0.9	95	10.7	N
19	31	F	24.8	2.7	1.13	90	12	128	64	49	66.2	6.5	3.8	28	24	0.75	98	10.1	N
20	24	F	34.2	0.5	0.84	89	10.2	183	164	58	92.2	7.3	4.1	24	26	0.65	103	12.3	N
21	32	F	31	3.9	1.2	96	8	196	138	55	113.4	6.9	3.8	23	29	0.82	109	10.4	N
22	25	F	28.8	0.5	0.93	116	9.8	205	100	62	123	6.7	3.8	20	21	0.93	79	11.2	N
23	30	F	29.2	3.2	1.04	110	12.3	121	111	61	37.8	6.9	4	24	19	0.81	86	10.8	N
24	18	F	20.1	2.3	0.86	56	11.9	142	122	46	71.6	7.1	3.9	18	17	0.91	74	10.2	N
25	37	F	20.3	1.6	0.92	65	13	135	128	58	51.4	6.9	3.9	16	21	0.72	79	9.7	N
26	17	F	22.1	2.6	0.87	108	9.2	176	136	54	94.8	7	4	26	18	0.82	86	9.4	N
27	29	F	22.1	0.5	0.98	109	11	187	125	57	105	6.8	4	28	20	0.8	88	10.2	N
28	31	F	35.6	1.2	0.99	56	10.9	203	167	46	123.6	6.5	3.8	30	19	0.8	100	12.1	N
29	29	F	23.9	0.9	0.82	63	10.8	214	185	47	130	6.7	3.9	32	18	0.9	104	10.9	N
30	33	F	21.2	2.3	1.05	54	11.3	189	100	56	113	7.2	4.1	27	24	1.1	113	9.8	N

MASTER CHART

Characteristics and Biochemical parameters of controls

S No	Age	Sex	BMI	TSH (µIU/ml)	T3 (ng/ml)	T4 (ng/ml)	Hcy (µMol/l)	Chol (mg/dl)	TGL (mg/dl)	HDL (mg/dl)	LDL (mg/dl)	Total protein (g/dl)	Alb (g/dl)	SGPT (IU/L)	Urea (mg/dl)	creat (mg/dl)	sugar (mg/dl)	HB (g/dl)	Peripheral smear
1	28	F	20.1	3.2	0.89	69	8.9	148	142	58	61.6	6.8	3.6	20	30	0.91	96	9.8	N
2	35	M	21.3	0.65	0.79	82	9.2	146	133	87	32.4	7	3.9	15	34	1.1	104	9.6	N
3	45	F	19.4	1.4	0.86	76	10.4	130	127	66	38.6	7.4	4.2	21	17	1	112	10.6	N
4	32	M	20	1.9	1.2	57	9.6	160	106	64	74.8	7.8	4.5	22	20	0.92	96	10.3	N
5	30	F	19.6	2.7	0.93	86	7.3	154	99	62	72.2	6.7	4.1	32	18	0.86	98	9.7	N
6	32	M	21.2	0.5	0.82	90	12	140	77	57	67.6	6.2	4	30	24	0.85	107	9.5	N
7	28	F	20.9	3.2	0.94	91	9	134	98	52	62.4	6.5	3.8	27	22	0.92	103	11.3	N
8	28	F	20.1	2.3	0.9	96	11.2	126	87	49	59.6	6.7	3.7	24	22	1.2	92	12.4	N
9	29	F	20.3	1.6	0.83	64	9.4	101	60	54	35	6.9	3.7	36	18	1.02	86	9.6	N
10	28	F	19.9	3.1	1.3	61	10	132	136	64	40.8	7.1	3.8	24	17	0.92	98	9.8	N
11	25	M	22.6	3.2	1.4	55	10.6	175	101	59	95.8	6	3.6	21	20	0.74	78	10.7	N
12	25	F	20.7	4	1.5	77	9.8	111	82	66	28.6	6.3	3.8	31	32	0.69	105	10.5	N
13	25	F	22.1	2.7	0.94	76	9.4	154	90	56	80	6.4	3.7	32	28	0.72	97	12.4	N
14	24	F	21.6	0.5	0.8	65	10.6	100	51	49	40.8	6.1	3.6	30	25	0.81	92	9.7	N
15	24	F	20.9	3.9	0.96	54	10.1	145	88	64	63.4	6.8	4	24	18	0.75	104	9.8	N
16	33	F	19.8	2.3	1.1	83	8.2	125	61	51	61.8	6.5	4.1	20	25	0.82	112	10.2	N
17	27	F	21	3.3	0.94	85	12.8	147	101	60	66.8	6.4	3.9	21	24	0.73	120	9.6	N
18	27	F	18.7	3.6	0.89	68	10.1	182	71	54	113.8	6.5	3.7	22	19	0.9	94	9	N
19	45	F	21.8	0.7	0.79	95	11.1	176	82	62	97.6	7	4	28	15	0.91	85	10.7	N
20	21	F	20.8	3	0.87	57	7.2	174	132	58	89.6	7.2	4.2	37	17	0.82	94	10.1	N
21	18	F	21.2	3.2	0.99	58	12	123	46	52	61.8	6.8	3.9	34	21	0.76	83	12.3	N
22	35	F	25.3	0.92	0.86	82	6.4	151	54	56	84.2	6.9	4.2	38	20	0.72	100	12.8	N
23	33	F	23.2	1.5	0.84	69	9.1	169	140	58	83	6.5	3.9	18	19	0.87	79	10.2	N
24	26	F	20.9	1.9	0.85	73	9	134	98	52	62.4	6.8	3.7	24	21	0.9	74	13.1	N
25	24	F	20.1	2.7	0.87	78	11.2	126	87	49	59.6	7.1	4.2	31	19	1.1	97	10.9	N
26	27	F	20.3	0.5	0.78	102	9.4	101	60	54	35	7.5	4.3	24	32	1	104	9.9	N
27	28	F	20.8	3.2	1.2	54	7.2	174	132	58	89.6	7.2	4	27	31	0.9	95	9.4	N
28	27	F	21.2	2.3	0.92	65	12	123	46	52	61.8	6.8	3.9	31	30	0.8	114	9.8	N
29	22	F	25.3	1.6	0.87	58	6.4	151	54	56	84.2	6.5	3.8	30	17	0.8	79	10.7	N
30	26	F	23.2	2.2	1.2	80	9.1	169	140	58	83	6.6	4	26	16	0.74	86	10.2	N

RESULTS

TABLE 5 : CHARACTERISTICS OF CONTROLS, TREATED HYPOTHYROIDISM AND RECENTLY DIAGNOSED HYPOTHYROIDISM

PARAMETERS		CONTROLS	TREATED HYPOTHYROIDISM	RECENTLY DIAGNOSED HYPOTHYROIDISM	P VALUE
AGE		28.57 ± 5.85	29.27 ± 6.3	30.57 ± 7.26	0.496 NS
SEX	MALE	4 [13.3 %]	1 [3.3 %]	4 [13.3 %]	
	FEMALE	26 [86.7 %]	29 [96.7 %]	26 [86.7 %]	
BODY MASS INDEX		21.14 ± 1.53	26.64 ± 5.04	26.8 ± 5.9	<0.001**
TSH [µIU/L]		2.26 ± 1.07	2.26 ± 1.3	45.4 ± 51.02	<0.001**
TOTAL CHOLESTEROL [mg/dl]		142.7 ± 23.62	159.6 ± 19.83	170.9 ± 35.5	<0.001**
TRIGLYCERIDES [mg/dl]		92.7 ± 31.27	145.77 ± 57.32	159.2 ± 74.98	<0.001**
LDL-c [mg/dl]		66.26 ± 21.57	74.31 ± 17.94	76.99 ± 30.5	<0.001**
HDL –c [mg/dl]		57.9 ± 7.45	56.13 ± 6.14	62.1 ± 14.52	0.068
HOMOCYSTEINE [µmol /L]		9.62 ± 1.65	9.84 ± 1.97	11.64 ± 3.72	**0.006***

NS – Non significant; * - significant; ** - Highly significant

Table - 5 shows baseline characteristics and biochemical parameters of the controls, treated hypothyroidism and recently diagnosed hypothyroidism cases.

No significant difference were found in the distribution of age and sex among the study groups. This shows that the study is age and sex matched.

Highly significant difference was observed between the study groups in Body mass index, total cholesterol, triglycerides, LDL-cholesterol, TSH with the p value < 0.001.

There is no significant difference in HDL-cholesterol between the study groups.

There was significant difference in total plasma homocysteine level between study groups with the p value 0.006.

FIGURE 10 : MEAN TSH VALUES IN THREE STUDY GROUPS

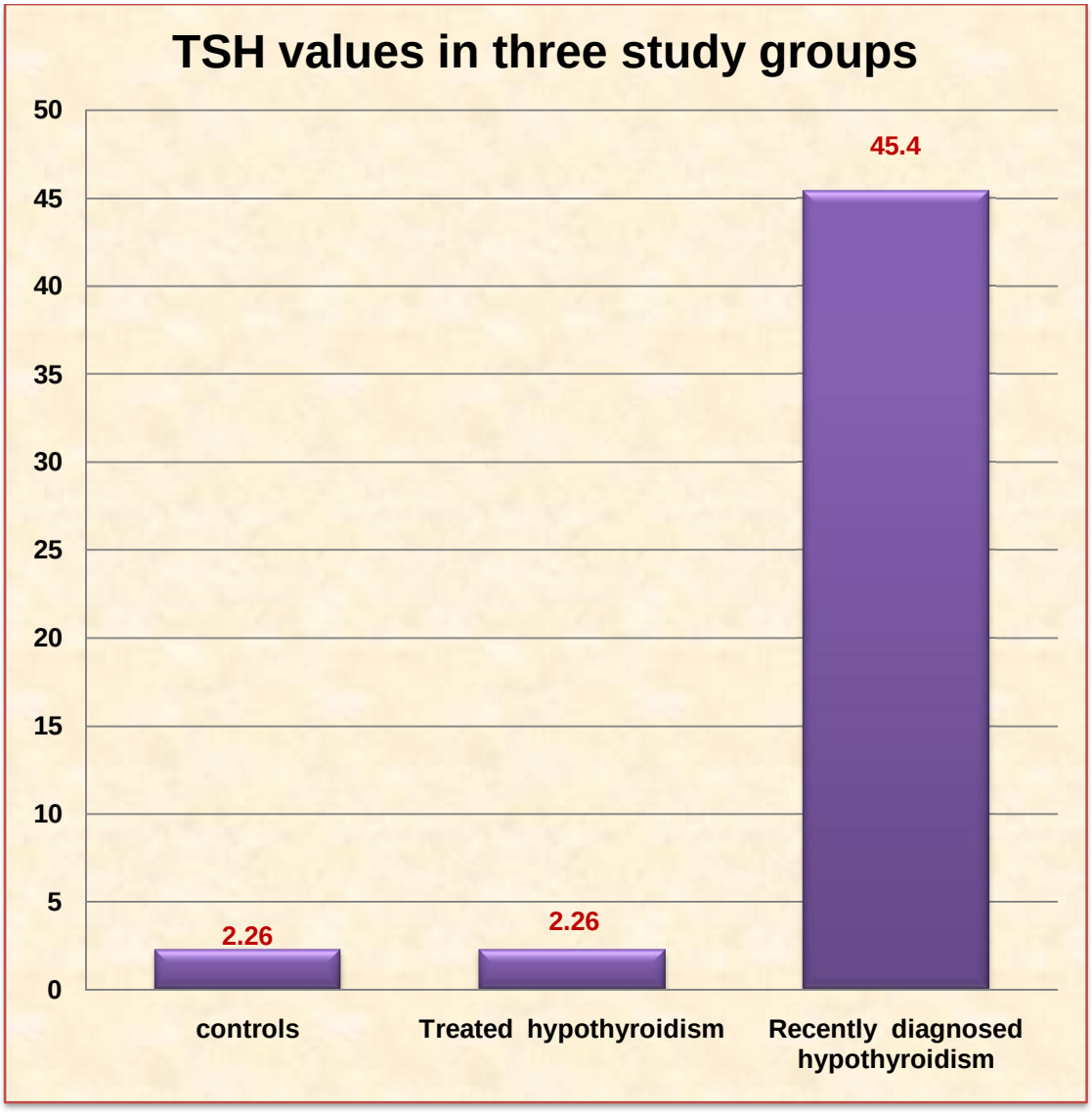


Figure 10: shows mean TSH values among three groups. TSH values are same for controls and treated hypothyroidism. Treated hypothyroidism subjects are included in this study after they become euthyroid. TSH value is more in recently diagnosed hypothyroidism cases compared to controls and treated hypothyroidism.

FIGURE 11 : MEAN TOTAL CHOLESTEROL LEVELS IN THREE STUDY GROUPS

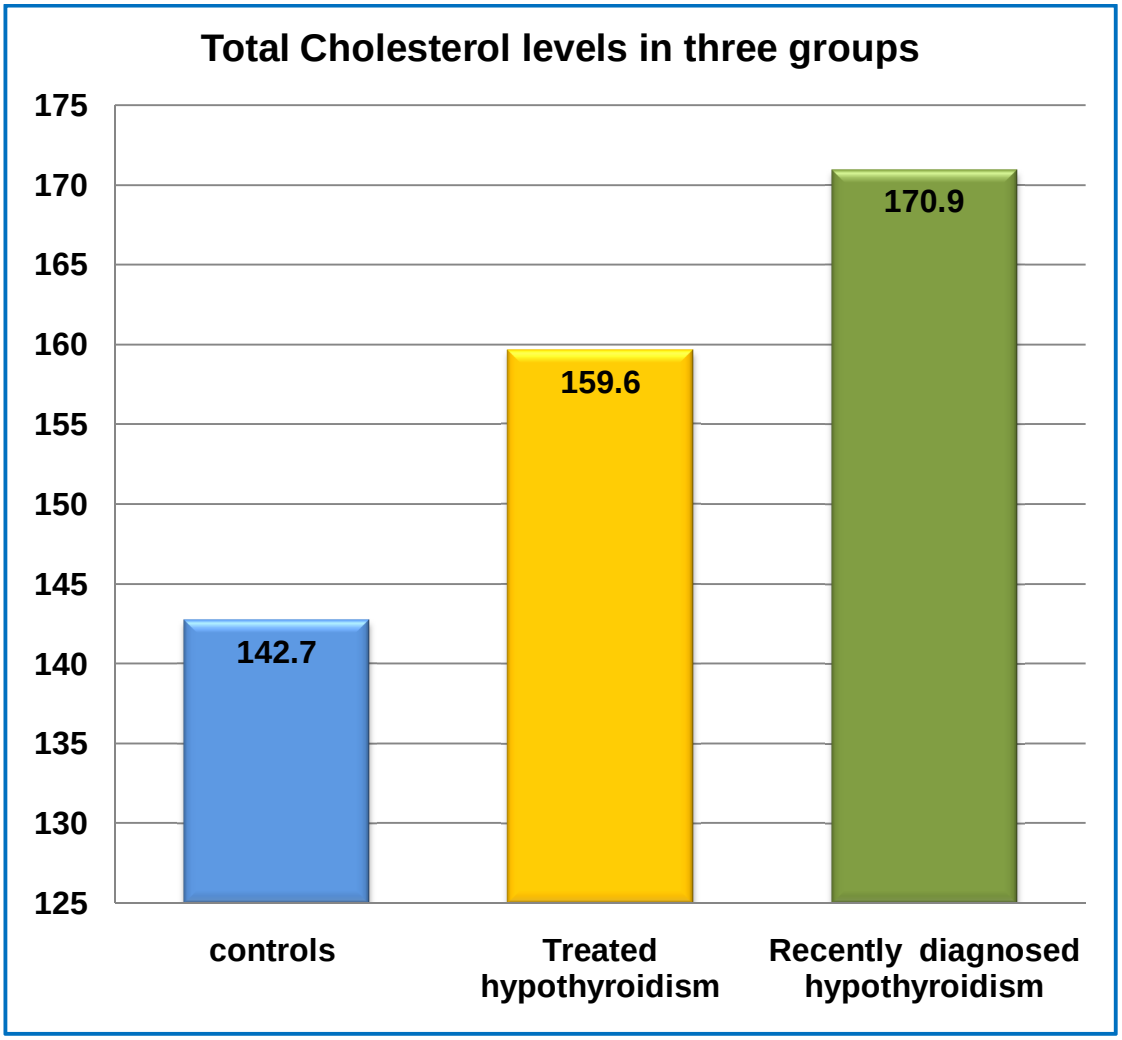


Figure 11: shows mean total cholesterol levels among three study groups. Recently diagnosed hypothyroidism cases showed high cholesterol levels compared to controls and treated hypothyroidism.

FIGURE 12 : MEAN LDL LEVELS IN THREE STUDY GROUPS

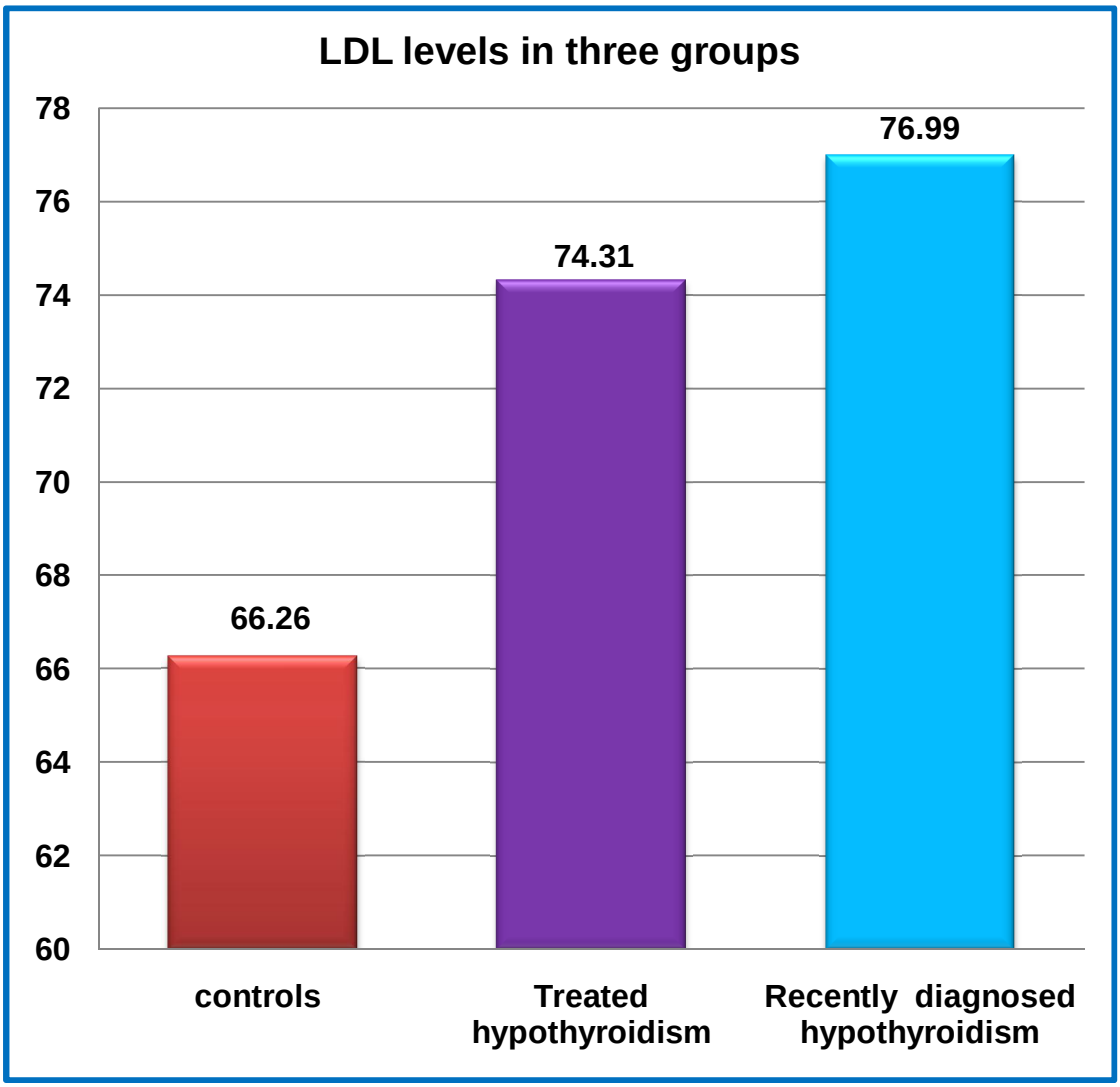
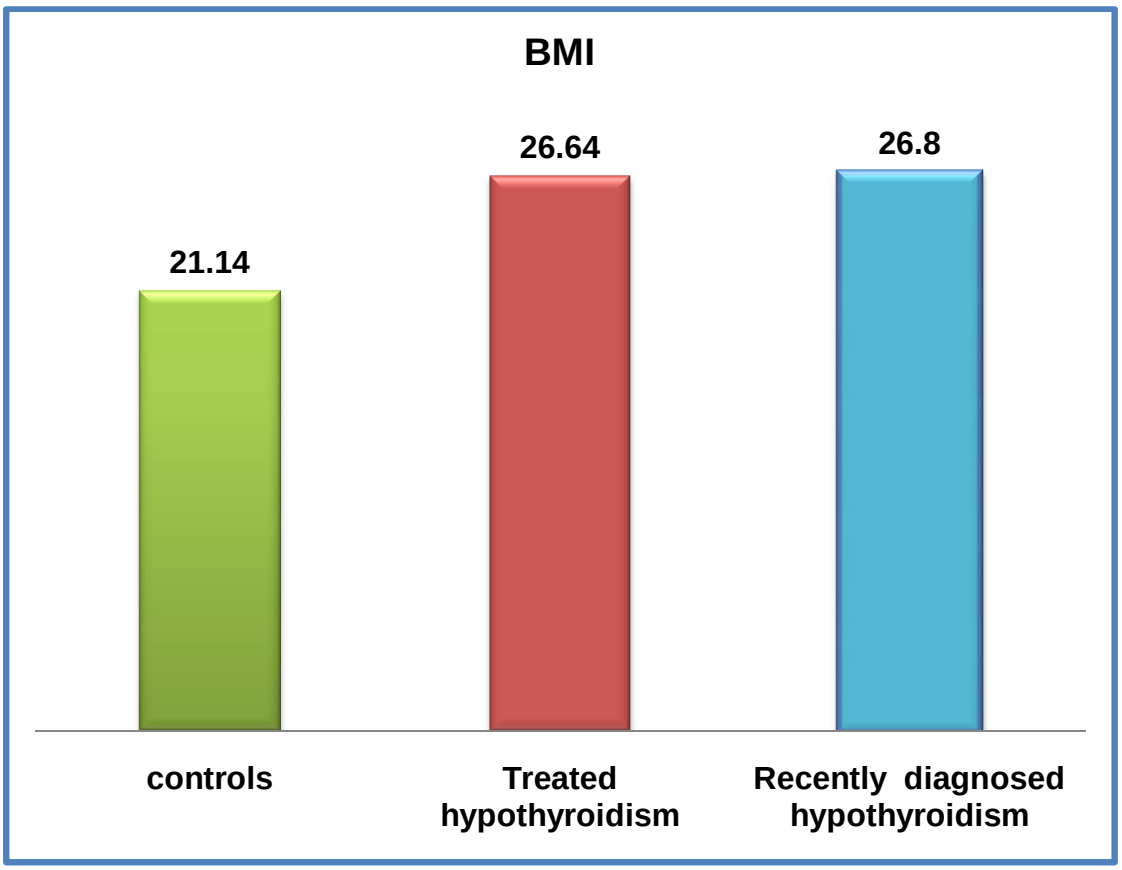


Figure 12: shows mean Low Density Lipoprotein – cholesterol among study groups. LDL-cholesterol is more in recently diagnosed hypothyroidism cases compared to treated hypothyroidism and controls.

FIGURE 13 : SHOWS MEAN BODY MASS INDEX

BETWEEN STUDY GROUPS



WHO classification:

Normal BMI = 18.5 to 25 Kg/m^2

Over weight = 25 to 30 Kg/m^2

Obese class I (moderately obese) = 30 to 35 Kg/m^2

Obese class II (severely obese) = 35 - 40 Kg/m^2

Obese class III (very severely obese) = > 40 Kg/m^2

Recently diagnosed and treated hypothyroidism were in stage 2 - Over weight. In this study Control group was in normal BMI.

TABLE 6 :COMPARISON OF CHARACTERISTICS BETWEEN CONTROLS AND RECENTLY DIAGNOSED HYPOTHYROIDISM

Parameters	Controls	Recently diagnosed hypothyroidism	p value
AGE	28.57 ± 5.85	30.57 ± 7.26	0.244 NS
SEX MALE	4 [13.3 %]	4 [13.3 %]	
FEMALE	26 [86.7 %]	26 [86.7 %]	
BODY MASS INDEX	21.14 ± 1.53	26.8 ± 5.9	< 0.001 **
TSH [µIU/L]	2.26 ± 1.07	45.4 ± 51.02	< 0.001 **
TOTAL CHOLESTEROL [mg/dl]	142.7 ± 23.62	170.9 ± 35.5	0.001 **
TRIGLYCERIDES [mg/dl]	92.7 ± 31.27	159.2 ± 74.98	< 0.001 **
LDL-c [mg/dl]	66.26 ± 21.57	76.99 ± 30.5	0.032 *
HDL –c [mg/dl]	57.9 ± 7.45	62.1 ± 14.52	0.11
HOMOCYSTEINE [µmol /L]	9.62 ± 1.65	11.64 ± 3.72	**0.004 ***

NS – Non significant *** - Significant** **** - Highly significant**

Table 6 shows the comparison of characteristics between controls and recently diagnosed hypothyroidism.

The two groups showed significantly different in body mass index, total cholesterol, triglycerides, TSH with the p value 0.001.

The two groups were significantly different in LDL-cholesterol with p value < 0.05.

No significant difference was observed between these two groups in age, sex, HDL-cholesterol.

Total plasma homocysteine levels were significantly more in recently diagnosed hypothyroidism [11.64 ± 3.72] than controls [9.62 ± 1.65] with p value of 0.004.

FIGURE 14 : GENDER DISTRIBUTION IN RECENTLY DIAGNOSED HYPOTYROIDISM AND CONTROLS

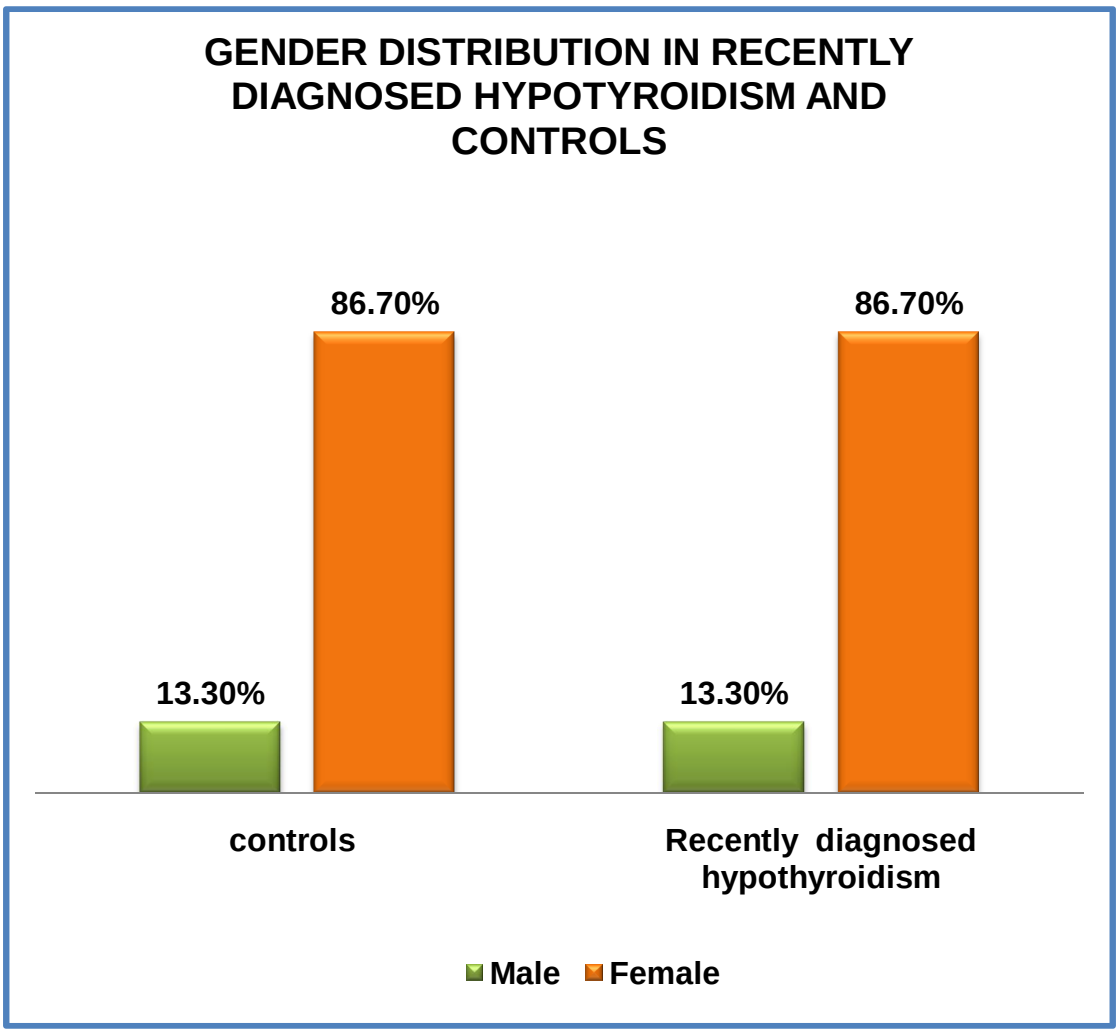


Figure 14 : shows gender distribution between controls and recently diagnosed hypothyroidism.

The incidence of hypothyroidism is higher in females compared to males.

TABLE 7 : COMPARISON OF CHARACTERISTICS BETWEEN RECENTLY DIAGNOSED HYPOTHYROIDISM AND TREATED HYPOTHYROIDISM

PARAMETERS	RECENTLY DIAGNOSED HYPOTHYROIDISM	TREATED HYPOTHYROIDISM	P VALUE
AGE	30.57 ± 7.26	29.27 ± 6.3	0.448 NS
SEX MALE	4 [13.3 %]	1 [3.3 %]	
FEMALE	26 [86.7 %]	29 [96.7 %]	
BODY MASS INDEX	26.8 ± 5.9	26.64 ± 5.04	0.919 NS
TSH [µIU/L]	45.4 ± 51.02	2.26 ± 1.3	< 0.001 **
TOTAL CHOLESTEROL[mg/dl]	170.9 ± 35.5	159.6 ± 19.83	0.005 *
TRIGLYCERIDES [mg/dl]	159.2 ± 74.98	145.77 ± 57.32	0.366 NS
LDL-c [mg/dl]	76.99 ± 30.5	74.31 ± 17.94	0.085 NS
HDL –c [mg/dl]	62.1 ± 14.52	56.13 ± 6.14	0.024 *
HOMOCYSTEINE [µmol /L]	11.64 ± 3.72	9.84 ± 1.97	0.009 *

NS – Non significant *** - Significant** **** - Highly significant**

Table 7 shows comparison of characteristics between recently diagnosed hypothyroidism and treated hypothyroidism.

Among these two study groups Total cholesterol , HDL-cholesterol and TSH levels were significant.

Triglycerides, LDL-cholesterol and Body mass index were found to be not statistically significant between these two study groups.

Total plasma Homocysteine levels were significantly increased in recently diagnosed hypothyroidism compared to treated hypothyroidism with p value of 0.009.

TABLE 8 : COMPARISON OF CHARACTERISTICS BETWEEN CONTROLS AND TREATED HYPOTHYROIDISM

PARAMETERS	CONTROLS	TREATED HYPOTHYROIDISM	P VALUE
AGE	28.57 ± 5.85	29.27 ± 6.3	0.487 NS
SEX MALE	4 [13.3 %]	1 [3.3 %]	
FEMALE	26 [86.7 %]	29 [96.7 %]	
BODY MASS INDEX	21.14 ± 1.53	26.64 ± 5.04	< 0.001 **
TSH [μIU/L]	2.26 ± 1.07	2.26 ± 1.3	1.00 NS
TOTAL CHOLESTEROL[mg/dl]	142.7 ± 23.62	159.6 ± 19.83	0.634 NS
TRIGLYCERIDES [mg/dl]	92.7 ± 31.27	145.77 ± 57.32	0.001 **
LDL-c [mg/dl]	66.26 ± 21.57	74.31 ± 17.94	0.664 NS
HDL –c [mg/dl]	57.9 ± 7.45	56.13 ± 6.14	0.499 NS
HOMOCYSTEINE [μmol /L]	9.62 ± 1.65	9.84 ± 1.97	0.749 NS

NS – Non significant *** - Significant** **** - Highly significant**

Table 8 shows the comparison of characteristics between apparently healthy individuals and treated hypothyroidism.

The subjects in these two groups showed highly significant difference in body mass index and triglycerides with p value of 0.001.

But these two groups were not significantly different in total cholesterol, LDL-cholesterol, HDL-cholesterol and homocysteine levels showing that treatment on thyroxine decreases total plasma homocysteine and lipid profile levels.

TABLE 9 : TOTAL PLASMA HOMOCYSTEINE LEVELS BETWEEN STUDY GROUPS

ANALYTE	CONTROLS	TREATED HYPOTHYROIDISM	RECENTLY DIAGNOSED HYPOTHYROIDISM	P VALUE
Total plasma homocysteine [µmol/L]	9.62 ± 1.65	9.84 ± 1.97	11.64 ± 3.72	0.006

Table 9 shows the comparison of mean total plasma homocysteine levels among controls, recently diagnosed and treated hypothyroidism patients.

Total plasma homocysteine levels showed significantly more in recently diagnosed hypothyroidism[11.64 ± 3.72] compared to treated hypothyroidism [9.84 ± 1.97] and with compared to controls [9.62 ± 1.65] with p value of 0.006.

FIGURE 15: MEAN HOMOCYSTEINE LEVELS

IN THREE STUDY GROUPS

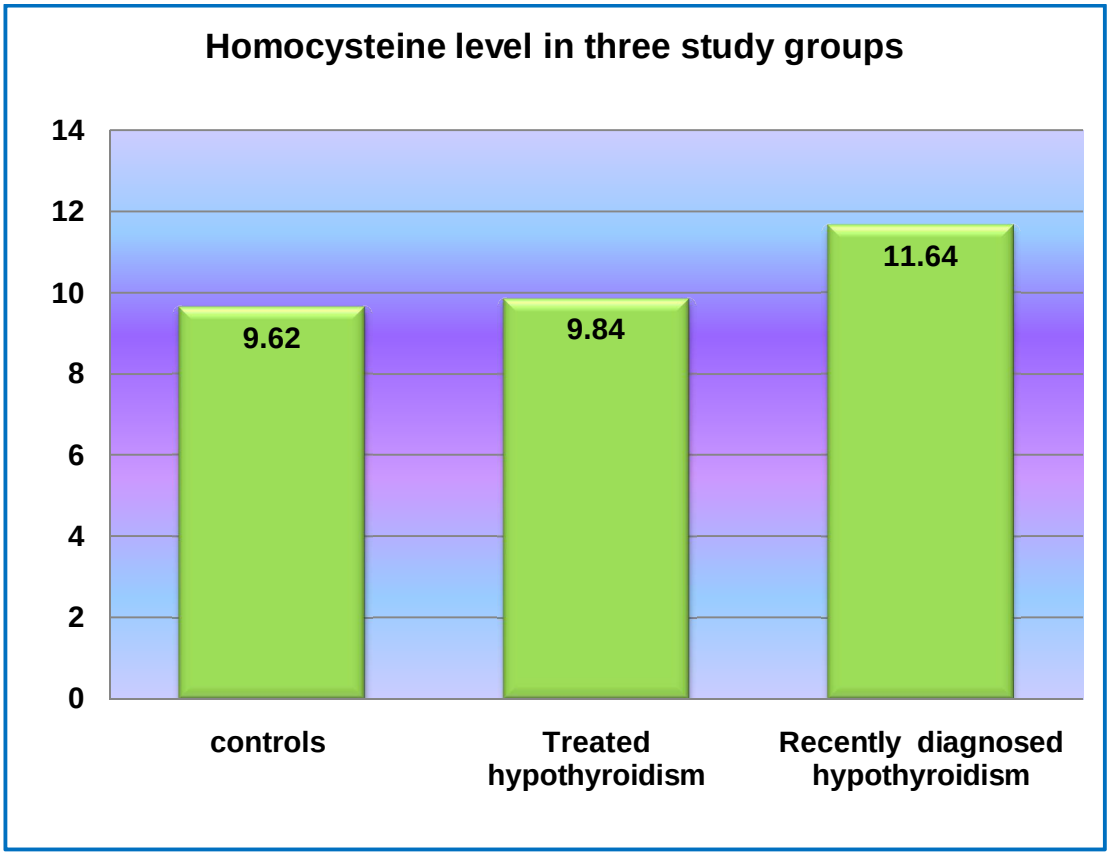


Figure 15: shows mean total plasma homocysteine levels among study groups. Homocysteine mean value of controls and treated hypothyroidism are almost same and within the normal range. Treatment with thyroxine has impact on the homocysteine levels.

FIGURE 16 : COMPARISON OF TSH AND HOMOCYSTEINE AMONG THE STUDY GROUPS

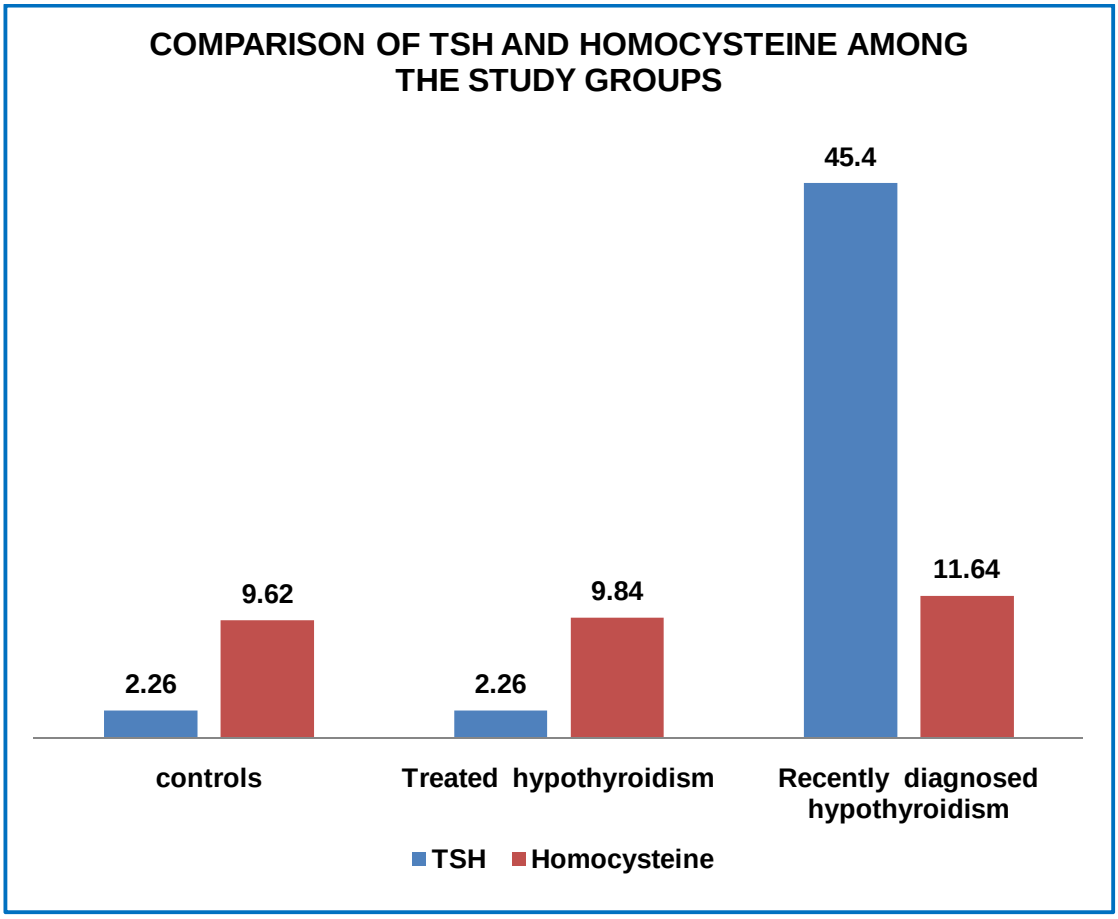


Figure 16 : shows comparison between TSH and total plasma homocysteine levels in study groups. TSH and homocysteine levels are almost similar in controls and treated hypothyroidism. In recently diagnosed hypothyroidism cases TSH and homocysteine shows a parallel rise.

FIGURE 17 : COMPARISON OF MEAN VALUES OF TSH AND CHOLESTEROL IN THREE STUDY GROUPS

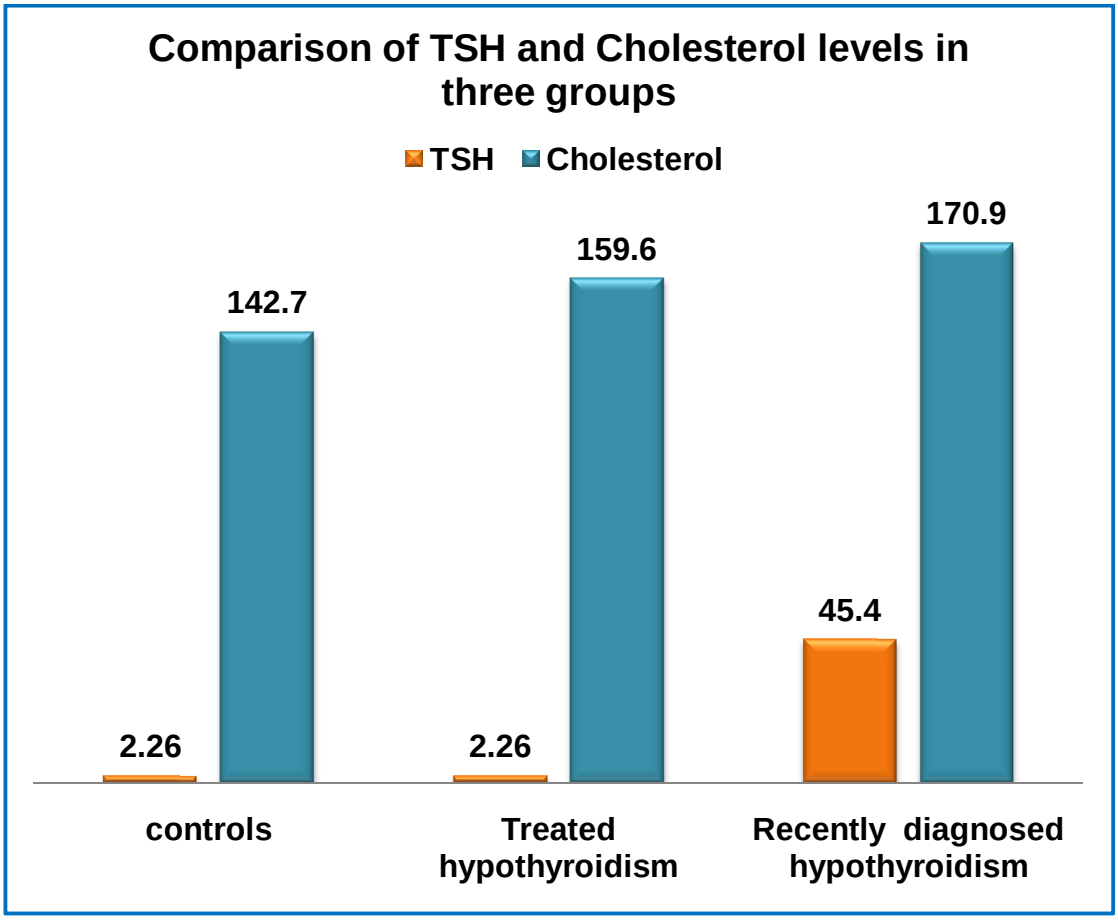


Figure 17 : shows comparison of TSH and total cholesterol levels among three study groups. Both are high in recently diagnosed hypothyroidism compared to controls and treated hypothyroidism.

FIGURE 18 : COMPARISON OF MEAN VALUES OF HOMOCYSTEINE AND CHOLESTEROL AMONG THE STUDY GROUPS

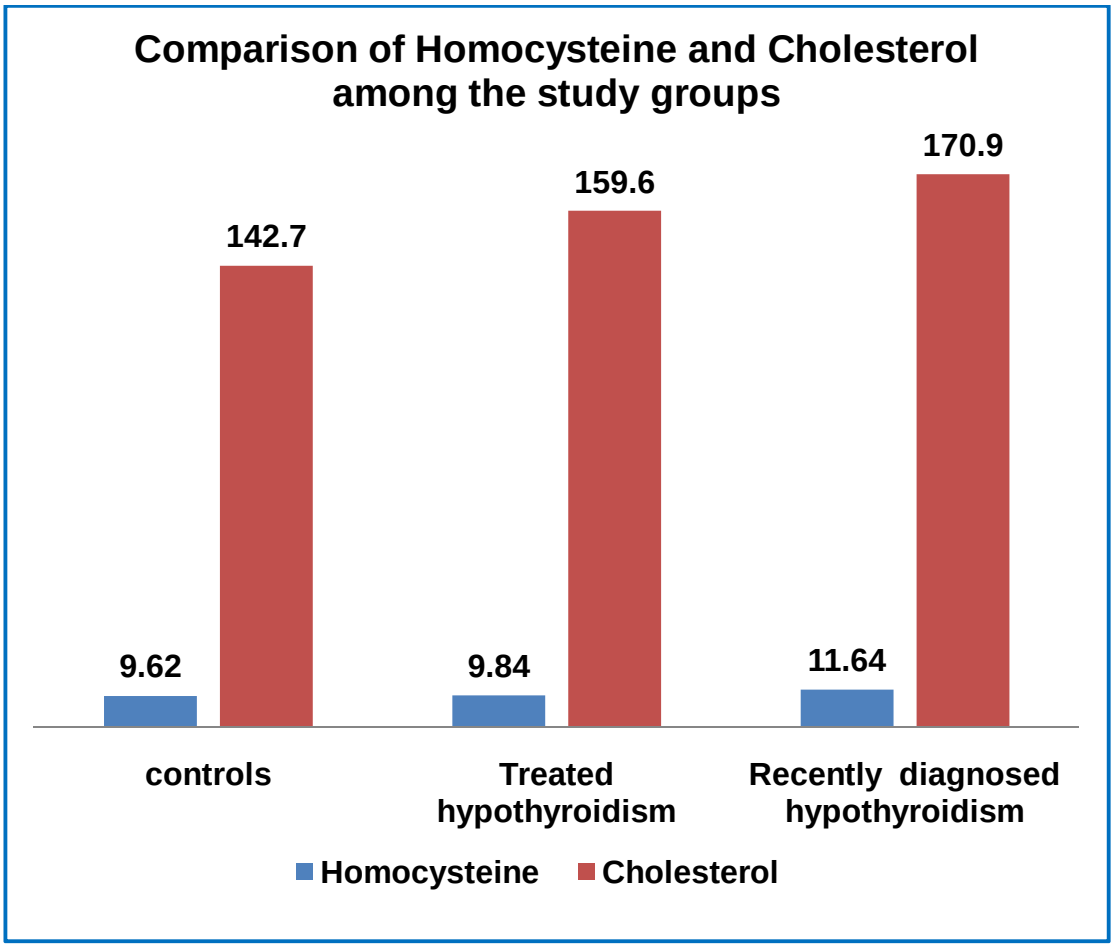


Figure 18 : shows comparison between total plasma homocysteine and total cholesterol levels in study groups. Both are high in recently diagnosed hypothyroidism compared to controls and treated hypothyroidism.

TABLE 10 : CORRELATION OF TSH WITH HOMOCYSTEINE AND OTHER PARAMETERS IN STUDY GROUPS

		TSH
Body mass index	Pearson Correlation	.028
	Sig.[2-tailed]	.79
	N	90
Homocysteine	Pearson Correlation	.174
	Sig.[2-tailed]	.10
	N	90
Total Cholesterol	Pearson Correlation	.018
	Sig.[2-tailed]	.87
	N	90
Triglycerides	Pearson Correlation	.13
	Sig.[2-tailed]	.22
	N	90
HDL	Pearson Correlation	.106
	Sig.[2-tailed]	.32
	N	90

Table 10: shows Pearson Correlation Coefficient. It was done on variables like Body mass index, Homocysteine, Total cholesterol, Triglycerides and HDL cholesterol with TSH in order to measure the strength of linear relationship between variables.

It is observed that as TSH increases, the concentration of homocysteine also increases. This is indicated by weak positive correlation with $r = 0.174$.

It is also observed that increase in TSH concentration has positive correlation with Body mass index, Total cholesterol, Triglycerides and HDL cholesterol. It indicates that as concentration of TSH increases, all the above variables also increases in the study population.

FIGURE 19 : COMPARISON OF VARIOUS PARAMETERS AMONG THE STUDY GROUPS

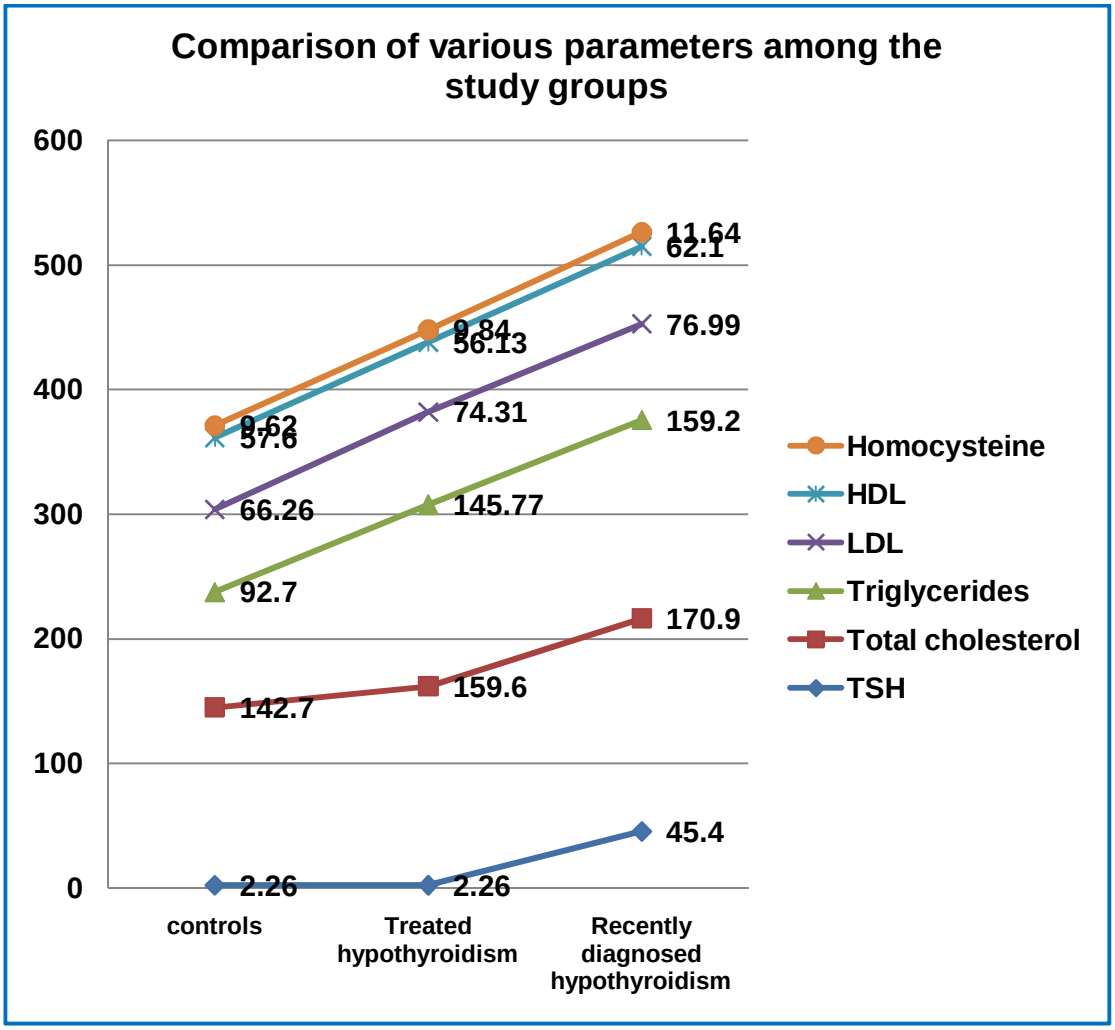


Figure 19: shows comparison of various parameters like TSH, Homocysteine, Total cholesterol, Triglycerides, HDL and LDL cholesterol among the three study groups.

DISCUSSION

DISCUSSION

This study was conducted to measure homocysteine level in newly diagnosed hypothyroid individuals and to correlate them with treated and control groups. Also in this study homocysteine was correlated with lipid profile and evaluated whether it can be used as a risk marker for atherosclerosis.

This study includes three groups.

- Group 1- 30 individuals with recently diagnosed hypothyroidism
- Group 2 – 30 patients with treated hypothyroidism
- Group 3 – 30 Apparently healthy individuals

In group 1, we selected newly diagnosed hypothyroidism based on thyroid profile with high TSH and low T3,T4 after excluding smoking, diabetes, liver disease, renal disease and megaloblastic anemia.

In group 2, we selected hypothyroid patients who were treated for a period of 3 months to 1 year duration were included. This is to know whether treatment with thyroxine decreases homocysteine level. This group was selected based on normal thyroid profile after excluding the above factors.

In group 3 , age and sex matched thirty apparently healthy individuals were included .

ANOVA analysis showed that there was statistically significant difference in total plasma homocysteine level between the three study groups. Total plasma homocysteine level was high in newly diagnosed hypothyroidism compared to treated hypothyroidism and controls with p value of 0.006.

Post-Hoc test was used to show the difference of total plasma homocysteine level between the two study groups. Homocysteine was high in recently diagnosed hypothyroidism [11.64 ± 3.72] compared to controls [9.62 ± 1.65] with p value of 0.004. . Homocysteine was high in recently diagnosed hypothyroidism [11.64 ± 3.72] when compared to treated hypothyroidism [9.84 ± 1.97] with p value of 0.009.

From the above we confirmed that hypothyroidism is associated with hyperhomocysteinemia. The causes for hyperhomocysteinemia are :

- In hypothyroidism the activity of flavokinase is decreased which has an effect on the activity of flavoprotein enzyme methylene tetrahydrofolate reductase.
- This decreases the conversion of methylene tetrahydrofolate to methyl tetrahydrofolate which is one of the cofactor for the conversion of homocysteine to methionine, thus leading to increase in homocysteine level in hypothyroidism.

- While on treatment with thyroxine this enzyme activity is regained and homocysteine is converted back to methionine.

The similar results were obtained by Bjorn G.Nedrebo et al [47], Homocysteine level was high in recently diagnosed hypothyroidism and decreased in treated hypothyroidism. So early diagnosis and treatment of hypothyroidism may reduce homocysteine levels, which is one of the cardiovascular risk factor.

- Prolonged exposure to high homocysteine is toxic because the levels of homocysteine exceeds the endothelial cell capacity to produce S-nitrosohomocysteine which has some effects of nitric oxide in smooth muscle relaxation.

- In hyperhomocysteinemia , homocysteine stimulates protease endothelial cell activator of factor V and directly activates coagulation in the absence of thrombin.

- Mild hyperhomocysteinemia favours binding of lipoprotein[a] to fibrin thus reduces plasminogen activation and inhibits fibrinolysis.

- Homocysteine thiolactone causes LDL cholesterol to aggregate and then phagocytosed by vascular macrophages to form foam cells. Homocysteine thiolactone released from foam cells which produces free radicals and causes endothelial cell damage.

3-9 months of treatment on thyroxine with appropriate dose decreases homocysteine level [43].

Homocysteine has weak positive correlation with TSH [r = 0.174]. This indicates that both TSH and homocysteine were in parallel rise in the study groups.

By ANOVA we observed that total cholesterol was highly significant between three groups with p value of < 0.001. Total cholesterol was high in recently diagnosed hypothyroidism. Comparing two groups by Post-Hoc test showed that recently diagnosed hypothyroidism [170.9 ± 35.5] has high total cholesterol compared to controls [142.7 ± 23.62] with p value of 0.001. Also recently diagnosed hypothyroidism has high cholesterol when compared to treated hypothyroidism [159.6 ± 19.83] with p value of 0.005.

- In hypothyroidism increased total cholesterol is secondary to downregulation of LDL receptors [93] that leads to increase in LDL cholesterol. LDL contains more cholesterol than other lipoproteins, so the total cholesterol is also increased in hypothyroidism.
- Thyroid hormone upregulates LDL receptors [82] and thus decreasing LDL cholesterol and also the total cholesterol.

- Thyroid hormone increases cholesterol conversion to bile acids and its excretion through bile.

The same results were observed by Ravi shekhar et al [93], with statistically significant difference of total cholesterol between the study groups. Total cholesterol was high in newly diagnosed hypothyroidism and decreased in treated hypothyroidism.

Also it has weak positive correlation with TSH [r = 0.018]. It indicates that total cholesterol increases with an increase in TSH in the study groups.

Triglyceride levels are highly significant as acquired by ANOVA between the three groups with p value of < 0.001.TGL level is high in recently diagnosed hypothyroidism cases compared to controls and treated hypothyroidism.

By analyzing the difference between two groups by Post-Hoc test it is high in recently diagnosed hypothyroidism [159.2 ± 74.98] compared to controls [92.7 ± 31.27] with p value of < 0.001. It is high in recently diagnosed hypothyroidism [159.2 ± 74.98] when compared to treated hypothyroidism [145.77 ± 57.32] with p value of 0.366. Triglycerides levels was decreased in treated hypothyroid patients but did not reach the control level.

- Thyroid hormone stimulates lipoprotein lipase activity which metabolizes triglyceride containing lipoproteins like Chylomicrons, VLDL and IDL.

- Thus hypothyroidism decreases the activity of lipoprotein lipase and leads to hypertriglyceridemia.

The Similar results were obtained by Diekman et al [94] .

It has weak positive correlation with TSH [r = 0.13], indicating that there is a parallel increase in the study groups.

LDL cholesterol variation is highly significant obtained by ANOVA with p value of < 0.001. It is high in recently diagnosed hypothyroidism [76.99 ± 30.5] compared to controls [66.26 ± 21.57] with p value of < 0.001 and also high compared to treated hypothyroidism [74.31 ± 17.94] with p value of 0.366 by Post-Hoc test.

- Hypothyroidism causes downregulation of LDL receptors, leads to increase in LDL cholesterol.

- Thyroid hormone increases LDL receptor gene expression by stimulating promoter region of LDL receptor gene which contains thyroid response element and upregulates the LDL receptors and increases its clearance[82].

The similar results were observed by M.C.Das et al [93], LDL cholesterol was decreased in treated hypothyroidism but not attained to the level of the control group.

In this study, by analyzing with ANOVA, HDL cholesterol is not statistically significant between three study groups with p value of 0.068. Comparison of recently diagnosed hypothyroidism [62.1 ± 14.52] with controls [57.9 ± 7.45] by Post-Hoc test with the p value of 0.11 and this is not statistically significant. By comparison of recently diagnosed hypothyroidism [62.1 ± 14.52] with treated hypothyroidism [56.13 ± 6.14] by Post-Hoc test with the p value of 0.024 and this difference is statistically significant.

- Thyroid hormone stimulates hepatic lipase and cholesteryl ester transfer protein[80,81].
- Hepatic lipase converts HDL_2 to HDL_3, which has low cholesteryl ester and accepts free cholesterol.
- CETP exchanges cholesteryl ester of HDL and triglycerides of VLDL/IDL. Because of this exchanging, HDL has low cholesteryl ester and accepts more cholesterol from peripheral tissues and converts to cholesteryl ester.

- In hypothyroidism hepatic lipase and CETP activity is decreased. Thus leads to increase in HDL concentration probably HDL_2 [98] which has more cholesteryl ester and it is not exchanged with VLDL, so it cannot accept further free cholesterol from peripheral tissues. So it also leads to increase in total cholesterol.

- Treatment with thyroxine HDL levels was decreased and attained the control group levels.

Similar results were observed by Srinivas et al [93]. HDL cholesterol is high in recently diagnosed hypothyroidism compared to controls and treated hypothyroidism.

In this study, by analyzing with ANOVA Body mass index was highly significant between the three groups with the p value of < 0.001.

By analyzing with Post-Hoc test BMI was significantly different in recently diagnosed hypothyroidism [26.8 ± 5.9] compared to controls [21.14 ± 1.53] with p value of <0.001 and not statistically significant difference when compared to treated hypothyroidism [26.64 ± 5.04] with p value of 0.919. Indicating that newly diagnosed and treated hypothyroidism were over weight and the weight was not reduced after treatment within one year of duration.

Analyzing TSH by using ANOVA was highly significant between the study groups with p value of < 0.001.

By analyzing TSH with Post-Hoc test there was significant difference in recently diagnosed hypothyroidism [45.4 ± 51.02] compared to controls [2.26 ± 1.07] with p value of <0.001. But it was not statistically significant when the values of recently diagnosed hypothyroidism was compared with treated hypothyroidism [2.26 ± 1.3] with p value of 1.00. Indicating that controls and treated hypothyroidism were in euthyroid state.

CONCLUSION

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This study was conducted to measure Homocysteine level in newly diagnosed hypothyroid individuals and to correlate with treated and control groups. Also in this study Homocysteine were correlated with lipid profile and evaluated whether it can be used as a risk marker for atherosclerosis.

This study includes three groups.

- Group 1- 30 individuals with Recently diagnosed hypothyroidism
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In group 2, we selected hypothyroid patients who were treated for a period of 3 months to 1 year duration were included. This is to know whether treatment with thyroxine decreases homocysteine level. This group was selected based on normal thyroid profile after excluding the above factors.

In group 3, age and sex matched thirty apparently healthy individuals were included .

In this study we found that,

- Total plasma Homocysteine level was significantly increased in recently diagnosed hypothyroidism.
- Total plasma Homocysteine level was near normal in treated hypothyroidism.
- Total plasma Homocysteine level was normal in control groups.
- Lipid profile was significantly altered in recently diagnosed hypothyroidism. The parameters like total cholesterol, TGL, LDL cholesterol and HDL were in higher range as compared to controls and treated hypothyroidism.

❖ In this study we found that hypothyroidism is associated with hyperhomocysteinemia which causes premature atherosclerosis.

❖ Hypothyroidism is one of the treatable cause for hyperhomocysteinemia.

❖ Abnormal lipid profile and hyperhomocysteinemia has synergestic effect over atherosclerosis [95] .

- Early diagnosis and treatment of hypothyroidism plays a major role in preventing the metabolic abnormality and several complications like infertility, birth defects, cardiovascular disease etc.
- During treatment with thyroxine hypothyroid patients should be monitored for 1) lipid profile 2)Total plasma homocysteine levels.
- Measurement of total plasma homocysteine level for screening cardiovascular risk factors and to avoid untoward complications in hypothyroidism cases is necessary.
- Patients with unexplained hyperhomocysteinemia should be screened for thyroid status[14].

LIMITATION

LIMITATIONS OF THIS STUDY

- Because of the financial limits, Vitamin B12 and Folic acid were not measured in the study groups to rule out its deficiency and Megaloblastic anemia.

SCOPE FOR

FURTHER STUDY

SCOPE FOR FURTHER STUDY

- Methylene tetrahydrofolate reductase enzyme activity can be measured and correlated with plasma Homocysteine level.
- Gene polymorphism of Methylene tetrahydrofolate reductase enzyme can be studied.
- Oxidized LDL can be estimated and correlated with plasma homocysteine level.
- Homocysteine level and thyroid function test in coronary artery disease
- Homocysteine level and thyroid function test in cerebro vascular disease.

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ANNEXURES

PROFORMA

Name	:	Sample no. :	
Age	:	Date of collection :	
Sex	:	Ht :	Wt :
Add & Phone.no. :		LCB:	
Duration of symptoms :		LMP:	

Wt gain	YES/NO	Menstrual irregularity	YES/NO
Fatigue/tiredness	YES/NO	Constipation	YES/NO
Infertility	YES/NO	H/o other symptoms-	
Renal disease	YES/NO	Liver disease	YES/NO
CVD	YES/NO	DM	YES/NO
Smoking	YES/NO	HT	YES/NO
Epilepsy	YES/NO	Pregnancy	YES/NO

H/O drug intake- Metformin, Thiazides, Phenytoin, Carbamazepine, B complex

If Thyroxine: duration- dose-

Other system examination:

CVS : RS:

Abdomen: CNS:

Skin:

INV Details:

1. TSH
2. T3
3. T4
4. LFT-T.Protein
5. Albumin
6. SGPT
7. T.Cholesterol
8. TGL
9. HDL
10. LDL
11. RFT-Urea
12. Creatinine
13. Sugar
14. Hb%
15. Peripheral smear
16. Homocysteine

INFORMATION SHEET

Title of the study **:**

A STUDY ON ALTERED TOTAL PLASMA HOMOCYSTEINE LEVEL AND LIPID PROFILE IN NEWLY DIAGNOSED HYPOTHYROID INDIVIDUALS

Name : Date :

Age : OP No :

Sex : Project Patient No :

Institution :

- Your venous blood sample of 5ml has been accepted.
- The purpose of this study is to identify occurance of increase Homocysteine level in Hypothyroidism with the help of certain ELISA tests.
- We are selecting certain cases and if your blood sample is found eligible, we may be using your blood sample to perform extra tests and special studies which in any way do not affect your final report or management.
- The privacy of the patients in the research will be maintained throughout the study. In the event of any publication or presentation resulting from the research, no personally identifiable information will be shared.
- Taking part in this study is voluntary. You are free to decide whether to participate in this study or to withdraw at any time; your decision will not result in any loss of benefits to which you are otherwise entitled.
- The results of the special study may be intimated to you at the end of the study period or during the study if anything is found abnormal which may aid in the management or treatment.

Signature of investigator **Signature of participant**

PATIENT CONSENT FORM

Title of the study:

A STUDY ON ALTERED TOTAL PLASMA HOMOCYSTEINE LEVEL AND LIPID PROFILE IN NEWLY DIAGNOSED HYPOTHYROID INDIVIDUALS

Name : Date :

Age : OP No :

Sex : Project Patient No :

Institution :

- The details of the study have been provided to me in writing and explained to me in my own language.
- I confirm that I have understood the above study and had the opportunity to ask questions.
- I understand that my participation in the study is voluntary and that I am free to withdraw at any time, without giving any reason, without the medical care that will normally be provided by the hospital being affected.
- I agree not to restrict the use of any data or results that arise from this study provided such a use is only for scientific purpose(s).
- I have been given an information sheet giving details of the study.
- I fully consent to participate in the above study.

Signature of investigator **Signature of participant**

ஆராய்ச்சி ஒப்புதல் கடிதம்

ஆராய்ச்சி தலைப்பு: புதிதாக கண்டுபிடிக்கப்பட்ட தைராய்டு சுரப்பி குறைபாடு உள்ளவர்களின் இரத்தத்தில் ஹோமோசிஸ்டீன் மற்றும் கொழுப்பு சத்தின் மாறுபாடுகளை கண்டறிம் ஆராய்ச்சி.

பெயர் : தேதி :

வயது : புறநோயாளி எண் :

பால் : ஆராய்ச்சி சேர்க்கை எண் :

முகவரி : இடம் :

- இந்த ஆராய்ச்சியின் விவரங்களும் அதன் நோக்கங்களும் முழுமையாக எனக்கு தெளிவாக விளக்கப்பட்டது.
- எனக்கு விளக்கப்பட்ட விஷயங்களை புரிந்து கொண்டு நான் எனது சம்மதத்தைத் தெரிவிக்கிறேன்.
- எனக்கு இரத்த/சிறுநீர் பரிசோதனை செய்து கொள்ள சம்மதம்.
- நான் புதிதாக கண்டுபிடிக்கப்பட்ட தைராய்டு சுரப்பி குறைபாடு உள்ளவர்களின் இரத்தத்தில் ஹோமோசிஸ்டீன் மற்றும் கொழுப்பு சத்தின் மாறுபாடுகளை கண்டறிம் இந்த ஆராய்ச்சியின் விபரங்களைக் கொண்ட தகவல் தாளைப் பெற்றுக் கொண்டேன்.

- இதன் மூலம் எந்த பின்விளைவும் வராது என மருத்துவர் மூலம் தெரிந்து கொண்டு என்னுடைய சுயநினைவுடன் மற்றும் முழு சுதந்திரத்துடன் இந்த மருத்துவ ஆராய்ச்சியில் என்னை சேர்த்துக்கொள்ள சம்மதிக்கிறேன் .

ஆராய்ச்சியாளர் கையொப்பம் பங்கேற்பாளர் கையொப்பம்

ஆராய்ச்சி தகவல் தாள்

ஆராய்ச்சி தலைப்பு: புதிதாக கண்டுபிடிக்கப்பட்ட தைராய்டு சுரப்பி குறைபாடு உள்ளவர்களின் இரத்தத்தில் ஹோமோசிஸ்டீன் மற்றும் கொழுப்பு சத்தின் மாறுபாடுகளை கண்டறிம் ஆராய்ச்சி.

பெயர் : தேதி :

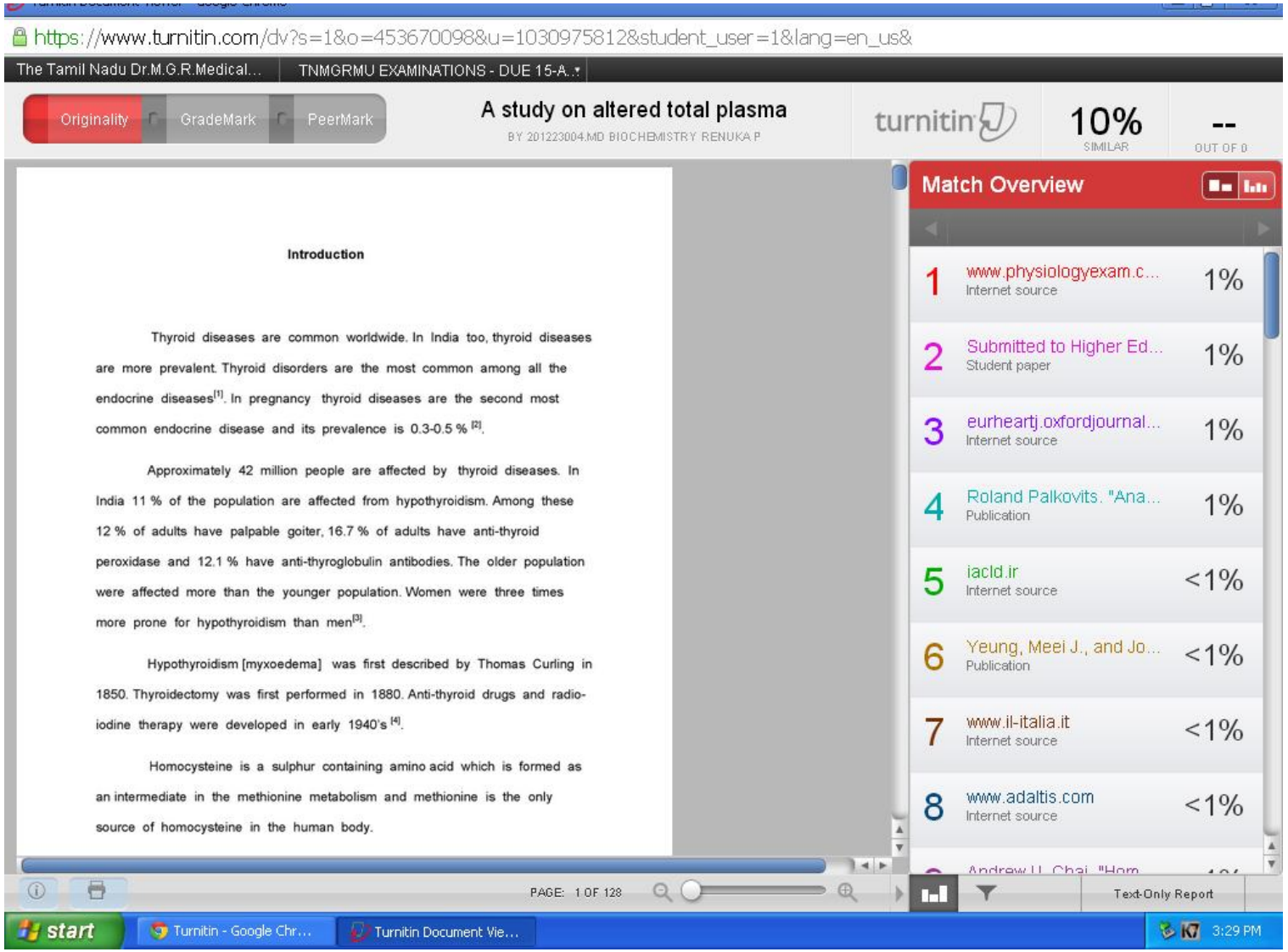
வயது : புறநோயாளி எண் :

பால் : ஆராய்ச்சி சேர்க்கை எண் :

இடம் :

- தங்களது இரத்தம் இங்கு பெற்றுக் கொள்ளப்பட்டது.
- புதிதாக கண்டுபிடிக்கப்பட்ட தைராய்டு சுரப்பி குறைபாடு
- உள்ளவர்களின் இரத்தத்தில் ஹோமோசிஸ்டீன் மற்றும் கொழுப்பு சத்தின் மாறுபாடுகளை கண்டறிம் இந்த ஆராய்ச்சி இங்கு நடைபெற்று வருகின்றது.
- நீங்களும் இந்த ஆராய்ச்சியில் பங்கேற்க நாங்கள் விரும்பிகிறோம் .இந்த ஆராய்ச்சியில் உங்களுடைய இரத்தம்/சிறுநீர் எடுத்து சில சிறப்புப் பரிசோதனைக்கு உட்படுத்தி அதன் தகவல்களை ஆராய்வோம். அதனால் தங்களது நோயின் ஆய்வறிக்கையோ அல்லது சிகிச்சையோ பாதிப்புக்கு உள்ளாகாது என்பதையும் தெரிவித்துக் கொள்கிறோம்.
- முடிவுகளை அல்லது கருத்துக்களை வெளியிடும் போதோ அல்லது ஆராய்ச்சியின் போதோ தங்களது பெயரையோ அல்லது அடையாளங்களையோ வெளியிடமாட்டோம் என்பதையும் தெரிவித்துக் கொள்கிறோம்
- இந்த ஆராய்ச்சியில் பங்கேற்பது தங்களுடைய விருப்பத்தின் பேரில் தான் இருக்கிறது.மேலும் நீங்கள் எந்நேரமும் இந்த ஆராய்ச்சியிலிருந்து பின்வாங்கலாம் என்பதையும் தெரிவித்துகொள்கிறோம்.
- இந்த சிறப்பு பரிசோதனைகளின் முடிவுகளை ஆராய்ச்சியின் போது அல்லது ஆராய்ச்சியின் முடிவின் போது தங்களுக்கு அறிவிப்போம் என்பதையும் தெரிவித்துக்கொள்கிறோம்.

ஆராய்ச்சியாளர் கையொப்பம் பங்கேற்பாளர் கையொப்பம்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Introduction
Thyroid diseases are common worldwide. In India too, thyroid diseases are more prevalent. Thyroid disorders are the most common among all the endocrine diseases[1]. In pregnancy thyroid diseases are the second most common endocrine disease and its prevalence is 0.3-0.5 % [2].
Approximately 42 million people are affected by thyroid diseases. In India 11 % of the population are affected from hypothyroidism. Among these 12 % of adults have palpable goiter, 16.7 % of adults have anti-thyroid peroxidase and 12.1 % have anti-thyroglobulin antibodies. The older population were affected more than the younger population. Women were three times more prone for hypothyroidism than men[3].
Hypothyroidism [myxoedema] was first described by Thomas Curling in 1850. Thyroidectomy was first performed in 1880. Anti-thyroid drugs and radio-iodine therapy were developed in early 1940's [4].
Homocysteine is a sulphur containing amino acid which is formed as an intermediate in the methionine metabolism and methionine is the only source of homocysteine in the human body.
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