" THE CORRELATION BETWEEN SERUM HOMCYSTEINE LEVELS AND HYPOTHYROIDISM- A ONE YEAR CASE CONTROL STUDY."

By

REG NO.BG0114011

Dissertation

Submitted to the
KLE University, Belagavi, Karnataka

In Partial Fulfillment
of the requirements for the degree of

M. D.
in
GENERAL MEDICINE

DEPARTMENT OF MEDICINE,

JAWAHARLAL NEHRU MEDICAL COLLEGE,

BELAGAVI, KARNATAKA

APRIL – 2017

KLE UNIVERSITY, BELAGAVI, KARNATAKA

ENDORSEMENT

This is to certify that the dissertation entitled **"THE CORRELATION BETWEEN SERUM HOMCYSTEINE LEVELS AND HYPOTHYROIDISM- A ONE YEAR CASE CONTROL STUDY IN KLES HOSPITAL BELAGAVI"** is a bonafide research work done by **REG NO.BG0114011.**

Dr. Rekha Patil MD
Professor and Head,
Department of Medicine,
J. N. Medical College,
Nehru Nagar, Belagavi – 10

Date:
Place: Belagavi.

Dr. N. S. Mahantshetti MD
Principal,
J. N. Medical College,
Nehru Nagar, Belagavi – 10

Date:
Place: Belagavi.

LIST OF ABBREVIATIONS USED

TSH	-	Thyroid Stimulating Hormone
fT3	-	free T3
fT4	-	free T4
Hcy	-	Homocysteine
BMI	-	Body Mass Index.
FAD	-	Flavin Adenine Dinucleotide
LDL	-	Low Density Lipoprotein
HDL	-	High Density Lipoprotein
Hhcy	-	Hyperhomocysteinemia
DIT	-	Diiodotyrosine
MIT	-	Monoiodotyrosine
TBG	-	Thyroid Binding Globulin
SAM	-	S-Adenosyl Methionine
ATP	-	Adenosine Tri Phosphate
MTHFR	-	Methylene Tetrahydrofolate Reductase
CVD	-	Cardiovascular disease
IDD	-	Iodine Deficiency Disorders

ABSTRACT

Background and Objective:

Thyroid gland is critical to the proper functioning and regulation of various physiological pathways and processes. It has been postulated that the decreased levels of hepatic enzymes involved in the remethylation pathway of homocysteine and concurrent changes in renal function leads to hyperhomocysteinemia. Also the thyroid hormones affect the metabolism of folic acid and riboflavin, by stimulating flavokinase, and thus reduces the flavin mononucleotides and flavin adenine dinucleotide (FAD). FAD- dependent methylene tetrahydrofolate reductase is reduced in hypothyroidism which leads to hyperhomocysteinemia.

Homocysteine has been shown to be a strong independent risk factor for cardiovascular disease. The objectives of the study are to study the incidence of hyperhomocysteinemia in patients with hypothyroidism, and the relation of homocysteine and cholesterol levels to thyroid hormones free T3, free T4 and TSH in newly detected hypothyroid patients.

Methodology

This was a hospital based case-control study, Which recruited 30 newly detected hypothyroid patients and 30 euthyroid controls, at the KLES Dr Prabhakar Kore Charitable Hospital and Medical Research Center, Belagavi. After initial clinical detection of new hypothyroid patients, the patient was subjected to clinical and blood investigations. Similar method was employed in the control group after matching with the case group. These findings were noted on a predesigned and pretested proforma.

Results

A total of 30 hypothyroid patients and 30 euthyroid controls were included in the study. In the study the majority of the patients were females,42 (70%) with a male: female ratio of 3:7. the mean age of patients in the hypothyroid group was 40.60 years, and the mean age of patients in the normal group was 41.40 years with a p value of 0.7502, with a study mean age of 41 years. The mean TSH levels in the hypothyroid group was 46.95 mU/L , and that in the normal group was 2.27 mU/L , with a p value of 0.0001. The mean Free T3 levels in the hypothyroid group was 1.41 µg/l , and that in the normal group was 1.80 µg/l, which was not statistically significant (p value 0.1148). The mean FT4 levels in the hypothyroid group was 4.69 ng/l , and that in the normal group was 5.75 ng/l , which was not statistically significant (p value 0.2677). The mean serum homocysteine levels in the hypothyroid group was 25.02 mcmol/l , and that in the normal group was 16.65 mcmol/l, which was statistically significant (p value 0.0064). The mean cholesterol levels in the hypothyroid population was 193.03 mg/dl, and that in the normal group was 165.90 mg/dl, which was statistically significant (p value 0.0066). On correlating the serum homocysteine levels in the hypothyyroid group with TSH, fT3 and fT4 and serum cholesterol levels, there was no statistically significant correlation (p values of 0.7548,0.4989,0.5590 and 0.8360 respectively) . On correlating the serum homocysteine levels in the normal group with TSH, fT3 and fT4 and serum cholesterol levels, there was no statistically significant correlation (p values of 0.9356, 0.5106 and 0.9429 respectively). On correlating the serum homocysteine levels with TSH , fT3 and fT3 and cholesterol levels in the total sample, the

correlation was not statistically significant (p values of 0.2278,0.1665,0.9441 and 0.0801 respectively).

Conclusion

- In conclusion, the study observed elevation of serum homocysteine and total cholesterol levels in hypothyroid patients. Hyperhomocysteinemia and increased cholesterol levels contributes to a greater cardiovascular risk. Hyperhomocysteinemia, along with hypercholesterolemia, can explain the progression of atherosclerosis in hypothyroid patients and we would recommend screening of serum homocysteine levels in hypothyroid patients , in view of the risk of accelerated atherosclerosis and cardiovascular disease. Further studies are needed on large populations.

Key Words: Homocysteine ; Hypothyroidism ; Cholesterol

CONTENTS

LIST OF TABLES

LIST OF GRAPHS

LIST OF FIGURES

INTRODUCTION

The thyroid gland is important for the regulation and proper functioning of numerous physiological pathways in the body. It is directly stimulated by the TSH from the anterior pituitary.[1,2] The synthesis and release of TSH is regulated by the TRH (Thyroid Releasing Hormone) from the hypothalamus. The TSH stimulates the thyroid to release T3 and T4.[2] T3 and T4 cause a negative feedback on the hypothalamus and anterior pituitary to reduce further release of TRH and TSH respectively.T here is evidence that hypothyroidism causes an increase in cardiovascular risk, via a rise in the circulating low density lipoprotein(LDL) cholesterol, induction of diastolic hypertension, altered coagulability, and direct effects on vasuclar smooth muscle. In recent years, newer risk factors for atherosclerotic cardiovascular disease have been identified, including homocysteine, raised C-reactive proetein (CRP), endothelial dysfunction and insulin resistance. A small number of studies have related these factors to thyroid status.[1-3]

Homocysteine (Hcy) is a sulphur containing amino acid which is produced by the metabolism of the methionine, an essential amino acid. [4] In 1969, Mc Cully for the first time proposed the "Homocysteine hypothesis of atherosclerosis". Wilchen and Wilcken gave evidence for the same in 1976.[5] A study of literature reveals a relation between coronary events and hyperhomocysteinemia. Various physiological and pathological processes which lead to accumulation of homocysteine over time correlate with thrombosis and coronary risk. [6] It was found that that a 5 mcmol/L increase in Hcy was associated with an increase in the all cause mortality of 49%,and the corresponding value for cardiovascular death was 50% .[5]

Overt hypothyroidism is a medical condition where the TSH is raised to more than 10 mu/L.Hypothyroidism occurs in 0.1% men and 0.4% women.[7] However the overall prevalance of hypothyroidism is not well documanted in Indian studies.One particular study done in AIIMS Delhi showed that the prevalance of hypothyroidism which was due to iodine deficiency was 5.4%, that due to autoimmune causes was 7.5%, in the general population.[8] In another study which was done by Francoise Barbe et al.,elevated Hcy was found in 22% of hypothyroid patients.[9] The thyroid hormones affect the metabolism of folic acid,affect riboflavin metabolism, mainly by the stimulation of flavokinase and thereby the formation of flavin adenine dinucleotide (FAD). FAD- dependent methylene tetrahydrofolate reductase is reduced in hypothyroidism which leads to hyperhomocysteinemia. [10] The decreased levels of the hepatic enzymes involved in the remethylation pathway of homocysteine and concurrent changes in the renal function in hypothyroidism can lead to hyperhomocysteinemia.[9] The cardiovascular morbidity in hypothyroid patients is related to elevated low density lipoprotein cholesterol,increased peripheral resistance and mostly diastolic hypertension,reduced myocardial contractility,reduced stroke volume and bradycardia. [11]

Thyroid hormones have an important effect on the lipid profile. [12] .The thyroid hormones cause induction of 3-hydroxy-3 methylyglutaryl Coenzyme A (HMG-COA) reductase,which is the first step in cholesterol biosynthesis.Also, T3(Triiodothyronine) causes upregulation of the LDL receptors by modulating the LDL receptor gene activation.This action of T3 resulting in gene activation is the result of the direct binding of T3 to specific Thyroid Hormone Responsive Elements(TREs). [13] Also,it has been shown that T3 has an effect on the sterol regulatory-element bindng protein-2 (SREBP-2) ,which in turn regulates the LDL

receptors gene expression. [14] .Also, T3 has been shown to have a protective effect on LDL from oxidation. [15]

The thyroid hormones have an important influence on the serum HDL levels by promoting the activity of cholesteryl ester transfer protein (CETP) , which exchanges cholesteryl esters to the Very Low Density Lipoproteins (VLDL) from HDL and TGs to the opposite direction. [16] Also thyroid hormones cause stimulation of the Lipoprotein Lipase (LPL), which catabolises the TG-rich lipoproteins, and also the Hepatic Lipase (HL), which hydrolyzes HDL_2 to HDL_3 and also causes the conversion of the intermediate density lipoproteins (IDL) to LDL and in turn the LDL to small dense LDL(sdLDL). [17,18] T3 has also shown to cause upregulation of the Apolipoprotein AV (ApoAV), which plays an important role in TG regulation. [19] Increased levels of ApoAV has been shown to correlate with a redcued level of TGs. [20] One of the mechanisms which have been proposed for this a reduction of hepatic VLDL-TG production and also an increase in the plasma LDL levels and activity, which cause an increase in the lipoprotein remnant generation due to increased LPL-mediated lipolysis of VLDL-TG. [21]

Thus due to the metabolic correlation between thyroid hormones, homocysteine levels and lipid profile,we study the Hcy and cholesterol levels in newly detected hypothyroid patients versus that in euthyroid population, to assess if there is a correlation.

OBJECTIVES

Objectives of this study are:

Primary objective - To study the correlation between serum homocysteine levels and hypothyroidism

Secondary Objective - To study the relation of thyroid hormones free T3,free T4 and TSH in newly detected hypothyroid patients to serum homocysteine levels and total cholesterol levels

REVIEW OF LITERATURE

Historical perspectives

In ancient times, numerous references about enlarged thyroid glands have been mentioned as far as 2700 BC.[22] This is probably because of a high prevalence during a period when its exact etiology and was unknown. It is noted that the Chinese physician Tshui Chin-thi, in 85 AD, distinguished between solid tumors of the neck which were incurable and movable (benign) tumors which were deemed to be curable.[23] However it is not known whether he knew that it was the thyroid gland which was involved.

In India, early Ayurvedic physicians who studied in detail about the thyroid gland, right from the time of 1400 BC, called goiters as 'galaganda' and described them in detail. [23] They classified thyroid diseases into three types; Vataja (hyperthyroid), Kaphaja (hypothyroid) and Medaja (thyroidal cyst). They have described in detail the symptoms of these diseases in ayurvedic medicine. It has been shown to closely be correlating the medical findings of the modern world.

Charaka was one of the main ayurvedic physicians who described in detail regarding the thyroid gland. He said that it cud be prevented by intake of appropriate quantities of milk, rice, green grams, sugarcane juice and cucumber. Charaka discouraged people from eating sour foods as they were told to aggravate the diseases of the thyroid. Kaanchanara (Bauhinia veriegata) and Bladderwrack (Fucus Vesiculosus) were the main ones which were suggested for for all sorts of thyroid problems.

Guggulu (Commiphora mukul) was used in order to to increase the basal metabolic rate in patients with hypothyroidism . Punarnava (boerhaavia diffusa Linn) was another excellent diuretic that was given to reduce the swelling in people with thyroid disorders.

For a patient with hyperthyroidism, Bugleweed [*Lycopus*], gypsywort [*Lycopus*], water horehound [*Lycopus americanus*], gromwell [*Lithospermum*] and *European gromwell* [*Lithospermum officinale*] were also used. Ancient Indian physicians were also able to distinguish between pitting and non-pitting edema, and also between facial and pedal edema.However they said that it was the edema which caused goiter rather than the thyroid gland being responsible. [24]

In ancient Greece,it was Plato and Hippocrates who first described the thyroid gland.They described the thyroid gland using the distinction between salivary and thymus glands. [25] Plato and Hippocrates said that these glands were spongy in nature and helped to lubricate the respiratory passageways. [26] However Galen,who was a renowned physician at the time,was of a different view. Galen said that because this gland was spongy in nature it was better suited for absorption rather than secretion. [26]

Galen supported his argument by saying that secretion of the gland could not enter the respiratory passage. Galen also labeled the thyroid gland as an important connection between the heart and brain. He later made an important finding in the anatomy of the third ventricle during the 2nd century [27] Galen also mentioned that the pituitary gland is located in the sella turcica and is surrounded by a vascular network. He was of the opinion that the ‘heat’ of the body travels through these vascular networks and is converted to nerve impulses which affected the ‘substance

of the neck', or the thyroid gland. Hippocrates and Gaius Plinius Secundus of Pliny regarded goiter to be related entirely to the dirnking of snow water. [23,28,29] Earlier it was thought that goitre was not thought to be an enlargement of the thyroid, but rather it was believed to be a herniation of larynx. Pedanius

Dioscorides, another 1st century AD Greek physician, and Galen later about the 2nd century AD supported this view. Leonardo da Vinci went on to sketch the thyroid gland in 1511 during his anatomical studies. Even though da Vinci was aware of its exact anatomical structure, he could not understand its function and assumed that it was created to fill the gap between muscles of the neck and to hold the trachea away from the sternum. [28]

Chinese doctors were known to use burnt sponges and seaweeds for the treatment of goiter as early as the 1600 BC. [30] However, they were not aware about what caused the disease. The medical treatment for enlarged thyroid was also described by Dioscorides in the 1st century AD. [31] It was noted that except for shells from marine organisms, iodine-rich substances were not mentioned. Numerous plants and animals, even lizards , were used in treatment of goiter.

The Byzantine physicians used a variety of things to treat goiter ,mostly calcium, copper, sulfur and ammonium salts. Roger Frugardi, in the 12th century,opined that goiter is treatable by drinking a tincture made with barren walnut leaves and roots, boiled in good wine with a bit of pepper added. [32] An alternative pharmacological treatment used in ancient Italy was a powder obtained from burnt and dried marine sponges. [33] This mixture was rich in silica, calcium phosphate, sodium chloride, sulfur chloride, iodine, bromine, magnesium carbonate and calcium carbonate. Due to the fact that it was rich in iodine, goiter may well have been cured.

Eventhough Robert Graves and Carl von Basedow are credited with modern description of thyroid related disorders, history reveals that older civilizations were well aware of this condition. The definitions and findings by Graves and Basedow came in the 19th century while mentions of this disease are found in works of Aristotle and Xenophon from 5th century BC. [34]

Goiter has also been described in the writings of the ancient Gandhara civilization. This dates long into the 1st or 2nd century AD. [35] However,doubts surround the matter whether the appearances related to the God Bes of ancient Egypt and Cleopatra are related to thyroid diseases. There is no definite evidence available, still, quite possibly their depictions may have been affected by thyroid disorders.

In 1656, it was the famous Thomas Wharton, an anatomist, who discovered the exact anatomical structure of the thyroid gland, along with the other glands of the body. He explained that production and secretion was a gland's primary function. He assigned specific roles to each gland.

He mentioned that the thyroid gland was responsible for heating the thyroid cartilage, which is generally cold due to its superficial position, lubricate the structures in the neck and to give rotundity and beauty to the neck. [36]

It is odd that even though Galen had already told how it is unlikely for the thyroid to be responsible for lubricating the neck, Wharton still followed the earlier belief. The gland was called thyroid, not because of its shape, but as it closely approximated the shield shape of the thyroid cartilage of the larynx.

De Bordeau said that each organ in the body gives off certain 'emanations' which are necessary for the body functioning. [37] He found out that goiter was

common in the Western part of the Pyrenees mountains (located between France and Spain). He also said that women had larger thyroids, and were the ones who suffered more frequently from goiter. De Bordeu through his studies, associated goitre with hoarseness of voice. In 1776, von Haller who hailed from Bern (Switzerland), grouped the thyroid with the thymus and spleen, together as ductless glands which poured their special secretions into the bloodstream. [38]

In India,various surveys conducted by the Central and State Health Directorates, Indian Council of Medical Research (ICMR) and medical institutes since 1950s have clearly demonstrated that IDD is a public health problem in all States and union territories in India. Among the 325 districts surveyed in India so far, 263 districts are IDD-endemic, *i.e.* the prevalence of IDD is above 10 per cent in the population [39] .

A survey which was conducted by the National Nutrition Monitoring Board (NNMB) in 2000-2001 in rural areas of Kerala, Tamil Nadu, Karnataka, Andhra Pradesh, Maharashtra, Madhya Pradesh, Orissa and West Bengal, showed that the overall prevalence of total goitre rate (TGR) among childred aged between six to twelve years old was about 4 per cent [40] It was shown that the prevalence of goitre was highest in Maharashtra (11.9%) and West Bengal (9%).

Globally, India is the country with the largest number of children born vulnerable to iodine-deficiency [41]. India, being one of the countries which is part of the UNGASS on children had committed to the goal of IDD elimination by 2005. However, India subsequently had to change the IDD control goal in 2006. The IDD control goal in India aimed to decrease the prevalance of IDD (*i.e.* total goitre rate) below 10 per cent in the entire country by 2012 . [42]

Anatomy and development of the thyroid gland During the development of the embryo, at about 3–4 weeks of gestational age, the thyroid appears as an epithelial proliferation in the floor of the pharynx ,basically at the base of the tongue in between the tuberculum impar and the copula linguae. The copula soon gets covered by the hypopharyngeal eminence [43], at a point which becomes later indicated by the foramen caecum. The thyroid after this stage descends in front of the pharyngeal gut as a bilobed diverticulum through the thyroglossal duct.

Over the next few weeks, the thyroid gland gradually migrates to the base of the neck, passing in front of the hyoid bone. During migration, the thyroid anatomically remains connected to the tongue by a narrow canal, the thyroglossal duct.

By the end of the fifth week the thyroglossal duct gradually degenerates and the detached thyroid continues on to its final position over the following two weeks.

In the fetus, the hypothalamus and pituitary start producing thyrotropin releasing hormone (TRH) and thyroid-stimulating hormone (TSH) at 18- 20 weeks, and the production of thyroxine (T_4) reaches a significant level by this time. [44] Fetal levels of triiodothyronine (T_3) remains low, less than 15 ng/dL (nanograms per decilitre) until 30 weeks, and then gradually increases to 50 ng/dL at full-term. The fetus needs to be self- sufficient in the levels of thyroid hormones to be protected against neurodevelopmental disorders that would arise from maternal hypothyroidism. [45] It is preterm neonates who are at risk of these disorders as their thyroid glands are insufficiently developed to meet their postnatal needs. [46]

The neuroendocrine parafollicular cells,also called as C cells, and are responsible for the production of calcitonin, are derived from neural crest cells, which then migrate to the pharyngeal arches.This is the part of gland which later first forms as the ultimopharyngeal body, which begins in the ventral fourth pharyngeal pouch and then later joins the primordial thyroid during its descent upto its final location in the anterior neck. Whenever there is any variation in prenatal development can result in various forms of thyroid dysgenesis and can lead to congenital hypothyroidism, and if untreated this can result in cretinism.

Anatomy of the thyroid gland The thyroid is a butterfly-shaped gland and consists of two lobes, one on the right and the left which are the wings, and the narrow connecting isthmus as the body. [47]

The thyroid is considered as one of the larger endocrine glands, weighing 2-3 grams in neonates and 25 grams in adults, and the size increases in pregnancy. [47] Each individual lobe of the thyroid is about 5 cm long, 3 cm wide and 2 cm thick The lobes of the thyroid are asymmetrical, with the right lobe usually larger. The gland generally is usually larger in women. [48]

The isthmus of the thyroid gland is the part which connects together the lower thirds of the right and left lobes of the thyroid gland. The isthmus of the thyroid measures about 1.25 cm in breadth, and the same in depth, and covers the second and third rings of the trachea. There are, however, many variations in its situation and size of the isthmus.

The isthmus lies at the front of the neck and is covered in front by the skin and fascia, and near the middle line, on either side, by the sternothyroid muscles. On

its upper border runs an anastomotic branch uniting the two superior thyroid arteries; and at its lower border are the inferior thyroid veins.

The thyroid is positioned near the front of the neck, lying against and around the front of the larynx and trachea. The top of the thyroid lies directly below the thyroid cartilage (just below the laryngeal prominence, or *Adam's apple*, and generally extends to the fifth or sixth tracheal ring.

It is generally difficult to demarcate the gland's upper and lower border with vertebral levels because it moves position in relation to these during swallowing. [49]

However the thyroid usually spans from C5 to C7.The thyroid gland is covered on the outside by a thin fibrous sheath, the capsule of the thyroid. [47] The external layer of this covering is anteriorly continuous with the pretracheal fascia and posterolaterally continuous with the carotid sheath. The capsule covering the thyroid extrudes into the gland itself and forms the septae that divides the thyroid tissue into microscopic lobules. [47]

The thyroid gland is covered anteriorly with infrahyoid muscles and laterally with the sternocleidomastoid muscle. On the posterior side of the thyroid,it is fixed to the cricoid and tracheal cartilages and cricopharyngeus by a thickening of the fascia to form the posterior suspensory ligament of thyroid gland also known as Berry's ligament. [50] The thyroid gland's firm attachment to the underlying trachea explains the reason behind its movement with swallowing. [51]

In this region of the thyroid, the recurrent laryngeal nerve and the inferior thyroid artery pass next to or in the ligament, and where present is the Zuckerkandl's

tubercle. [51] The two parathyroid glands lie on either side between the two layers of the capsule, at the back of the thyroid lobes.

Bood,Lymph and nerve supply The blood supply of the thyroid is from the superior thyroid artery, a branch of the external carotid artery, and the inferior thyroid artery, a branch of the thyrocervical trunk, and sometimes by the thyroid ima artery, branching directly from the subclavian artery. [47] The superior thyroid artery divides into anterior and posterior branches supplying the thyroid, and the inferior thyroid artery divides into superior and inferior branches. [47] The venous drainage is through the superior and middle thyroid veins, which drain to the internal jugular vein, and via the inferior thyroid veins. The inferior thyroid veins begin from a network of veins and drain into the left and right brachiocephalic veins.

Lymphatic drainage of the thyroid passes frequently the deep lateral cervical lymph nodes, and the pretracheal and paratracheal lymph nodes. The thyroid is supplied by parasympathetic nerve input from the superior laryngeal nerve and the recurrent laryngeal nerve.

The Thyroid hormones

The thyroid hormones, namely triiodothyronine (T_3) and its prohormone, thyroxine (T_4), are tyrosine-based hormones produced by the thyroid gland that are primarily responsible for regulation of metabolism. T_3 and T_4 are partially composed of iodine. Whenever there is a deficiency of iodine it leads to decreased production of T_3 and T_4, enlarges the thyroid tissue and will cause the disease known as simple goitre. The main form of thyroid hormone in the blood is thyroxine (T_4), which has a longer half-life than T_3. [53] In human beings, the ratio of T_4 to T_3 released into the blood is between 14:1 and 20:1. T_4 is converted to the active form T_3 (three to four

times more potent than T_4) within cells by deiodinases (5'-iodinase). These undergo further processing by decarboxylation and deiodination to produce iodothyronamine (**T_1a**) and thyronamine (**T_0a**). All the three isoforms of the deiodinases contain selenium, thus dietary selenium is essential for T_3 production.

Synthesis of the Thyroid hormones

The sysnthesis of thyroxine and triiodothyronine is primarily regulated by thyroid-stimulating hormone (TSH), released by the anterior pituitary gland. TSH release in turn is controlled by the thyrotropin releasing hormone (TRH) from the hypothalamus. The thyroid hormones provide negative feedback back to the thyrotropes TSH and TRH: when the thyroid hormones levels are eaised, TSH production is suppressed. This negative feedback also happens when levels of TSH are high, causing TRH production to be suppressed. [54]

TRH is secreted at a higher rate in situations such as cold exposure (to stimulate thermogenesis) which is important in case of infants. TSH production is reduced by dopamine and somatostatin which act as local regulators at the level of the pituitary, in response to higher levels of glucocorticoids and sex hormones (estrogen and testosterone), and excessively high blood iodide concentration

Hormone production

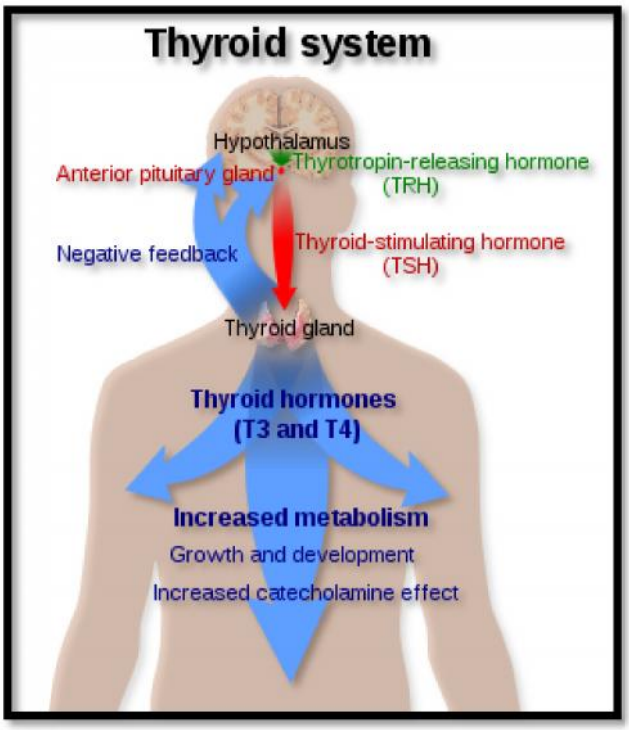


Figure 1 : The system of the thyroid hormones T_3 and T_4

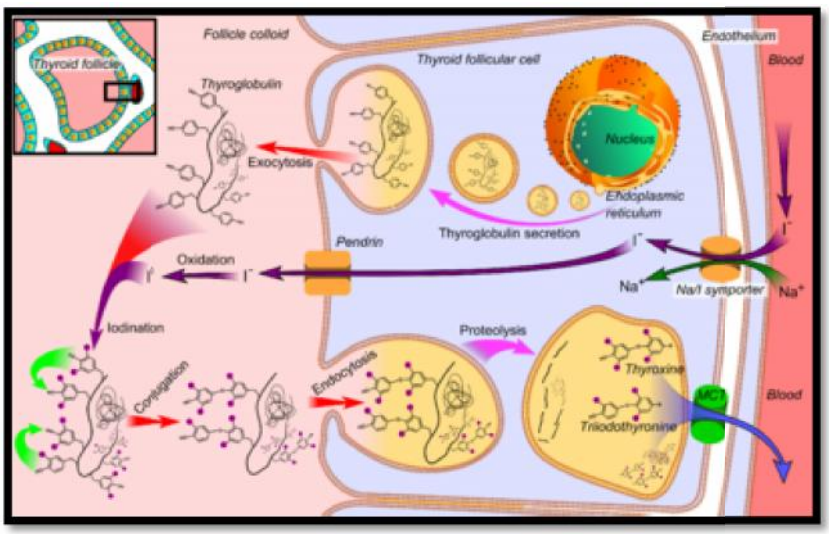


Figure 2 : Synthesis of the thyroid hormones, as seen on an individual thyroid follicular cell

Thyroglobulin is synthesized in the rough endoplasmic reticulum and follows the secretory pathway to enter the colloid in the lumen of the thyroid follicle by exocytosis.

At the same time, there is a sodium-iodide (Na/I) symporter which pumps iodide (I^-) actively into the cell, which earlier has crossed the endothelium by largely unknown ways.

This iodide present in the cytoplasm enters the follicular lumen by the transporter pendrin, which is by a passive manner.

In the colloid, iodide (I^-) undergoes oxidisation to iodine (I^0) by an enzyme named thyroid peroxidase.

Iodine (I^0) is a very reactive component and it iodinates the thyroglobulin at tyrosyl residues in its protein chain (which totally contains approximately 120 tyrosyl residues).

In *conjugation*, adjacent tyrosyl residues are paired together. The entire complex re-enters the follicular cell by endocytosis. Proteolysis by various proteases releases thyroxine and triiodothyronine molecules, which is then transported into the blood by largely unknown mechanisms.

The thyroid hormones are formed from the addition of succesive iodide ions to thyroglobulin, a precursor molecule, Iodide from the circulation is taken up by follicular cells through the sodium-iodide symporter. This is an ion channel on the cell membrane which transports two sodium ions and an iodide ion into the cell.From within the cell iodide is then transported to the follicular space from the cell via the iodide-chloride antiporter pendrin. In the follicular space, the iodide is then oxidized

to iodine, following which it is attached to thyroglobulin by the enzyme thyroid peroxidase. This is the precursors of the hormones monoiodotyrosine (MIT), and diiodotyrosine (DIT).

Thyroid hormones (T_4 and T_3) are produced by the follicular cells of the thyroid gland and are regulated by TSH made by the thyrotropes of the anterior pituitary gland. The effects of T_4 in vivo are mediated via T_3 (T_4 is converted to T_3 in target tissues). T_3 is 3- to 5- fold more active than T_4.Thyroxine (3,5,3',5'-tetraiodothyronine) is produced by follicular cells of the thyroid gland. It is produced as the precursor thyroglobulin (this is *not* the same as TBG), which is cleaved by enzymes to produce active T_4.

The steps in this process are as follows:

1. The Na^+/I^- symporter transports two sodium ions across the basement membrane of the follicular cells along with an iodide ion. This is a secondary active transporter that utilises the concentration gradient of Na^+ to move I^- against its concentration gradient.

2. I^- is moved across the apical membrane into the colloid of the follicle.

3. Thyroperoxidase oxidises two I^- to form I_2. The Iodide is non-reactive, and only the more reactive iodine is required for the subsequent step.

4. The thyroperoxidase then iodinates the tyrosyl residues of the thyroglobulin which is within the colloid. This thyroglobulin was produced in the ER of the follicular cell and secreted into the colloid.

5. Iodinated Thyroglobulin binds megalin for endocytosis back into cell.

6. The TSH released from the adenohypophysis binds the TSH receptor (a G_s protein-coupled receptor) on the basolateral membrane of the cell and this causes the endocytosis of the colloid.

7. The endocytosed vesicles fuse with the lysosomes of the follicular cell. The lysosomal enzymes cleave the T_4 from the iodinated thyroglobulin.

8. The thyroid hormones cross the follicular cell membrane towards the blood vessels by an unknown mechanism. [55] Text books have stated that diffusion is the main means of transport. [56] but recent studies indicate that monocarboxylate transporter (MCT) 8 and 10 play major roles in the efflux of the thyroid hormones from the thyroid cells.

Thyroglobulin (Tg) weighs 660 kDa, and is a dimeric protein produced by the follicular cells of the thyroid and used entirely within the thyroid gland.Thyroxine is produced by the attachment of iodine atoms to the ring structures of this protein's tyrosine residues; thyroxine (T_4) contains four iodine atoms, while triiodothyronine (T_3), otherwise identical to T_4, has one less iodine atom per molecule.The thyroglobulin protein accounts for nearly half of the protein content of the thyroid gland.Each thyroglobulin molecule contains about 100-120 tyrosine residues, a small number of which (<20) are iodinated catalysed by thyroperoxidase. [57]

The same enzyme then leads to the coupling of one modified tyrosine with another, via a free radical-mediated reaction, and when these iodinated bicyclic molecules are released by hydrolysis of the protein, T3 and T4 are formed. hence, each Tg protein molecule finally yields very small amounts of thyroid hormone (experimentally observed to be on the order of 5-6 molecules of either T4 or T3 per original molecule of Tg).The monoatomic, anionic form of iodine, iodide, is actively

absorbed from the bloodstream by a process called iodide trapping. During this process, sodium is cotransported with iodide from the basolateral side of the membrane into the cell, which is then concentrated in the thyroid follicles to about thirty times its concentration in the blood.Later, in the first reaction catalysed by the enzyme thyroperoxidase, tyrosine residues in the protein thyroglobulin undergo iodination on their phenol rings, at one or both of the positions *ortho* to the phenolic hydroxyl group, yielding monoiodotyrosine (MIT) and diiodotyrosine (DIT), respectively. This introduces 1-2 atoms of the element iodine, covalently bound, per tyrosine residue. Then further coupling reactions yield two fully iodinated tyrosine residues, also catalysed by thyroperoxidase, yields the peptidic (still peptide-bound) precursor of thyroxine, and coupling one molecule of MIT and one molecule of DIT yields the comparable precursor of triiodothyronin.

- peptidic MIT + peptidic DIT → peptidic triiodothyronine (eventually released as triiodothyronine, T_3)
- 2 peptidic DITs → peptidic thyroxine (eventually released as thyroxin, T_4)

Thyroxine is considered to be a prohormone and a reservoir for the most active and main thyroid hormone T_3. T_4 is converted as required in the tissues by iodothyronine deiodinase. Whenever there is deficiency of deiodinase it can mimic an iodine deficiency. T_3 is the more active form than T_4 and is the final form of the hormone, though it is present in less quantity than T_4.

Whenever there is a deficiency of dietary iodine, the thyroid will not be able to produce thyroid hormone. When there is a lack of thyroid hormone it will lead to decreased negative feedback on the pituitary, leading to increased production of thyroid-stimulating hormone, which causes the enlargement of the thyroid .

This results in increasing the thyroid's ability to trap more iodide, compensating for the iodine deficiency and allowing it to produce adequate amounts of thyroid hormone.

Diseases of the thyroid gland

Hyperthyroidism

Whenever there is overproduction of the thyroid hormone due to an overactive thyroid it is called hyperthyroidism, which is most commonly a result of Graves' disease, a toxic multinodular goitre, a solitary thyroid adenoma, and inflammation. Other frequent causes include drug-induced excess of iodine, particularly due to the use of amiodarone,which is an antiarrhythmic medication; high levels of iodine caused by the preferential uptake of iodine by the thyroid following iodinated contrast imaging, or from pituitary adenomas which may cause an overproduction of TSH. [58] Hyperthyroidism leads to the development of a variety of non-specific symptoms including weight loss, increased appetite, insomnia, decreased tolerance of heat, tremor, palpitations, anxiety and nervousness. Very often it can cause chest pain, diarrhoea, hair loss and muscle weakness. [59] Such symptoms can be managed temporarily with drugs such as beta blockers.

Long-term management of hyperthyroidism may consists of drugs that suppress thyroid function such as propylthiouracil, carbimazole and methimazole. [60] Radioactive iodine-131 can destroy the thyroid tissue. Radioactive iodine when used on the gland,is selectively taken up by the thyroid, which over time destroys the cells involved in its uptake. The choice of treatment evetually will depend on the individual and on the country where being treated. Surgical procedures to remove the thyroid can sometimes be performed as a transoral thyroidectomy, a minimally-invasive

procedure. However surgical procedures carry a risk of damage to the parathyroid glands and the nerves controlling the vocal cords. If the whole thyroid gland is removed, hypothyroidism will naturally result, and hormone therapy will be needed

Hypothyroidism

Whenever there is an underactive thyroid gland it results in hypothyroidism. Typical symptoms of hypothyroidism are abnormal weight gain, tiredness, constipation, heavy menstrual bleeding, baldness, cold intolerance, and a slow heart rate.Hypothyroidism may occur as a result of autoimmune disease such as Hashimoto's thyroiditis; iodine deficiency; due to medical treatments such as surgical removal or radioablation of the thyroid, amiodarone and lithium; due to congenital thyroid abnormalities; or from other diseases such as amyloidosis or sarcoidosis or because of transient inflammation of the thyroid. [61] There are some forms of hypothyroidism which can result in myxedema and severe cases can result in myxedema coma. [62]

Hypothyroidism is treated with the replacement of the hormone thyroxine. Thyroxine is usually given daily as an oral supplement, and may take a few weeks to become effective.Certain other causes of hypothyroidism, such as Postpartum thyroiditis and Subacute thyroiditis may be transient and pass over time and may be rectified with dietary supplementation.

Thyroid Nodules

Thyroid nodules are often found on the thyroid gland, with a prevalence rate of 4-7% [63] However most of the nodules do not cause any symptoms and are non-cancerous.Non-cancerous nodules of the thyroid include simple cysts, colloid nodules, and thyroid adenomas. Malignant nodules of the thyroid, which only occur in about

5% of nodules, include follicular, papillary, medullary carcinomas and metastases from other sites. [64] Thyroid nodules are more likely in females, those who are exposed to radiation, and in those who are iodine deficient.When a nodule is detected in the gland, thyroid function tests are performed and reveal whether a person has a normal about of thyroid hormones ("euthyroid") or an excess of hormones.

If the thyroid function tests are normal, an ultrasound can be used to investigate the nodule, and provide information such as whether the nodule is fluid-filled or a solid mass, and whether the appearance is suggestive of a benign or malignant cancer.A needle aspiration biopsy can be performed, and the sample sent for cytology, in which the appearance of cells is viewed to determine whether they resemble normal or cancerous cell.

There can sometimes be many nodules, which is termed a multinodular goiter, and this can sometimes be a toxic multinodular goiter

Blood tests that are used to test the function of the thyroid:

Test	Abbreviation	Normal ranges [65]
Serum thyrotropin/thyroid-stimulating hormone	TSH	0.27–4.20 μIU/ml
Free thyroxine	fT_4	0.93–1.7 ng/dl
Free triiodothyronine	fT_3	2.0 – 4.4 pg/ml
Radioactive iodine-123 uptake	RAIU	10–30%
Radioiodine scan (gamma camera)	N/A	N/A - thyroid contrasted images
Serum thyroxine	T_4	46–120 μg/l = 4.6–12.0 μg/dl

Thyroxine-binding globulin	TBG	12–20 ug/dl T4 +1.8 µg
TRH stimulation test	Peak TSH	9–30 µIU/ml at 20–30 min.
Serum thyroglobulin l	Tg	0-30 ng/m
Thyroid microsomal antibody titer	TMAb	Varies with method
Thyroglobulin antibody titer	TgAb	Varies with method

- µU/ml = mU/l, microunit per milliliter
- ng/dl, nanograms per deciliter
- µg, micrograms
- pg/d, picograms per day
- µIU/ml = mIU/l, micro-international unit per milliliter

Homocysteine metabolism and Hyperhomocysteinemia

Increased plasma homocysteine is an independent risk factor for atherosclerotic vascular disease as well as for Alzheimer's disease and fractures. Because homocysteine is formed as a result of *S*- adenosylmethionine-dependent methylation reactions, the major methyltransferases play an important role in determining the plasma level of this molecule; these consist of methyltransferases involved in the synthesis of phosphatidylcholine and creatine. The clearance of homocysteine is affected either by the remethylation pathway (which converts homocysteine back to methionine) or by the transsulfuration pathway, which produces cysteine. A number of B vitamins (especially folic acid, pyridoxal, and vitamin B12) are involved in homocysteine metabolism so that deficiencies of these vitamins are among the most common causes of hyperhomocysteinemia. Decreased renal function is also an important contributor to hyperhomocysteinemia.

Although the term homocysteine is used generically, plasma contains several different forms of this particular amino acid. Plasma Hcy is made up of free and protein-bound homocysteine. The free homocysteine encompasses homocysteine,homocystine, and cysteine–homocysteine-mixed disulfides [65] and also a protein bound fraction is linked to proteins by disulfide linkage, principally to cysteine 34 of albumin . Generally, in humans, the protein-bound fraction makes up the bulk of plasma Hcy accounting for >70%, homocystine and cysteine–homocysteine-mixed disulfides make up 5–15% each, while only trace amounts (≤1%) are found as free reduced homocysteine [66].

These different forms of homocysteine along with reduced, free oxidized, and protein-bound forms of cysteine and cysteinylglycine form a dynamic system [66] . A change in any one of these species leads to alterations in the thiol redox status. Despite the presence of these different forms, typically the parameter measured is "total plasma homocysteine," since assays for the separate forms are not yet satisfactory for routine clinical measurement. Hyperhomocysteinemia is classified as being moderate, intermediate, or severe with basal Hcy values of between 15 and 30, between 31 and 100, and greater than 100 nmol/mL, respectively [65] . Homocysteine is produced via demethylation of dietary methionine, which is abundant in animal protein [68,69] . The liver and kidney remove excess Hcy from the blood. People who have hyperhomocysteinemia get blood clots in their veins and arteries (e.g., deep vein thrombosis and pulmonary embolism). Hcy is an important determinant of the methylation cycle. Homocysteine is metabolized either by remethylation pathway to methionine or the transsulfuration pathway to cysteine. The combination of methionine with adenosine triphosphate (ATP), leads to the formation of s-adenosylmethionine (SAM), a principle methyl donor for all methylation reactions in

cells [5,6]. The demethylation leads to the formation of s-adenosylhomocysteine (SAH) which further leads to the formation of Hcy and adenosine. The first pathway of Hcy metabolism is dependent on the proper functioning of methylene tetrahydrofolate reductase (MTHFR) enzyme, methionine synthetase, Vitamin B12, and folic acid. The later pathway is dependent on the enzymes cystathionine beta-synthetase and MTHFR [70] .Homocysteine elevation occurs because of poor diet (absence of essential food components). If a person consumes high protein rich diet daily, i.e., meat, egg, chicken, milk, etc., he should also incorporate certain vital amines in his diet. It is the absence of these vital amines which causes hyperhomocysteinemia [71] .Smoking is associated with vascular disease and many complicationsrelated to Hhcy. Nicotine has a direct effect on the methylation and catabolises folate cycle. Physical activity plays an important role in lifesince it is a way of strengthening our health and reduces therisk of cardiovascular diseases (CVD). Physical inactivity increases the concentrations of total plasma Hcy, and thus increases the probability of developing CVD in healthy and already sick people.

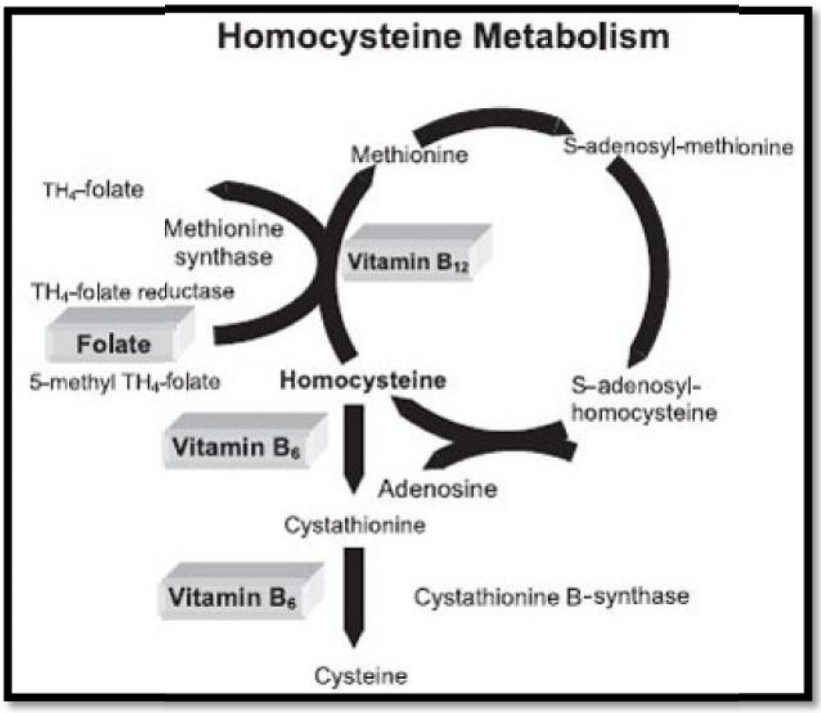
Vitamin B6, B12, and folic acid are required for the enzymes involved in Hcy metabolism; high protein intake seems to lower Hcy levels. Strict vegetarians are at higher risk for Hhcy due to low plasma B12 levels. Coffee consumption (4 cups/day) is linked to mild elevations in Hcy, although this effect can be countered by supplementing with 200 mg/day of folic acid. Patients having problems such as dyslipidemia, diabetes, renal insufficiency, Alzheimer disease, and hypertension should be tested for Hhcy. Hcy is assayed by chromatographic method, immunoassay method, enzyme cycling method, capillary electrophoresis, and chemosensors. Blood vessels abnormalities, atherosclerosis, thrombosis, and bone loss are common symptoms of Hhcy [72,73].

Chronic consumption of alcohol interferes in the metabolism of folic acid and cyanocobalamin. It is associated with gastrointestinal disturbances, which cause a decreased absorption of vitamins and folic acid, thus contributing to elevated Hcy levels. It also inhibits methionine synthase and decreases hepatic uptake and increase excretion via urine.

Some drugs such as cholestyramine and metformin prevent vitamin absorption from the gut. Methotrexate, nicotinic acid, and fibric acid derivatives interfere with the metabolism of folate and Hcy . Oxcarbazepine and topiramate can cause Hhcy because of their capacity to activate hepatic enzymes. Patients with renal failure have extremely high Hcy levels due to less efficient renal clearance of Hcy. Patients with kidney disease have higher rates of cardiovascular morbidity and death . Hcy levels increase as renal function deteriorates. The exact cause of Hhcy in renal disease is not yet understood, although reduced plasma Hcy clearance is the most proximate cause [74,75].

The metabolism of homocysteine involves four steps. The first step is transmethylation pathway which includes the conversion of methionine to Hcy [76] . This pathway results in the formation of SAM, which transfers a methyl group to a number of several methyl acceptor molecules (proteins, DNA, neurotransmitters) and forms adenosylhomocysteine, which is subsequently converted to Hcy. The second step in metabolism is the transsulfuration pathway that involves the irreversible conversion of Hcy to cysteine in the presence of cystathione-β-synthase and Vitamin B6 as an essential cofactor. The third step of metabolism is the re-methylation pathway in which regeneration of methionine occurs from Hcy by methionine synthase along with MTHF – 5 Methyltetrahydrofolate and Vitamin B12 as essential

cofactors. The last step of Hcy metabolism is the regeneration of methylenetetrahydrofolate (MTHF) from THF, which is catabolized by enzyme 5, 10-methelenetetrahydrofolate reductase [77,78] .



[78]

Figure 3 : Homocysteine metabolism

The relationship between Hhcy and atherosclerosis was proposed by McCully in 1969. It is now widely accepted that Hhcy is a strong, independent risk factor for stroke, myocardial infarction, and other vascular events [79] . Hcy is an unstable amino acid, which undergoes auto oxidation and produces free oxygen radicals which further increases oxidative stress. It contributes to atherosclerosis in two mechanisms. The first one includes free oxygen radicals converts low-density lipoproteins of sub endothelial tissues to oxidized low-density lipoproteins (LDL). Oxidized LDL further acts as an important mediator of the inflammatory process in atherosclerosis. Oxidized LDL induces the release of vascular cell adhesion molecule and monocyte chemo attractant protein [80] . The monocytes then get converted to macrophages,

which take up oxidized LDL and get converted to foam cells. The foam cells get accumulated below the endothelium to form fatty streak. The latter part includes the suppression of nitric oxide activation by oxygen free radicals, which results in endothelial dysfunction and contributed to atherosclerosis [81] .

Hypothyroidism and effect of homocytsteine levels and lipid profile

The thyroid hormones affect the metabolism of folic acid,affect riboflavin metabolism,, mainly by the stimulation of flavokinase and thereby the synthesis of flavin mononucleotide and flavin adenine dinucleotide (FAD). FAD- dependent methylene tetrahydrofolate reductase is reduced in hypothyroidism which leads to hyperhomocysteinemia. (10) The decreased levels of the hepatic enzymes involved in the remethylation pathway of homocysteine and concurrent changes in the renal function in hypothyroidism can lead to hyperhomocysteinemia. (9) The increased cardiovascular morbidity in hypothyroid patients has been attributed to elevated low density lipoprotein cholesterol,increased peripheral resistance and mostly diastolic hypertension,reduced myocardial contractility,recued stroke volume and bradycardia. (11)

Thyroid hormones have an important effect on the lipid profile. (12) .The thyroid hormones cause induction of 3-hydroxy-3 methylyglutaryl Coenzyme A (HMG-COA) reductase,which is the first step in cholesterol biosynthesis.Also, T3(Triiodothyronine) causes upregulation of the LDL receptors by modulating the LDL receptor gene activation.This action of T3 resulting in gene activation is the result of the direct binding of T3 to specific Thyroid Hormone Responsive Elements(TREs).(13) Also,it has been shown that T3 has an effect on the sterol regulatory-element bindng protein-2 (SREBP-2) ,which in turn regulates the LDL

receptors gene expression. [14] .Also, T3 has been shown to have a protective effect on LDL from oxidation. [15]

The thyroid hormones have an important influence on the serum HDL levels and its metabolism by increasing the levels of cholesteryl ester transfer protein (CETP) activity, which exchanges cholesteryl esters from HDL to the Very Low Density Lipoproteins (VLDL) and TGs to the opposite direction. [16] Also thyroid hormones cause stimulation of the Lipoprotein Lipase (LPL), which catabolises the TG-rich lipoproteins, and also the Hepatic Lipase (HL), which hydrolyzes HDL_2 to HDL_3 and also causes the conversion of the intermediate density lipoproteins (IDL) to LDL and in turn the LDL to small dense LDL(sdLDL). [17,18] T3 has also shown to cause upregulation of the Apolipoprotein AV (ApoAV), which plays an important role in TG regulation. [19] Increased levels of ApoAV has been shown to correlate with a redcued level of TGs. [20] One of the mechanisms which have been proposed for this a recution of hepatic VLDL-TG production and also an increase in the plasma LPL levels and activity, which cause an increase in the lipoprotein remnant generation due to increased LPL-mediated lipolysis of VLDL-TG. [21]

METHODOLOGY

The present study was conducted in the Department of Medicine, KLES Dr. Prabhakar Kore Hospital and Medical Research Centre, Belagavi from January 2015 to December 2015.

Study design and duration

The study design was a one year case control study.

Study period

The present study was carried out from January 2015 to December 2015.

Source of Data

The study recruited newly detected hypothyroid patients and matched controls, from both the outpatients and inpatients of KLE Dr.Prabhakar Kore Hospital,Belagavi over a one year period.

Sample size

A total of 30 newly detected hypothyroid patients and 30 matched controls were included in the study.

Sampling procedure

The sample size is calculated by the formula

$$\frac{2(Z_{\alpha} + Z_{\beta})^2 (S_1 + S_2)^2}{(x_1 - x_2)^2}$$

Where based on the review article, $Z_{\alpha} = 1.96$, $Z_{\beta} = 0.84$, $S_1 = 5.5$, $S_2 = 3.03$,

Effect size $(x_1 - x_2)^2 = 5$, which on calculation gives a sample size of approximately 30 cases and 30 controls.

Selection criteria:

Inclusion Criteria:

- This study will include the case group include patients who are consenting for investigations and patients with a TSH of >4.78mU /L
- The control group will include euthyroid patients , with a TSH level <4.78mU/L

Exclusion Criteria:

- Cases not consenting for the study
- Patients with hemorrhagic, ischemic strokes, cardio embolic strokes, renal failure
- Patients with past history of coronary events or any cardiac interventions
- Patients with a History of DVT
- Patients with a history of hypertension or Type 2 Diabetes Mellitus

Cases and Controls were matched on basis of age, sex and body mass index.

Ethical clearance

Prior to the beginning the study was approved by the Institutional Ethics Committee, Jawaharlal Nehru Medical College, Belagavi.

Informed consent

The patients who fulfilled the selection criteria were informed about the nature of study in detail and a written informed consent was obtained (Annexure→I).

Data collection

After initial clinical detection of new hypothyroid patients at the KLES Dr Prabhakar Kore Charitable Hospital & Medical Research Center, Belgaum and written informed consent taken, the patient was subjected to clinical and blood investigations.Similar method was employed in the control group after matching with

the case group. These findings were noted on a predesigned and pretested proforma (Annexure-II).

Investigations

- Lipid profile- total cholesterol, Low density lipoprotein (LDL), High density lipoprotein (HDL), Triglycerides (TG)
- Thyroid profile- Thyroid stimulating hormone (TSH), free T3 and free T4 levels
- Fasting serum homocysteine levels
- Random blood sugar

Statistical methods

The data obtained was coded and entered into the Microsoft Excel Spreadsheet (Annexure III). The categorical data was expressed in terms of rates, ratios and percentages and continuous data was expressed as mean ± standard deviation.

RESULTS

This one year hospital based case control study was done at Department of Medicine and Endocrinology, KLES Dr. Prabhakar Kore Hospital and Medical Research Centre, Belagavi.

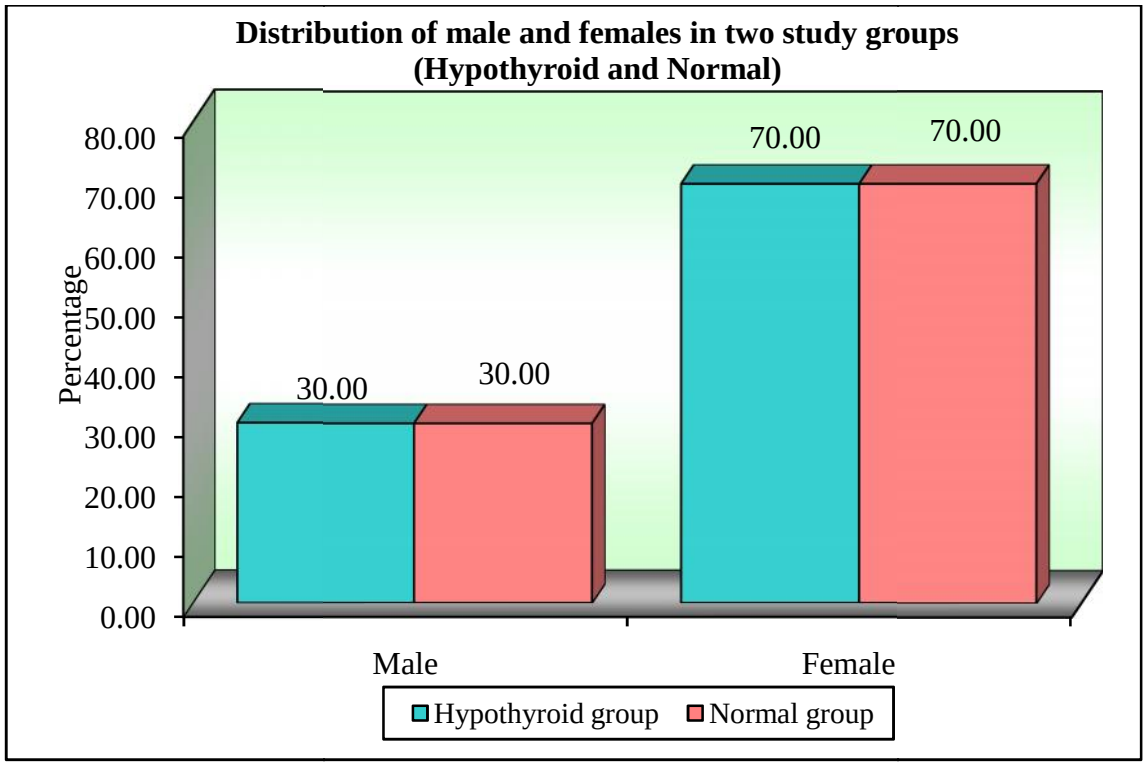
The study recruited 30 newly detected hypothyroid patients and 30 euthyroid patient at the KLES Dr Prabhakar Kore Charitable Hospital & Medical Research Center, Belagavi

The data obtained was coded and entered into the Microsoft Excel Spreadsheet (Annexure III). The data was analysed and the final results and observations were tabulated as follows:

Table 1: Distribution of male and females in two study groups (Hypothyroid and Normal)

Gender	Hypothyroid group	%	Normal group	%	Total	%
Male	9	30.00	9	30.00	18	30.00
Female	21	70.00	21	70.00	42	70.00
Total	30	100.00	30	100.00	60	100.00
Chi-square=0.0000 P = 1.0000						

Graph 1 : Distribution of males females in the two study groups

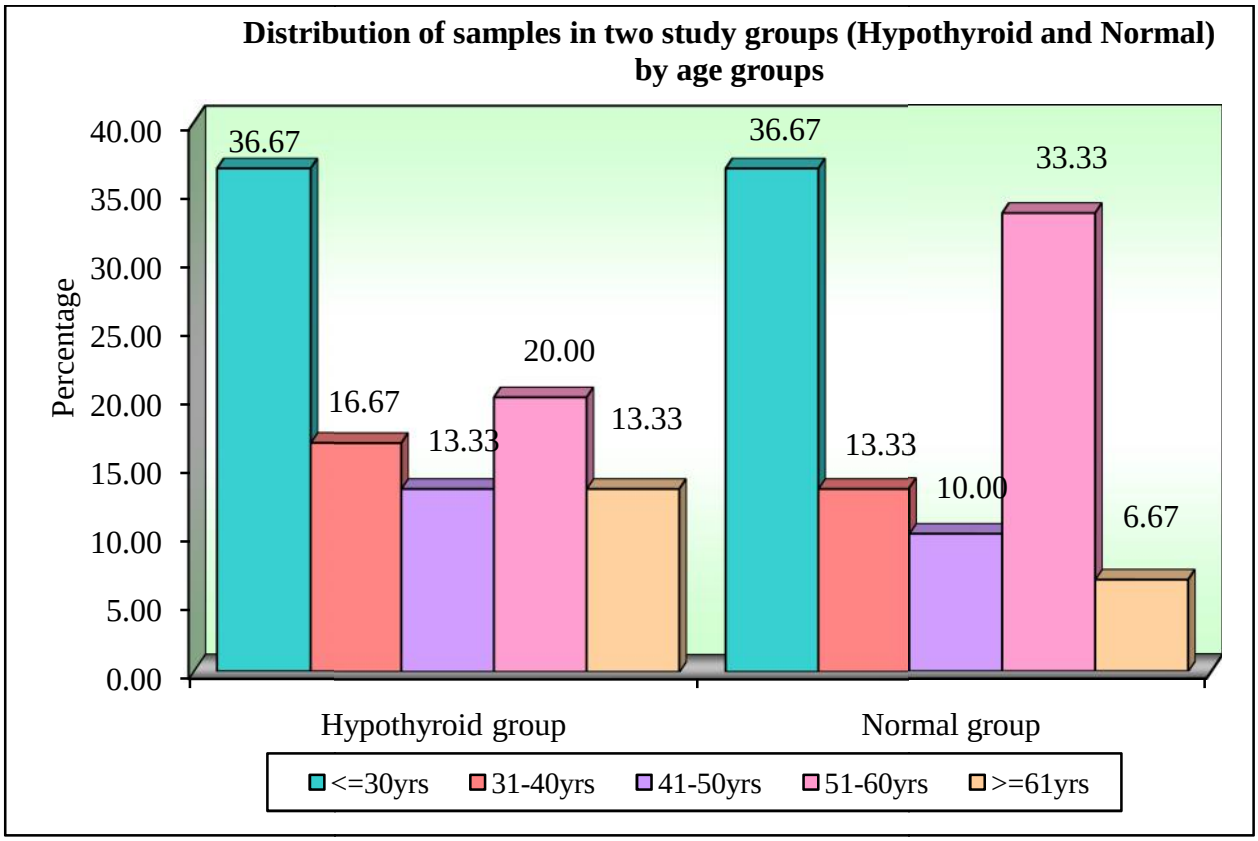


In the study the majority of the patients were females- 42 (70%) with a male:female ratio of 3:7.

Table 2: Distribution of samples in two study groups (Hypothyroid and Normal) by age groups

Age groups	Hypothyroid group	%	Normal group	%	Total	%
<=30yrs	11	36.67	11	36.67	22	36.67
31-40yrs	5	16.67	4	13.33	9	15.00
41-50yrs	4	13.33	3	10.00	7	11.67
51-60yrs	6	20.00	10	33.33	16	26.67
>=61yrs	4	13.33	2	6.67	6	10.00
Chi-square=1.9231 P = 0.7502						
Total	30	100.00	30	100.00	60	100.00
Mean age	40.60		41.40		41.00	
SD age	15.58		14.90		15.12	

Graph 2 : Disribution of samples in the two study groups by age group



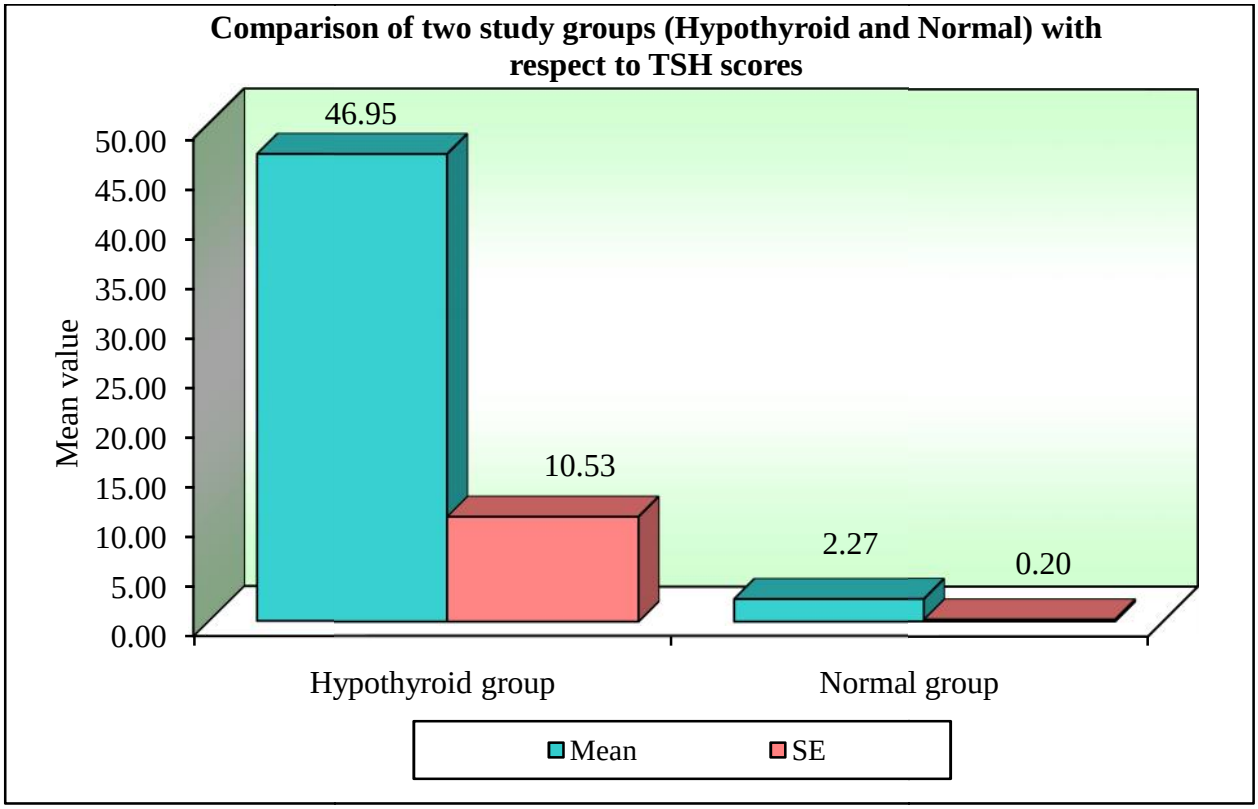
In the study , the mean age of patients in the hypothyroid group was 40.60 years, and the mean age of patients in the normal group was 41.40 years with a p value of 0.7502, with a study mean age of 41 years.

Table 3: Comparison of two study groups (Hypothyroid and Normal) with respect to TSH scores by t test (in mcIU/ml)

Groups	n	Mean (mcIU/ml)	SD	SE	t-value	P-value
Hypothyroid group	30	46.95	57.67	10.53	4.2431	0.0001*
Normal group	30	2.27	1.08	0.20		

*p<0.05

Graph 3 : Comparison of the two study groups with respect to TSH levels

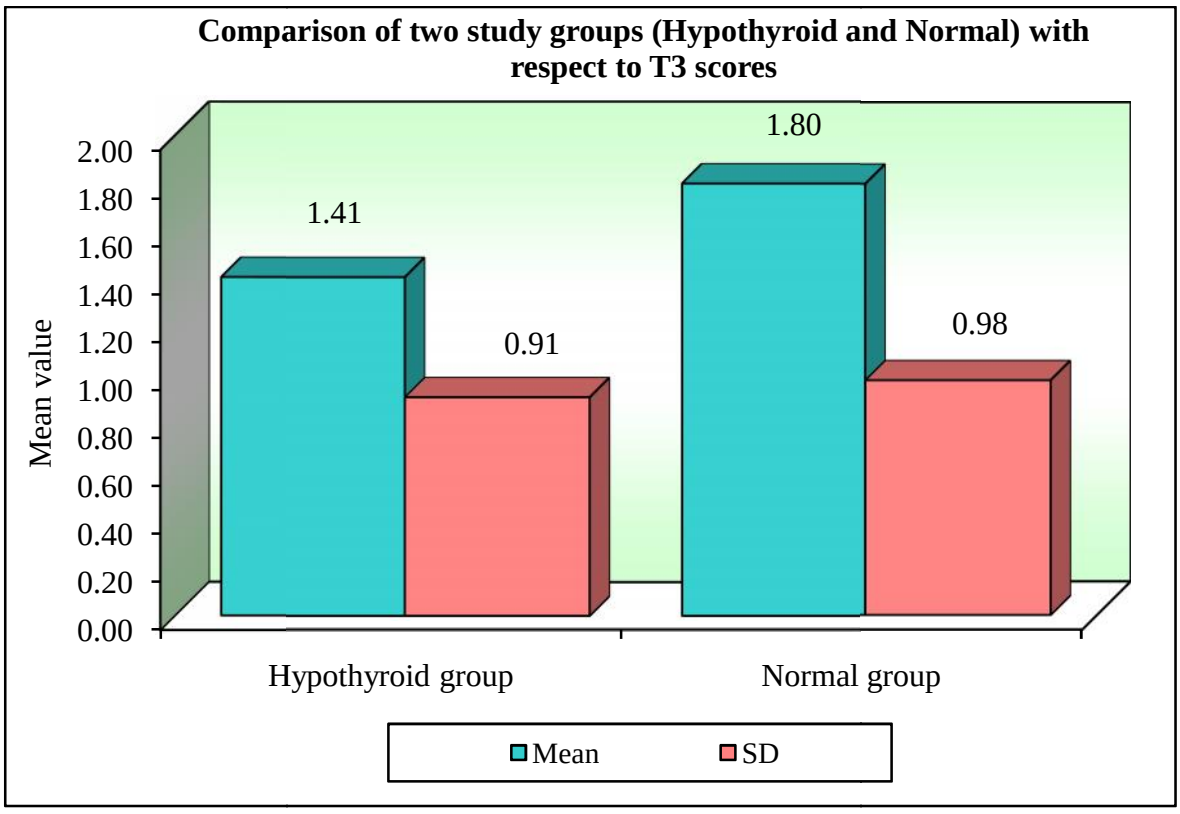


The mean TSH levels in the hypothyroid group was 46.95 mcIU/ml , and that in the normal group was 2.27 mcIU/ml , with a p value of 0.0001

Table 4: Comparison of two study groups (Hypothyroid and Normal) with respect to T3 scores by t test

Groups	n	Mean(mcg/dl)	SD	SE	t-value	P-value
Hypothyroid group	30	1.41	0.91	0.17	-1.6012	0.1148
Normal group	30	1.80	0.98	0.18		

Graph 4 : Comparison of the two study groups with respect to free T3 levels

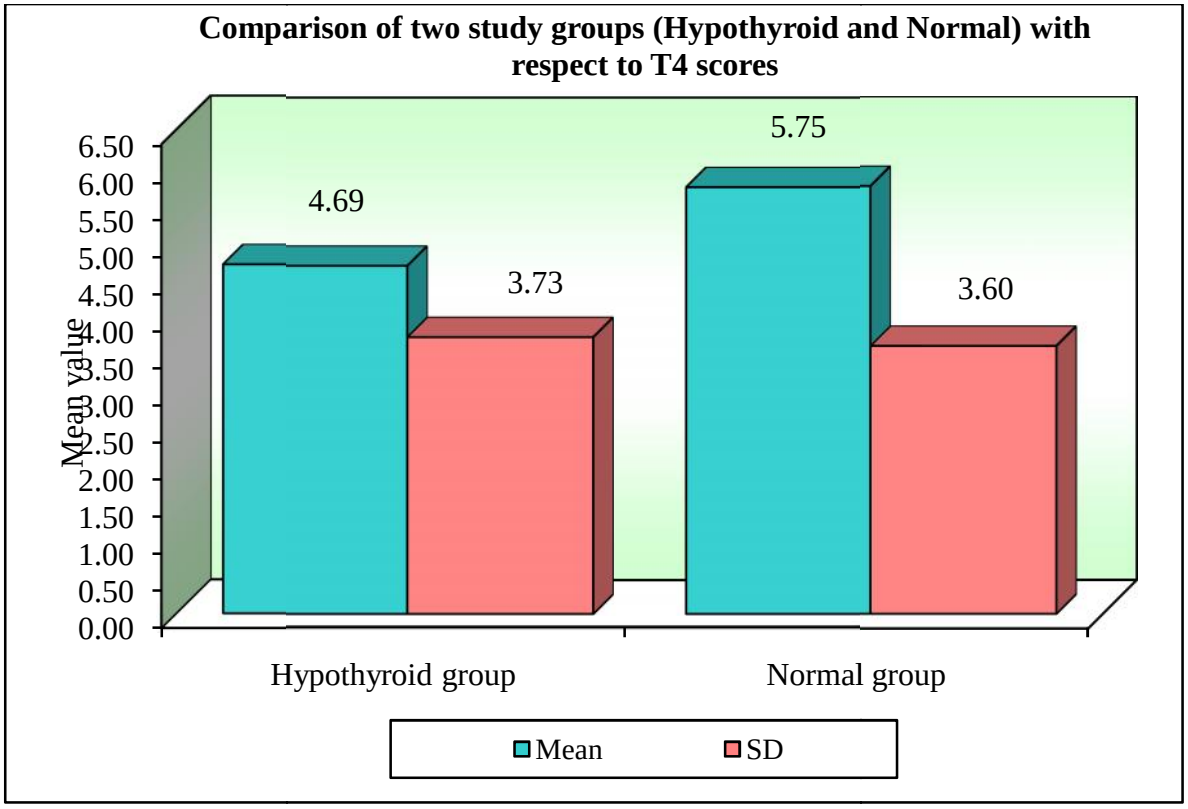


The mean Free T3 levels in the hypothyroid group was 1.41 µg/l , and that in the normal group was 1.80 µg/l, which was not statistically significant (p value 0.1148)

Table 5 : Comparison of two study groups (Hypothyroid and Normal) with respect to T4 scores by t test

Groups	n	Mean	SD	SE	t-value	P-value
Hypothyroid group	30	4.69	3.73	0.68	-1.1191	0.2677
Normal group	30	5.75	3.60	0.66		

Graph 5 : Comparison of the two study groups with respect to free T4 levels



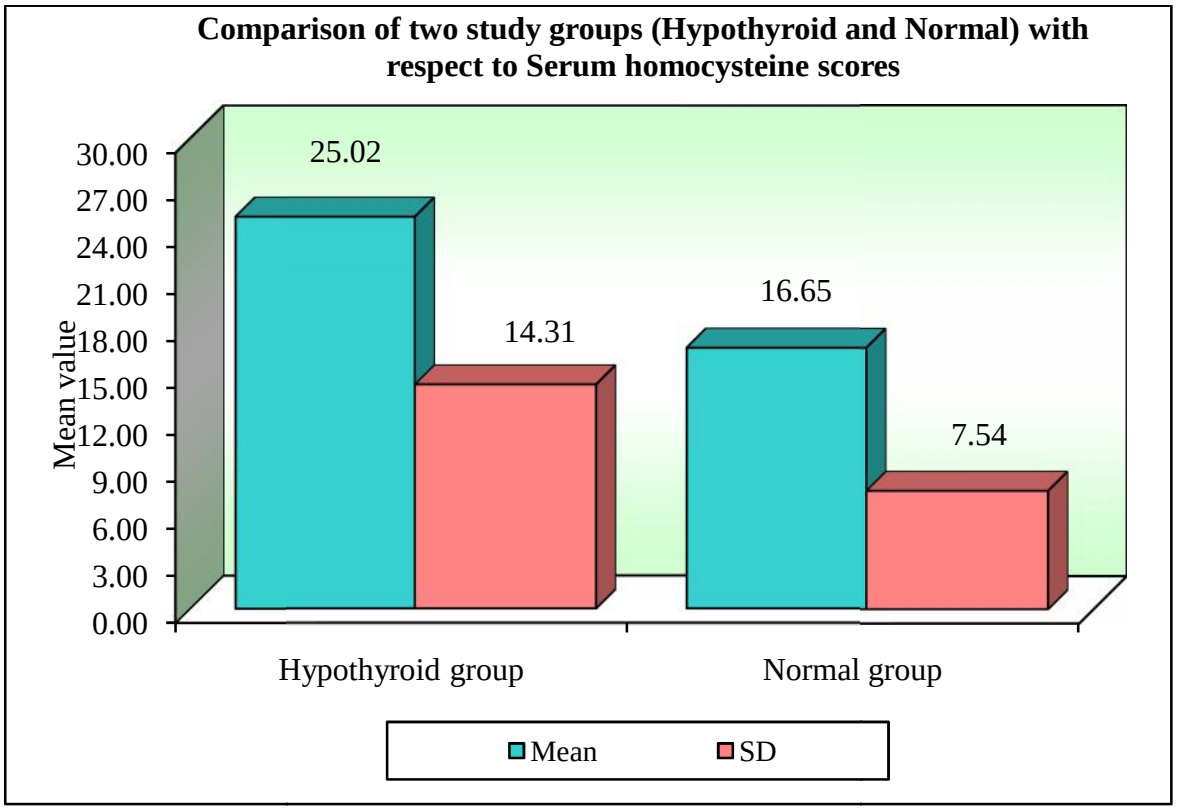
The mean FT4 levels in the hypothyroid group was 4.69 ng/l , and that in the normal group was 5.75 ng/l , which was not statistically significant (p value 0.2677)

Table 6: Comparison of two study groups (Hypothyroid and Normal) with respect to Serum homocysteine scores by t test

Groups	n	Mean(mcmol/L)	SD	SE	t-value	P-value
Hypothyroid group	30	25.02	14.31	2.61	2.8316	0.0064*
Normal group	30	16.65	7.54	1.38		

*p<0.05

Graph 6: Comparison of the two study groups with respect to serum homocysteine levels



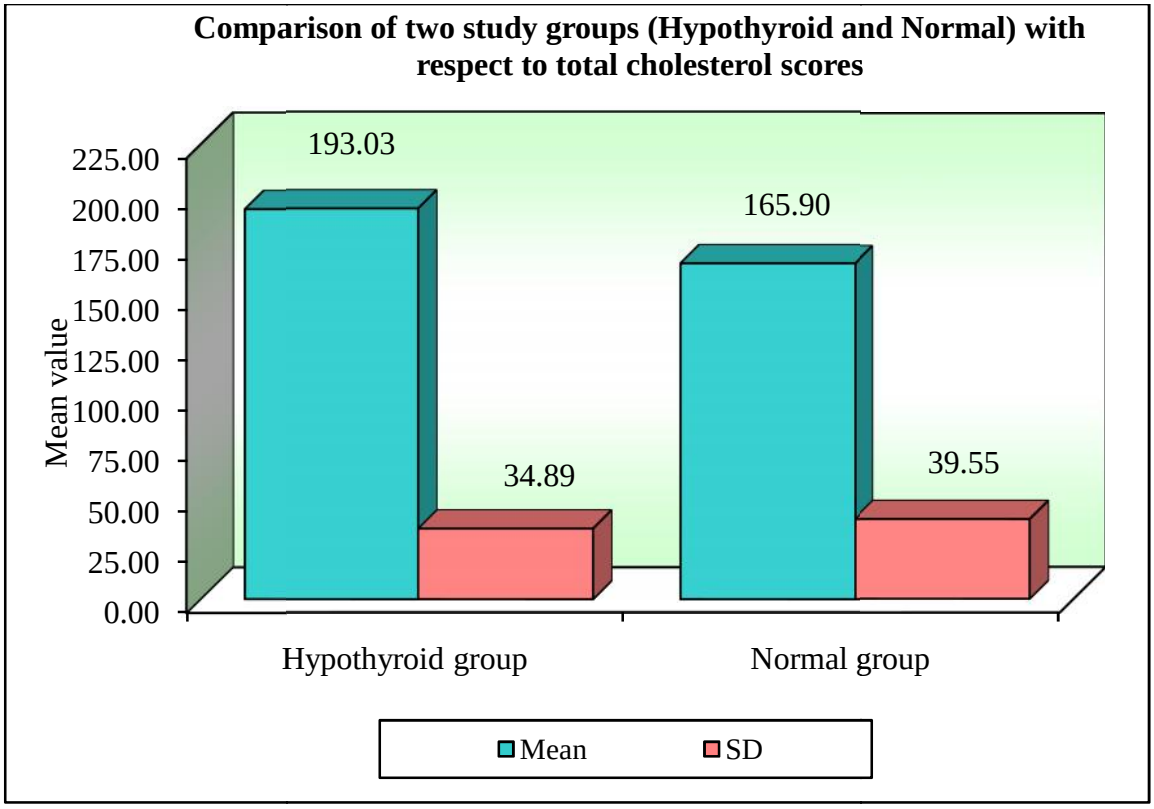
The mean serum homocysteine levels in the hypothyroid group was 25.02 mcmol/L , and that in the normal group was 16.65 mcmol/L, which was statistically significant (p value 0.006

Table 7: Comparison of two study groups (Hypothyroid and Normal) with respect to total cholesterol scores by t test

Groups	n	Mean(mg/dl)	SD	SE	t-value	P-value
Hypothyroid group	30	193.03	34.89	6.37	2.8177	0.0066*
Normal group	30	165.90	39.55	7.22		

*p<0.05

Graph 7: Comparison of the two groups with respect to serum cholesterol levels

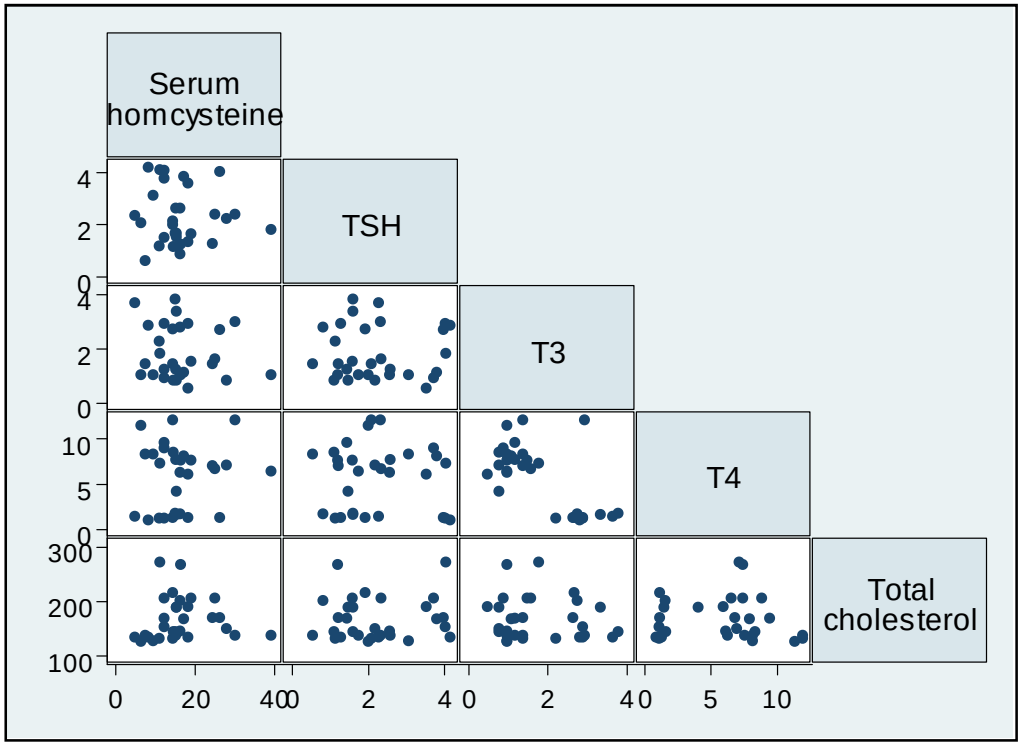


The mean cholesterol levels in the hypothyroid population was 193.03mg/dl , and that in the normal group was 165.90 mg/dl, which was statistically significant (p value 0.0066)

Table 8: Correlation between serum homocysteine with TSH, T3, T4 and total cholesterol scores in Hypothyroid samples by Karl Pearson's correlation method

Variables	Correlation between serum homocysteine with		
	r-value	t-value	p-value
TSH	-0.0595	-0.3154	0.7548
T3	-0.1284	-0.6852	0.4989
T4	0.1111	0.5913	0.5590
Total cholesterol	0.0395	0.2090	0.8360

Graph 8 : Correlation between serum homocysteine with TSH, T3, T4 and total cholesterol scores in Hypothyroid samples by Karl Pearson's correlation method

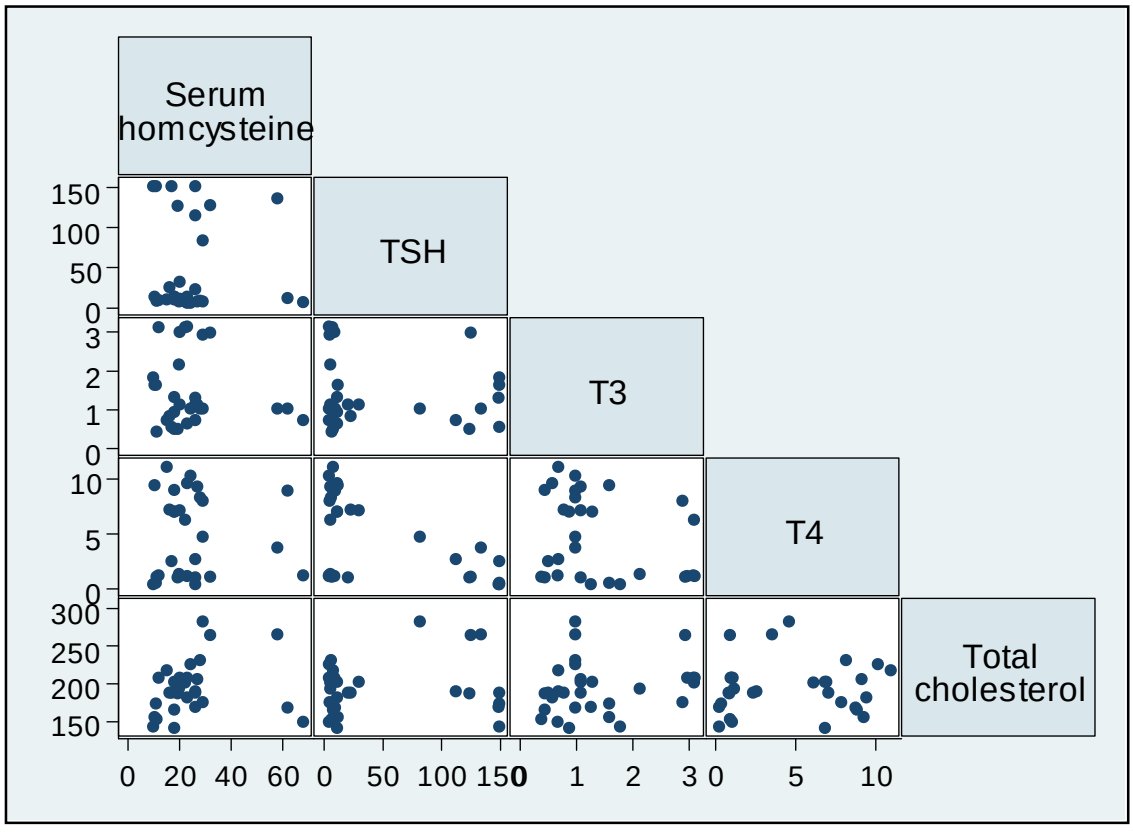


On correlating the serum homocysteine levels in the hypothyyroid group with TSH, fT3 and fT4 and serum cholesterol levels, there was no statistically significant correlation(p values of 0.7548,0.4989,0.5590 and 0.8360 respectively)

Table 9: Correlation between serum homocysteine with TSH, T3, T4 and total cholesterol scores in Normal samples by Karl Pearson's correlation method

Variables	Correlation between serum homocysteine with		
	r-value	t-value	p-value
TSH	-0.0154	-0.0816	0.9356
T3	-0.1250	-0.6665	0.5106
T4	0.0137	0.0723	0.9429
Total cholesterol	0.1841	0.9910	0.3302

Graph 9: Correlation between serum homocysteine with TSH, T3, T4 and total cholesterol scores in Normal samples by Karl Pearson's correlation method

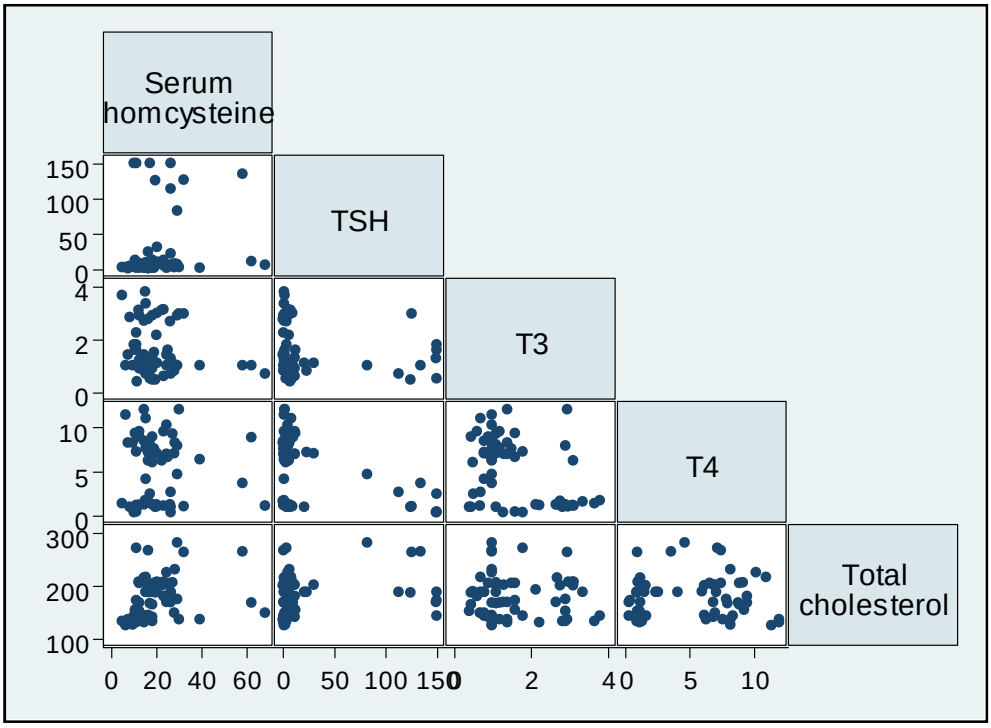


On correlating the serum homocysteine levels in the normal group with TSH, fT3 and fT4 and serum cholesterol levels, there was no statistically significant correlation(p values of 0.9356, 0.5106 and 0.9429 respectively)

Table 10: Correlation between serum homocysteine with TSH, fT3, fT4 and total cholesterol scores in total samples by Karl Pearson's correlation method

Variables	Correlation between serum homocysteine with		
	r-value	t-value	p-value
TSH	0.1580	1.2188	0.2278
T3	-0.1810	-1.4013	0.1665
T4	-0.0092	-0.0704	0.9441
Total cholesterol	0.2278	1.7814	0.0801

Graph 10: Correlation between serum homocysteine with TSH, fT3, fT4 and total cholesterol scores in total samples by Karl Pearson's correlation method



On correlating the serum homocysteine levels with TSH , fT3 and fT3 and cholesterol levels in the total sample, the correlation was not statistically significant (p values of 0.2278,0.1665,0.9441 and 0.0801 respectively)

DISCUSSION

- Thyroid gland is critical for the regulation and proper functioning of various physiological pathways and processes in the human body

- The increased cardiovascular morbidity in patients who are hypothyroid has been generally related to higher levels of cholesterol and low-density lipoprotein cholesterol (LDL-C). Lipid abnormalities in hypothyroid patients do not fully account for the cardiovascular risk.

- Certain prospective studies have shown a strong association between cardiovascular morbidity and hyperhomocysteinemia, and total homocysteine is recognised as an independent risk factor for cardiovascular disease. Homocysteine induces oxidative stress, endothelial injury and smooth muscle hypertrophy.

- The objectives are to study the incidence of hyperhomocysteinemia in patients with hypothyroidism, and the relation of homocysteine and cholesterol levels to thyroid hormones free T3, free T4 and TSH in newly detected hypothyroid patients.

In Saleh A Bamashmoos et al, thirty recently diagnosed overt hypothyroid patients (f=27, m=3) and twenty normal volunteers control (f=18, m=2) were compared . There were a significant increase of homocysteine, TSH, cholesterol and creatinine levels by 113%, 12 times, 58% and 54%, respectively, and a decrease of fT3 and fT4 levels by 56.4% and 49.6% , respectively, in hypothyroid as compared to control group. For tHcy (Mean, 24.45 μmol/l vs 11.48 μmol/l, respectively; $P < 0.001$). tHcy was significantly positively correlated with TSH, age and creatinine and

had a negative correlation with free thyroxine (fT4).There was no significant correlation with fT3 and cholesterol. The study concluded the observation of elevated serum homocysteine, cholesterol and creatinine levels in overt hypothyroidism and the presence of an inverse relationship between tHcy with fT4 and a also positive relation with serum TSH. [82]

C V Rizos et al showed that hypothyroidism has an adverse effect on the serum lipid profile, and regular screening of the lipid profile is warranted in all patients with hypothyroidism.

Turhan S et al studied fifty-three subclinical hypothyroid patients (serum thyrotropin [TSH] concentrations >4.12 mU/L) against a group of 50 euthyroid subjects whose sex,age and body mass indices were matched. TSH concentration was correlated with plasma homocysteine concentration (tHcy), low-density lipoprotein cholesterol (LDL), very low-density lipoprotein cholesterol (VLDL), high-density lipoprotein cholesterol (HDL), total cholesterol (TC) and triglycerides (TG). There was a significant statistical difference between the patient and control groups for normal free T4 (1.02 vs. 0.86, P<.001), TSH (1.64 vs. 6.62, P<.001), TC (185 vs. 206 , P=.01), TG (103 vs. 132, P=.04), LDL-C (114 vs. 127, P=.04), and TC/HDL-C (3.81 vs. 4.19, P=.04), respectively.

However there was no statistically significant difference between the two groups for HDL, VLDL, LDL/HDL, and homocysteine. Serum TSH was significantly correlated with plasma tHcy (P=.001), TC (P=.001), LDL-C (P=.001), TC/HDL-C (P=.002) and LDL-C/HDL-C (P=.004) across all participants.

Bjørn G. Nedrebø et al did a longitudinal study on tHcy in hyper- and hypothyroid patients through treatment for their thyroid status . Forty patients with hyperthyroidism and 12 detected with hypothyroidism were evaluated ,and showed that tHcy and total cholesterol were high in hypothyroidism and tends to be low in hyperthyroidism. [83]

- The current study was a case control study done from January 2015 to December 2015 at the KLES Dr Prabhakar Kore Charitable Hospital & Medical Research Center, Belgaum. A total of 30 newly detected hypothyroid patients and 30 euthyroid patients were included in the study. Patients with hemorrhagic, ischemic strokes, cardio embolic strokes, renal failure, past history of coronary events or any cardiac intervention, DVT, hypertension and Type 2 Diabetes Mellitus were excluded from the study. Cases and Controls were matched on basis of age, sex and body mass index.

Age and Sex Distribution

In the study, the mean age group of all the 60 recruits(case and control) was 41 years, with a standard deviation of 15.12.The mean age of the hypothyroid group was 40.60 years, and that of the control group was 41.40 years.Majority of the patients in the total study group was less than or equal to 30 years (n=22, 36.67%) followed by 51-60 years (n=16, 26.67%).The least number of recruits were in the age group ≥61 years (n=6, 10%).

Majority of the patients were females (n=42, 70%) as compared to males (n=18, 30) , with equal distribution of the respective sexes among the case and control groups,accounting for a final study population of 60,which was in accordance with

Bjørn G. Nedrebø et al (females 75%, males 25%) and with Saleh A Bamashmoos et al (females 90%,males 10%).

In comparison,in Bjørn G. Nedrebø et al, the median age of hypothyroid patients was 55 years,which was a higher median age.The study was comparable to Saleh A Bamashmoos et al,which had a median age of 37 years.

Comparison of the case and control group with respect to TSH levels

In the study, on comparing the mean TSH levels in the hypothyroid and the normal subjects, The mean TSH levels in the hypothyroid group was 46.95 mcIU/ml , and that in the normal group was 2.27 mcIU/ml , which was a significant difference with a p value of 0.0001. This was in accordance with Saleh A Bamashmoos et al, where there was a significant difference in the TSH levels between case and control groups(p value 0.001).

Comparison of the case and control groups with respect to Free T3 levels

On comparing the two groups with respect to Free T3 levels, The mean Free T3 levels in the hypothyroid group was 1.41 µg/l , and that in the normal group was 1.80 µg/l, which was not statistically significant (p value 0.1148). In contrast, in the study by Saleh A Bamashmoos et al, the mean fT3 in the hypothyroid group was 1.46 µg/l, and in the normal group was 3.35 µg/l ,which was a statistically significant difference (p value 0.001).

Comparison of the case and control groups with respect to Free T4 levels

On comparing the two groups with respect to free T4 levels, the mean FT4 levels in the hypothyroid group was 4.69 ng/l , and that in the normal group was 5.75 ng/l , which was not statistically significant (p value 0.2677). In contrast, in the study

by Saleh A Bamashmoos et al, the fT4 in case and control groups were 0.69 ng/l and 1.39 ng/l, which was statistically significant (p value 0.001).

Comparison of the case and control groups with respect to Serum Homocysteine levels

On comparing the two groups with respect to serum homocysteine levels, the mean homocysteine levels in the hypothyroid group was 25.02± 14.31mcmol/L, and that in the normal group was 16.65± 7.54 mcmol/L, which was statistically significant (p value 0.0064). This was in accordance with both the studies by Bjørn G. Nedrebø et al and Saleh A Bamashmoos et al.

Increased tHcy levels might be the result of two mechanisms either increased tHcy formation or decreased renal tHcy clearance due to direct effect of thyroid hormones on the tHcy metabolism in the liver and clearance in the kidney [84]. The former may be explained as thyroid hormone deficiency decreases hepatic levels of enzymes involved in the remethylation pathway of tHcy to methionine, methylenetetrahydrofolate reductase (MTHFR). Experimental studies have also indicated that MTHFR was decreased in hypothyroidism and increased in hyperthyroidism .[71]

Comparison of the case and control groups with respect to serum cholesterol levels

On comparing the two groups with respect to mean cholesterol levels ,the cholesterol levels in the hypothyroid population was 193.03 mg/dl , and that in the normal group was 165.90 mg/dl, which was statistically significant (p value 0.0066). This was in accordance with both the studies by Bjørn G. Nedrebø et al and Saleh A Bamashmoos et al.This can be explained by the fact that thyroid hormones cause

induction of 3-hydroxy-3 methylyglutaryl Coenzyme A (HMG-COA) reductase, which is the first step in cholesterol biosynthesis. Also, T3(Triiodothyronine) causes upregulation of the LDL receptors by modulating the LDL receptor gene activation. This action of T3 resulting in gene activation is the result of the direct binding of T3 to specific Thyroid Hormone Responsive Elements(TREs). [13] Also,it has been shown that T3 has an effect on the sterol regulatory-element bindng protein-2 (SREBP-2) ,which in turn regulates the LDL receptors gene expression. [14] .Also, T3 has been shown to have a protective effect on LDL from oxidation. [15]

Correlation between serum homocysteine with TSH, T3, T4 and total cholesterol scores in Hypothyroid samples by Karl Pearson's correlation method

On correlating the serum homocysteine levels with the TSH, free T3, free T4 and total cholesterol levels in the case (hypothyroid) group, the r values,t values and p values obtained were (-0.0595, -0.3154, 0.7548), (-0.1284, -0.6852, 0.4989), (0.1111, 0.5913, 0.5590), (0.0395, 0.2090, 0.8360) respectively, which was not statistically significant.This was in contrast to Saleh A Bamashmoos et al, where serum homocysteine levels had a significant correlation with serum TSH and free T4, but not with free T3 and cholesterol levels.

Correlation between serum homocysteine with TSH, T3, T4 and total cholesterol scores in Normal samples by Karl Pearson's correlation method

On correlating the serum homocysteine levels with the serum TSH, free T3, free T4 and cholesterol levels in the control group (euthyroid) , the r values, t values and p values obtained were (-0.0154, -0.0816, 0.9356), (-0.1250, -0.6665, 0.5106), (0.0137, 0.0723, 0.9429), (0.1841, 0.9910, 0.3302) respectively, none of which were statistically significant.

Correlation between serum homocysteine with TSH, fT3, fT4 and total cholesterol scores in total samples by Karl Pearson's correlation method

On correlating the serum homocysteine levels with the TSH, free T3, free T4 and total cholesterol levels in the total sample size (case plus control groups) , the r values, t values and p values obtained were (0.1580, 1.2188, 0.2278), (-0.1810, -1.4013, 0.1665), (-0.0092, -0.0704, 0.9441), (0.2278, 1.7814, 0.0801), none of which were statistically significant)

CONCLUSION

Based on the findings of the present study the prominent features are;

- The majority of the patients detected with hypothyroidism were females (70%) as compared to males (30%).
- The mean age of newly detected hypothyroid patients in the study was 40.60 years.
- The majority of the patients detected with hypothyroidism newly were aged less than or equal to 30 years (36.67%).
- On comparing the TSH levels in the hypothyroid and the normal groups,there was a statistically significant difference,with mean TSH levels being higher in the hypothyroid group.
- On comparing the two groups by free T3 levels, the was no statistically significant difference.
- On comparing the two groups by free T4 levels, there was no statistically significant difference between the two groups.
- There was a statistically significant difference on comparing the serum homocysteine levels in the hypothyroid group and the normal group, with mean serum homocysteine levels being higher in the hypothyroid group.
- On comparing the case and control groups with respect to serum cholesterol levels, there was a statistically significant difference between the two grups,with mean serum cholesterol levels being higher in the hypothyroid group.

- There was no statistically significant correlation of the serum homocysteine levels with serum TSH,free T3, free T4 and cholesterol levels in the hypothyroid population.
- There was no statistically significant correlation of the serum homocysteine levels with serum TSH, free T3,free T4 and cholesterol levels in the normal population.
- In conclusion,the study observed elevation of serum homocysteine and total cholesterol levels in hypothyroid patients. Hyperhomocysteinemia and increased cholesterol levels contributes to a greater cardiovascular risk. Hyperhomocysteinemia, along with hypercholesterolemia, can explain the progression of atherosclerosis in hypothyroid patients and we would recommend screening of serum homocysteine levels in hypothyroid patients , in view of the risk of accelerated atherosclerosis and cardiovascular disease.

SUMMARY

Hypothyroidism is known to have an important impact on cardiovascular risk factors, via an induction of diastolic hypertension, bradycardia, altered coagulability, direct effect on vascular smooth muscles and endothelial dysfunction. In this study, we aimed to assess the relationship between hypothyroidism and one of the newer risk factors for cardiovascular disease, serum homocysteine.

30 newly detected hypothyroid patients snd 30 euthyorid matched controls from the KLES Dr.Prabhakar Kore Hospital, Belagavi were compared with respect to serum homocysteine and cholesterol levels.

In this study, the majority of the patients detected to be hypothyroid were females (70%) as compared to men (30%).

In the study, the majority of patients detected with hypothyroidism were less than or equal to 30 years (36.67%).

In this study, there was a statistically significant difference on comapring the serum homocysteine levels in the hypothyroid and the normal groups, with the mean serum homocysteine levels in the hypothyroid group being 25.02 mcmol/L, and in the euthyroid group being 16.65 mcmol/L. There was also a statistically significant difference on comapring the serum cholesterol levels in the two groups, with the

mean cholesterol levels in the hypothyroid group being 193.03 mg/dl, and that in the euthyroid group being 165.90 mg/dl.

Hyperhomocysteinemia and increased cholesterol levels contributes to a greater cardiovascular risk. Hyperhomocysteinemia, along with hypercholesterolemia, can explain the progression of atherosclerosis in hypothyroid patients and we would recommend screening of serum homocysteine levels in hypothyroid patients , in view of the risk of accelerated atherosclerosis and cardiovascular disease.

BIBLIOGRAPHY

1. Surks MI,Ocampo E.Subclinical thyroid disease.Am J Med. 1996:100:217-23
2. Longo DL, Fauci AS, Kasper DL, Hauser SL, Jameson JL, Loscalzo J,et al.,editors. Harrison's Principles of internal medicine. Disorders of the thyroid gland 18th ed.United States of Americ: McGraw Hill:2012.p.2911-22
3. Cappola AR, Ladenson PW. Hypothyroidism and atherosclerosis. J Clin Endocrinol Metab. 2003 Jun;88(6):2438-44.
4. Ciaccio M, Bivona G, Bellia C. Therapeutical approach to plasma homocysteine and cardiovascular risk reduction. Ther Clin Risk Manag. 2008 Feb;4(1):219-24.
5. Vollset SE, Refsum H, Tverdal A, Nygård O, Nordrehaug JE, Tell GS, Ueland PM. Plasma total homocysteine and cardiovascular and noncardiovascular mortality: the Hordaland Homocysteine Study. Am J Clin Nutr. 2001 Jul;74(1):130-6.
6. Longo DL, Fauci AS, Kasper DL, Hauser SL, Jameson JL, Loscalzo J,et al.,editors. Harrison's Principles of internal medicine.Pathogenesis,treatment of atherosclerosis. 18th ed.United States of Americ: McGraw Hill:2012.p.1983-92.
7. Yajnik CS, Deshpande SS, Lubree HG, Naik SS, et al. Demystifying Relevance of Homocysteine Hypothesis in Native Asian Indian Population. J Assoc Physicians Ind 2006;54:775-82.
8. Kochupillai N. Clinical Endocrinology in India. Current science. 2000 Oct;79(8):1061-67.

9. Barbé F, Klein M, Chango A, Frémont S, Gérard P, Weryha G, Guéant JL, Nicolas JP. Homocysteine, folate, vitamin B12, and transcobalamins in patients undergoing successive hypo- and hyperthyroid states. J Clin Endocrinol Metab. 2001 Apr;86(4):1845-6.
10. Nair CP, Viswanathan G, Noronha JM. Folate-mediated incorporation of ring-2-carbon of histidine into nucleic acids: influence of thyroid hormone. Metabolism. 1994 Dec;43(12):1575-8.
11. ROBERT L. MASON, M.D., HAZEL M. HUNT, A.B., and LEWIS HURXTHAL, M.D. Blood Cholesterol Values in Hyperthyroidism and Hypothyroidism — Their Significance. N Engl J Med 1930; 203:1273-1278
12. C.V Rizos, M.S Elisaf, and E.N Liberopoulos . Effects of Thyroid Dysfunction on Lipid Profile. Open Cardiovasc Med J. 2011; 5: 76–84.
13. Bakker O, Hudig F, Meijssen S, Wiersinga WM. Effects of triiodothyronine and amiodarone on the promoter of the human LDL receptor gene. Biochem Biophys Res Commun. 1998;249:517–21
14. Shin DJ, Osborne TF. Thyroid hormone regulation and cholesterol metabolism are connected through Sterol Regulatory Element-Binding Protein-2 (SREBP-2) J Biol Chem. 2003;278:34114–8.
15. Faure P, Oziol L, Artur Y, Chomard P. Thyroid hormone (T3) and its acetic derivative (TA3) protect low-density lipoproteins from oxidation by different mechanisms. Biochimie. 2004;86:411–8.
16. Lagrost L. Regulation of cholesteryl ester transfer protein (CETP) activity: review of in vitro and in vivo studies. Biochim Biophys Acta. 1994;1215:209–36.

17. Kuusi T, Saarinen P, Nikkila EA. Evidence for the role of hepatic endothelial lipase in the metabolism of plasma high density lipoprotein2 in man. Atherosclerosis. 1980;36:589–93.
18. Santamarina-Fojo S, Gonzalez-Navarro H, Freeman L, Wagner E, Nong Z. Hepatic lipase, lipoprotein metabolism, and atherogenesis. Arterioscler Thromb Vasc Biol. 2004;24:1750–4.
19. Prieur X, Huby T, Coste H, Schaap FG, Chapman MJ, Rodriguez JC. Thyroid hormone regulates the hypotriglyceridemic gene APOA5. J Biol Chem. 2005;280:27533–43.
20. Rensen PC, van Dijk KW, Havekes LM. Apolipoprotein AV: low concentration, high impact. Arterioscler Thromb Vasc Biol. 2005;25:2445–7.
21. Iglesias P, Diez JJ. Influence of thyroid dysfunction on serum concentrations of adipocytokines. Cytokine. 2007;40:61–70.
22. Langer P. Vol. 44. Geneva: WHO; 1960. History of goiter. In: Endemic Goiter; p. 9.
23. Leoutsakos V. A short history of the thyroid gland. Hormones. 2004; 3: 268–71.
24. Kalra S. Endocrinology in Ayurveda: Modern Science, Ancient history. Indian J Endocrinol Metab. 2011 [In press]
25. Hippocratis M. Lipsiae. Opera Omnia. Carolus Gottlob Kunh. 1825-1827;3:658.
26. Schneider BA. Effects of feeding thyroid substance. Q Rev Biol. 1939;14:289–310.
27. Toni R. Ancient views on the hypothalamic-pituitary-thyroid axis: An historical and epistemological perspective. Pituitary. 2000;3:83–95.

28. O'Malley CD. New York: Dover; 1925. Leonardo on the human body.
29. Szpunar WE, Stoffer SS. A history of endocrinology. JAMA. 1983;249:415.
30. Haeger K. London: Harold Starke; 1988. The illustrated history of surgery.
31. Dioscoridis P. Lipsiae De material medica. Curtius Sprengel. 1829;1:83–4.
32. Frugardo R. Post Mundi Fabricam. Rogerina. XII-XII sec.
33. Bifulco M, Cavallo P. Thyroidology in the medieval medical school of Salerno. Thyroid. 2007;17:39–40.
34. Sawin CT. Theories of causation of Grave's disease: A historical perspective. Endocrinol Metab Clin North Am. 1998;27:63–72.
35. Blumberg BS. Goiter in Gandhara. JAMA. 1964;189:1008–12
36. Rolleston HD. Oxford University Press: 1936. The endocrine glands with an historical review; p. 142.
37. Rolleston HD. Oxford University Press: 1936. The Endocrine Organs in Health and Disease; p. 16.
38. Medvei VC. Lancaster: MTP Press; 1982. A History of Endocrinology; pp. 289–96.
39. New Delhi: Ministry of Health and Family Welfare, Government of India; 2011.Department of Health and Family Welfare. Annual Report 2010-2011
40. Prevalence of Micronutrient Deficiencies. Hyderabad: NNMB Technical Report No.22. ICMR, National Institute of Nutrition; 2000. National Nutrition Monitoring Bureau.
41. Iodine Network. Global scorecard 2010.
42. New Delhi: Directorate General of Health Services Ministry of Health & Family Welfare, Government of India; 2006. National Rural Health Mission

IDD & Nutrition Cell. Revised Policy Guidelines On National Iodine Deficiency Disorders Control Programme

43. Larsen, William J. (2001). Human embryology (3. ed.). Philadelphia, Pa.: Churchill Livingstone. pp. 372–374.
44. Page 493 (Table 33-3) in: Eugster, Erica A.; Pescovitz, Ora Hirsch (2004). Pediatric endocrinology: mechanisms, manifestations and management. Hagerstwon, MD: Lippincott Williams & Wilkins.
45. Zoeller RT (April 2003). "Transplacental thyroxine and fetal brain development". J. Clin. Invest. 111 (7): 954–7. doi:10.1172/JCI18236
46. Berbel P, Navarro D, Ausó E, Varea E, Rodríguez AE, Ballesta JJ, Salinas M, Flores E, Faura CC, et al. (2010). "Role of late maternal thyroid hormones in cerebral cortex development: an experimental model for human prematurity". Cereb Cortex. 20 (6): 1462–75
47. Gray's anatomy : the anatomical basis of clinical practice (40th ed.). London: Churchill Livingstone. pp. 462–464
48. "Thyroid, Parathyroid, Adrenal, Endocrine Surgery, Anatomy of the Thyroid Gland". www.endocrinesurgeon.co.uk.
49. Clinical Case - Anterior Triangle of the Neck
50. Yalçin B.; Ozan H. (February 2006). "Detailed investigation of the relationship between the inferior laryngeal nerve including laryngeal branches and ligament of Berry". Journal of the American College of Surgeons. 202 (2): 291–6.
51. Kamath, M. Aroon. "Are the ligaments of Berry the only reason why the thyroid moves up with deglutition?"

52. Page, C; Cuvelier, P; Biet, A; Boute, P; Laude, M; Strunski, V (July 2009). "Thyroid tubercle of Zuckerkandl: anatomical and surgical experience from 79 thyroidectomies.". The Journal of laryngology and otology. 123 (7): 768–71
53. Irizarry, Lisandro (23 April 2014). "Thyroid Hormone Toxicity".
54. Johannes W. Dietrich (2002). Der Hypophysen-Schilddrüsen-Regelkreis. Berlin, Germany: Logos-Verlag Berlin. ISBN 978-3-89722-850-4. OCLC 50451543
55. Synthesis of Thyroid Hormones" in: Walter F. Boron; Emile L. Boulpaep (2012). Medical Physiology (2nd ed.). Elsevier/Saunders.
56. Human Anatomy & Physiology, Sixth Edition. Benjamin Cummings
57. Boron, W.F. (2003). Medical Physiology: A Cellular And Molecular Approaoch. Elsevier/Saunders
58. Davidson's 2010, p. 738.
59. Davidson's 2010, p. 740.
60. Cury, A. N.; Meira, V. T.; Monte, O.; Marone, M.; Scalissi, N. M.; Kochi, C.; Calliari, L. E. P.; Longui, C. A. (24 October 2012). "Clinical experience with radioactive iodine in the treatment of childhood and adolescent Graves' disease". Endocrine Connections.
61. Davidson's 2010, p. 741.
62. Davidson's 2010, p. 741-3.
63. Dean, DS; Gharib, H (December 2008). "Epidemiology of thyroid nodules.". Best practice & research. Clinical endocrinology & metabolism. 22 (6): 901–11
64. Welker, Mary Jo; Orlov, Diane (February 1, 2003). "Thyroid Nodules". American Family Physician. 67 (3): 559–567.

65. Kang SS,Wong PW, Malinow MR: Hyperhomocyst(e)inemia as a risk factor for occlusive vascular disease. Annu Rev Nutr 12: 279–298, 1992.
66. Jacobsen DW: Practical chemistry of homocysteine and other thiols. In: Carmel R, Jacobsen DW (eds), Homocysteine in Health and Disease. Cambridge University Press, Cambridge, UK, 2001, pp 9–20.
67. Ueland PM, Mansoor MA, Guttormsen AB, Muller F, Aukrust P, Refsum H, Svardal AM: Reduced, oxidized and protein-bound forms of homocysteine and other aminothiols in plasma comprise the redox thiol status—a possible element of the extracellular antioxidant defense system. J Nutr 126: 1281S–1284S, 1996.
68. Ganguly P, Alam SF. Role of homocysteine in the development of cardiovascular disease. Nutr J 2015;14:6.
69. Boushey CJ, Beresford SA, Omenn GS, Motulsky AG. A quantitative assessment of plasma homocysteine as a risk factor for vascular disease. Probable benefits of increasing folic acid intakes. JAMA 1995; 274(13):1049-57.
70. Blom HJ, Smulders Y. Overview of homocysteine and folate metabolism. With special references to cardiovascular disease and neural tube defects. J Inherit Metab Dis 2011;34(1):75-81.
71. Friedman AN, Bostom AG, Selhub J, Levey AS, Rosenberg IH. The kidney and homocysteine metabolism. J Am Soc Nephrol 2001;12(10):2181-9.
72. Silbernagl S. The renal handling of amino acids and oligopeptides. Physiol Rev 1988;68(3):911-1007.
73. Brattström L, Wilcken DE. Homocysteine and cardiovascular disease: Cause or effect? Am J Clin Nutr 2000;72(2):315-23.

74. Gupta JK, Sana QS. Potential benefits of methylcobalamin: A review. Austin J Pharmacol Ther 2015;3(3):1-3.
75. Pisciotta L, Cortese C, Gnasso A, Liberatoscioli L, Pastore A, Mannucci L, et al. Serum homocysteine, methylenetetrahydrofolate reductase gene polymorphism and cardiovascular disease in heterozygous familial hypercholesterolemia. Atherosclerosis 2005;179(2):333-8.
76. Jakubowski H. An Overview of Homocysteine Metabolism. New York: Springer-Verlag Wien; 2013. p. 7-18.
77. Miller AL, Kelly GS. Methionine and homocysteine metabolism and the nutritional prevention of certain birth defects and complication of pregnancy. Altern Med Rev 1996;1(4):221-3.
78. Selhub J. Homocysteine metabolism. Annu Rev Nutr 1999;19:217-46.
79. Herrmann W. The importance of hyperhomocysteinemia as a risk factor for diseases: An overview. Clin Chem Lab Med 2001;39(8):666-74.
80. Wald DS, Law M, Morris JK. Homocysteine and cardiovascular disease: Evidence on causality from a meta-analysis. BMJ 2002;325(7374):1202.
81. Mattson MP, Shea TB. Folate and homocysteine metabolism in neural plasticity and neurodegenerative disorders. Trends Neurosci 2003;26(3):137-46.
82. Saleh A Bamashmoos1, Mohammed AK Al-Nuzaily1, Ali M Al-Meeri2 and Faisal HH Al, Relationship between total homocysteine, total cholesterol and creatinine levels in overt hypothyroid patients, SpringerPlus 2013
83. Bjørn G. Nedrebø, Ottar Nygård, Per M. Ueland, and Ernst A. Lien , Plasma Total Homocysteine in Hyper- and Hypothyroid Patients before and during 12 Months of Treatment, Clinical Chemistry , Vol. 47, Issue 9 September 2001

84. Orzechowska-Pawiłojć A, Lewczuk A, Sworczak K, The influence of thyroid hormones on homocysteine and atherosclerotic vascular disease, Endokrynol Pol. 2005 Mar-Apr;56(2):194-202.

ANNEXURE – I - INFORMED CONSENT

Title of research study: THE RELATIONSHIP BETWEEN SERUM HOMCYSTEINE LEVELS AND HYPOTHYROIDISM- A ONE YEAR CASE CONTROL STUDY IN KLES HOSPITAL BELGAUM

Principal investigator:-

Dr. _____

Post graduate student,

Department of general medicine,

J.N. Medical College, Belgaum.

Introduction and purpose:-

Thyroid gland is critical to the proper functioning and regulation of various physiological pathways and processes.

The increased cardiovascular morbidity in hypothyroid patients has been related to elevated levels of cholesterol and low-density lipoprotein cholesterol (LDL-C). Lipid abnormalities in hypothyroid patients do not fully account for the cardiovascular risk.

Some prospective studies showed a strong association between hyperhomocysteinemia and cardiovascular disease, and Total homocysteine is an independent risk factor for cardiovascular disease. Hyperhomocysteinemia induces endothelial injury, oxidative stress, smooth muscle hypertrophy and oxidation of LDL-cholesterol.

The objectives are to study the incidence of hyperhomocysteinemia in patients with hypothyroidism, and the relation of homocysteine and cholesterol levels to thyroid hormones free T3, free T4 and TSH in newly detected hypothyroid patients

Procedure:

If you agree to be part of the research study, you will be asked the relevant history and will be subjected to relevant clinical examination and investigations. You may have to give blood and urine samples for the necessary investigations.

Risks and benefits:

The only risk and possible discomfort you might get is while taking blood samples for the investigations. It may cause swelling, pain, redness bruising or infection(rarely happens) at the site from where the samples are drawn.

Benefit is recognising the well defined precipitating factors of hepatic encephalopathy in cirrhosis of liver to prevent mortality.

Alternatives:

Taking part in this study is voluntary. You may choose not to take part in this study, or if you decide to take part you can later change your mind and withdraw from the study. Your decision will not change the present or future health care or other services that you receive. The study doctor or sponsor may stop your participation in this study at any time. If you choose not to take part in the study, you will receive the standard treatment for patients with your condition.

Privacy and confidentiality:

All information collected about you during the course of this study will be kept confidential to the extent permitted by law. The code numbers will identify you

in this research record. Information from this study may be published but your identity will be confidential in any publication.

Institution/sponsors/compensation:

In case of any injury related to the study, treatment will be made available at KLES Dr Prabhakar Kore Hospital and Medical Research Centre, Belgaum. There is no compensation or payment for such medical treatment by law.

Financial incentives for participants:

You will not be paid/offered any gifts/incentives for participating in the study.

Authorisation to publish the results:

The results of the study would be forwarded to the KLE University, Belgaum as part of requirement towards the completion of MD degree, review and publishing.

Questions/contact details

In case of the queries during study you may contact following persons.

1) Dr_____ 2)Dr. _____

3) Dr. Ganga Pilli, Chairman,

J.N.M.C Ethical committee for human research

Phone no: 08312471350

Extn: 1527

Consent statement

I voluntarily agree to take part in this study by signing below. I may withdraw at any time. I am not giving up any of my legal rights by signing this form. My signature below indicates that I have read, or it has been read to me, this consent form, and have had all the questions answered.

Name of the participant:_____ Signature/Thumb print:_____

Name of the witness:_____ Signature/Thumb print:_____

Investigator name:_____ Signature:_____

Date: Place:

ANNEXURE – II - PROFORMA

CASE No:

NAME: **AGE/SEX:**

IP NO: **ADDRESS:**

OCCUPATION:

COMPLAINTS AT PRESENTATION:

PAST HISTORY:

TREATMENT HISTORY:

PHYSICAL EXAMINATION:

GENERAL CONDITION :

PALLOR: Yes/No

Icterus: Yes/No

Lymphadenopathy: Yes/No

Cyanosis: Yes/No

Clubbing: Yes/No

Edema : Yes/No

VITALS:

Temperature:

Pulse:

Respiratory rate:

Blood pressure:

Systemic examination:

R.S.:

CVS:

PA:

CNS:

INVESTIGATIONS: