DISSERTATION SUBMITTED FOR THE MASTER'S DEGREE IN

MEDICAL BIOCHEMISTRY

TITLE

EVALUATION OF VITAMIN D IN DIAGNOSED CASES OF HYPOTHYROIDISM AND CONTROL SUBJECTS

BY

SHANTI GUPTA

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DEPARTMENT OF BIOCHEMISTRY

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In partial fulfillment of the requirement for the award of degree of

Master of Science

In

Medical Biochemistry

By

SHANTI GUPTA

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CERTIFICATE

This is to certify that **Miss. Shanti Gupta**, student of M.Sc. Medical Biochemistry, Integral University has completed her dissertation titled **"Evaluation of Vitamin D in diagnosed cases of Hypothyroidism and control subjects"** successfully. She has completed this work in the Department of Biochemistry, Integral Institute of Medical Sciences and Research, Integral University under my supervision. The dissertation was a compulsory part of her M.Sc. degree.

I wish her good luck and a bright future.

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I wish her good luck and a bright future.

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I hereby declare that Integral Institute of Medical Sciences & Research, Integral University, Lucknow shall have the right to preserve, use and disseminate this dissertation in print/ electronic format for academic/ research purposes.

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Shanti Gupta

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Date:

Place: Lucknow **Shanti Gupta**

LIST OF ABBREVIATIONS

WHO	World Health Association
T3	Triiodothyronine
T4	Thyroxin
TSH	Thyroid-stimulating Hormones
25(OH)D	25-hydroxy vitamin D
1,25 $(OH)_2D$	1, 25-dihydroxy vitamin D
NFHS	National Family Health Survey
OPD	Outpatient Department
VDR	Vitamin D receptor
DIO	Deiodoniase
Tg	Thyroglobulin

CONTENTS

INTRODUCTION

The thyroid gland is classified as a prominent endocrine gland and generally exhibits a weight ranging from 15 to 20 grams in adult individuals (Hall et al., 2011). Its anatomical location is below the larynx, situated bilaterally, and positioned anteriorly to the trachea (Hall et al., 2011).this gland is divided into a pair of lobes that are joined by an isthmus(Pang et al., 2012).

In its typical state, the thyroid gland demonstrates measurements of approximately 0.5 cm in thickness, 2 cm in width, and a height ranging from 1 to 2 cm (Salomon et al., 2016).

The thyroid gland's primary component is made up of structures called thyroid follicles. In the centre of these follicles is a sticky fluid termed as colloid. These Thyroid hormones, which are essential for the body, are produced within the colloid.

Thyroid stimulating hormone, or TSH, regulates the production of thyroid hormones such as T4 (thyroxine) and triiodothyronine (T3). These hormones are predominantly linked to carrier proteins in the circulation, accounting for 99.97% of T4 and 99.7% of T3. A small percentage of these hormones, known as free T3 (fT3) and free T4 (fT4), are not attached to proteins and are thus physiologically active. In India, the prevalence rate of hypothyroidism is 10.95% (Sahana et al., 2020).

Anterior pituitary gland secretes TSH. It is made up of two separate chains: the α and β chains. TSH has an estimated molecular mass of 28,000 Dalton (Pirahanchi Y et al.,2023). The primary function of TSH is to regulate the release of two important hormones, T3 (triiodothyronine) and T4 (thyroxine), from the follicular cells of the thyroid gland. It should be noted that T4 accounts for around 80% of the total thyroid hormone released by the gland. However, T4 undergoes a process of de-iodination, where it is converted into T3, which is a more potent form of thyroid hormone. Interestingly, the thyroid gland produces only around 20% of the total T3 present in the human system. A specifically designed enzyme known as

deiodinase play role in peripheral conversion which release the remaining 80% of T3. (Delitala AP. et al.,2019).

TSH functions via a signalling route cAMP second messenger system. AMP is converted into cAMP in this mechanism. TSH also activates another signalling cascade known as the IP3 signalling cascade, which results in calcium being released from its stores. Both the cAMP and IP3/Ca2+ cascades have sequential effects that increase thyroid hormone synthesis and stimulate thyrocyte growth and development. These signalling pathways play an essential part in controlling the many physiological processes involved in production of T3 and T4. (Pirahanchi Y et.al., 2023).

Less than 1% of thyroid hormones are unbound in the bloodstream, while over 99% of them attach to thyroid-binding globulin, pre-albumin, and albumin (Delitala AP et. al.,2019). T4 and T3 absorption and outflow are mainly carried out by specialized transporters found on cell membrane. Only fT4 and fT3 can be transferred into thyroid hormone-target cells.

Iodine is necessary for thyroid production. Imbalance of iodine amount can also cause hypothyroidism; however, overt hypothyroidism is rare. T4 and T3 absorption and outflow are mainly carried out by specialized transporters found on cell membrane.

India is considered to have 'optimal' iodine dietary habits, with more than eighty-three percent of families in urban areas and 66.1% in rural region receiving appropriate iodized salt data given WHO report publish in 2004 (Unnikrishnan A.G. et al., 2013).

Hypothyroidism is a thyroid gland dysfunction characterized by decreased thyroid hormone T3 and T4 synthesis and release. Regardless of Thyroid hormone production, its action has been linked to clinical hypothyroidism. All organ systems, including the skin and appendages, cardiovascular, respiratory, gastrointestinal, central, and peripheral nervous systems,

skeletal (calcium and phosphorus metabolism), renal, pituitary, and energy metabolism, can be impacted by hypothyroidism. (Shlomon melmed et al., 2016).

The two basic kinds of hypothyroidism are primary and secondary (central).Iodine shortage is the most frequent cause of primary hypothyroidism, which occurs when the thyroid gland is unable to produce enough thyroid hormones. Impairment function of pituitary gland or hypothalamus is a root cause of central Hypothyroidism; however, thyroid gland is unaffected. Autoimmune thyroid diseases are the leading causes of hypothyroidism in the US and other iodine-sufficient nations. (Taylor, P. N. et al., 2018).

cholecalciferol or ergocalciferol, two forms of Vitamin D that primarily control the calcium and phosphate balance in the body, are induced under the effect of UVB light. Vitamin D3 is synthesized in the skin by a non-enzymatic way (Holick MF, 2007). The CYP27R1 enzyme converts vitamin D to 25-hydroxy vitamin D during hydroxylation in the liver. It is an inactive form with a 15- days half-life that has significance in measuring the serum level of vitamin D.

In the function of CYP27B1 enzyme, hydroxylation occurs in kidney to release the biologically active 1, 25-dihydroxy vitamin D which induces skin cells, osteoblasts, dendritic cells, and thyrocytes in target cells . A number of epithelial cells, immune system cells, and the parathyroid gland are examples of non-renal organs that also express CYP27B1 (Khammissa AG. et al., 2018). According to Bikle D. (2015), PTH stimulates 1,25-dihydroxy vitamin D production in the kidney while calcium, phosphate, and FGF23 suppress it.

Vitamin D act as a steroidal hormone that interacts with the vitamin D receptor (VDR) and triggers a variety of physiological reactions that can be genomic or non-genomic. Due to the fact that VDR is present in various bodily cells, it plays a significant part in the regulation of

vascular tone, activation of cell differentiation and proliferation, inhibition of cell growth, and also shows antioxidant effects. (Bidar et al., 2015; Wiseman, 1993).

It has been noted that vitamin D signaling is disturbed in autoimmune illnesses (Muscogiuri, et al., 2015).

- Vitamin D levels over 30 ng/ml are regarded as normal.
- Vitamin D level is insufficient at 20-29ng/ml (>50 nmol/L).
- Vitamin D levels below 10ng/ml (12.5nmol/L) indicate severe insufficiency. (2nd edition, William et al.).

Some studies found that High TSH levels found in hypothyroid patients are negatively correlated with vitamin D levels. (Zhang et al., 2014). Under normal circumstances, high TSH leads to increased thyroid hormone, which undergoes negative feedback regulation and favors the activity of the vitamin D receptor in thyrocytes, reducing iodine uptake. Thyrotropin-releasing hormone and thyroid-stimulating hormone secretion are regulated as a result of negative feedback (Dietrich et al., 2012). Therefore, it's possible that deficiencies in vitamin D raise the level of TSH in hypothyroidism.

REVIEW OF LITERATURE

Thyroid hormone, T3 and T4 plays an important role in enhancing cellular metabolic activity as well as the quantity and activity of mitochondria. Thyroid hormone promotes growth, particularly in children. Thyroid hormone stimulates ion transportation across cell membranes, cardiovascular function, the production of a vast variety of structural and transport proteins, and so on (Hall J.E. et al., 2012).

Insufficiency in synthesis and release of thyroid hormone manifest Hypothyroidism.

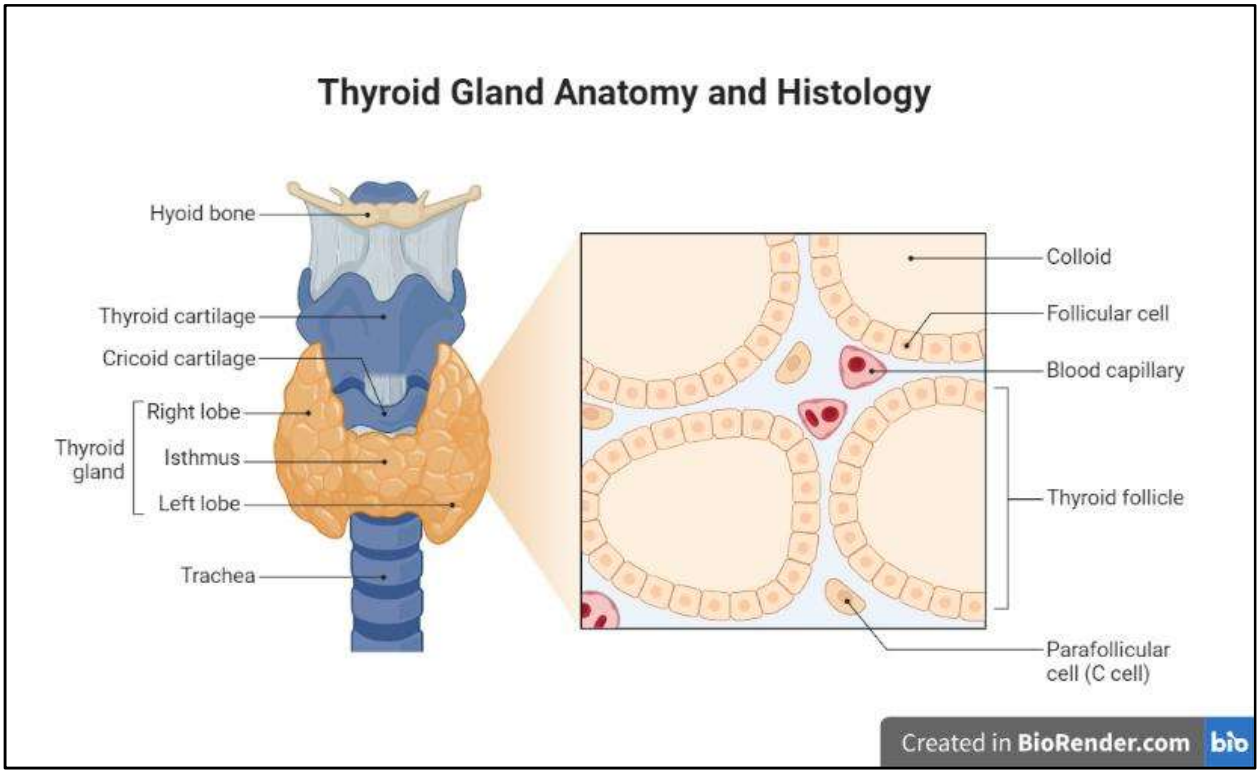


Figure 1- Structure of Thyroid Gland- *It is made up of small structures called follicles, which are surrounded by follicular cells and filled with a substance called colloid. These follicular cells play an important role in trapping iodine, which is then released into the follicle along with a protein called thyroglobulin (Boron W.F. et al., 2017).*

Hypothyroidism is a worldwide endocrine illness that affects about five percent of the population, with 99% of those affected suffering from primary hypothyroidism caused by iodine shortage in the thyrocytes, which causes Hashimoto's disease.

Thyroid hormones synthesis

1. **Synthesis of Thyroglobulin-** The confirmational essential for tyrosyl joining and Iodide organification in the formation of diamino acid thyroid hormones are supplied by a large precursor molecule term as, Thyroglobulin (Anthony P., 2012).
2. **Iodine uptake-** Triiodothyronine and Tetraiodothyronine need essential element iodine to be functional; they are synthesized as part of very big precursor molecules; and kept in a colloid; an intracellular reservoir (Chiovoto L et. al., 2019). Sodium-potassium ATPase are induces the function of Na+-I symporters have role to sent iodide in the thyrocytes which transportation regulated by phosphorylation of protein kinase A. Pendrin which is an iodide transporter helps to iodide reach to colloid mainly present on the apical side of cell . (Shahid, M. A. et al., 2018).
3. **Iodination of Tg -** Protein kinase A also phosphorylates and induces thyroid peroxidase, which performs the following roles: oxidation, organification, and coupling.
   (a) **Oxidation-** TPO uses hydrogen peroxide to oxidise iodide into I_2. H_2O_2 is generated by the apical enzyme NADPH-oxidase for thyroid peroxidase.
   (b) **Organification-** Thyroglobulin protein's tyrosine residues bind iodine through TPO. Monoiodotyrosine and diiodotyrosine are released as a result.Double residues of tyrosine are present on DIT,while MIT has only one tyrosine residue with iodine (Shahid, M. A. et al., 2018).Inefficient precursors, monoiodotyrosine (MIT) and diiodotyrosine (DIT), contribute to approximately 70% of the iodide in thyroglobulin.
4. **Coupling action-** thyroid peroxidase enzyme have role in combining of tyrosine residues with iodine to produce thyroid hormones. T4 gets formed when two DIT molecules interact, whereas T3 is synthesised when MIT and DIT both join together.

5. **Storage of thyroid hormone –** It is stored in lumen of follicle by binding to Tg**.**
6. **Release-** Thyrocytes release thyroid hormones in the manner described below into the interconnected system of capillaries:
   (a) Via endocytosis thyroglobulin iodinate form reaches to cell of thyroid gland.
   (b) Fusion occure between the lysosome and endosome that contains iodinated thyroglobulin.
   (c) Endolysosome proteolytic enzymes break thyroglobulin into T3, T4, MIT and DIT.
   (d) The MCT8 transporter transports 20% T3 and 80% of T4 into the thin capillaries (Schweizer, U. et al.,2013).
   (e) Deiodinase enzyme activity removes small amounts of iodine from diiodotyrosine and monoiodotyrosine. It is possible to recover iodine and add it to a reservoir of iodide inside the cell (Shahid, M. A. et al., 2018).

The basal metabolic rate (BMR) is increased by thyroid hormone. It enhances expression of Na+/K+ ATPase in several tissues, resulting in raised oxygen consumption, rate of respiration, and thermal regulation.

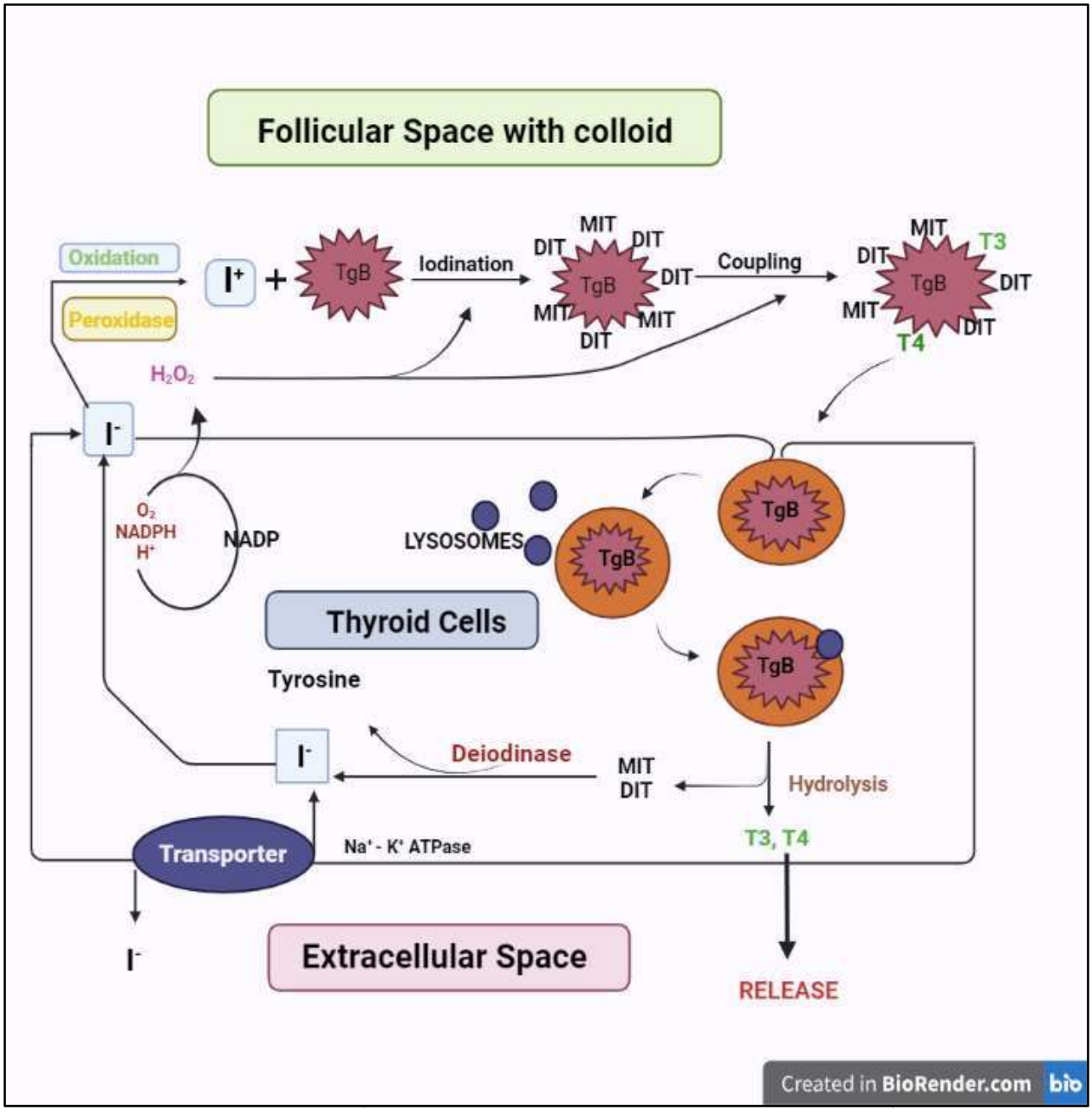


Figure 2- *Iodide mechanism in the production of Thyroid hormone (Anthony P., 2012).*

Regulation of thyroid hormones

TSH specifically regulates the release of T3 and T4 from the thyroid follicular cells. TSH promotes the amount of thyroid hormone secretion by increasing iodide absorption, thyroglobulin synthesis, and thyroperoxidase function. TSH additionally improves blood supply

to the thyroid gland and encourages hypertrophy as well as hyperplasia of thyroid follicular cells, causing the thyroid gland to enlarge (Rousset B. et. al., 2015).

Thyroid-releasing hormone, a hormone that triggers the rostral part of the pituitary thyrotrophs to produce Thyroid stimulating hormone, is secreted by axis of hypothalamus-pituitary which regulates the release of TSH. (Pirahanchi Y. et al.,2023). Thyroid- releasing hormone interacts to its receptors which present on the front part of pituitary gland, activating a sequential signal reaction controlled by serpentine Gpcr . Phospholipase C (PLC) activity is triggred when G alpha q protein is activated. PLC promotes PIP3 and DAG production , are second messengers, which promote intracellular calcium activity and stimulate protein kinase C, resulting in activation of genes and TSH production. TSH causes the follicular cells of the thyroid to secrete T4 about 80% T3 is approx 20%. However, Somatostatin is a hypothalamic hormone that suppresses the secretion of TSH from the anterior part of the pituitary. When T4 gets into circulation, it undergoes deiodination and is transformed into T3.

Mechanism of actions of Thyroid Hormones

T3 and T4 thyroid hormones inhibit hypothalamus synthesis of TRH and pituitary production of TSH. TSH promotes T3 and T4 synthesis. Thyroid epithelial cells wrap a proteinaceous colloid containing Thyroglobulin in order to form thyroid follicles.

Blood concentrations of T3 and T4 induce a compensatory feedback loop affecting the hypothalamus and pituitary gland. The negative feedback mechanism regulates the concentration of T3 and T4 hormone in the blood and helps the body achieve thyroid homeostasis.

TSH interacts with and activates the TSH receptor (TSHR) on the basolateral side of thyroid follicular cells, which is a G-protein coupled receptor (GPCR). TSHR is linked to both Gs and Gq G-proteins, triggering both the cAMP route (through G- stimulatory alpha) and the IP/Ca2+

(via Gq alpha) second messenger signaling cascades. Iodide absorption, thyroid hormone release, and gland growth and differentiation are all activated by the Gs pathway.

By increasing iodide organification, the Gq path limits Thyroid hormone production. Increase activity of TSH receptor and reduce activity of TSH receptor due to mutation, mainly cause Hyperthyroidism and hypothyroidism, respectively. (Pirahanchi Y et.al.,2023).

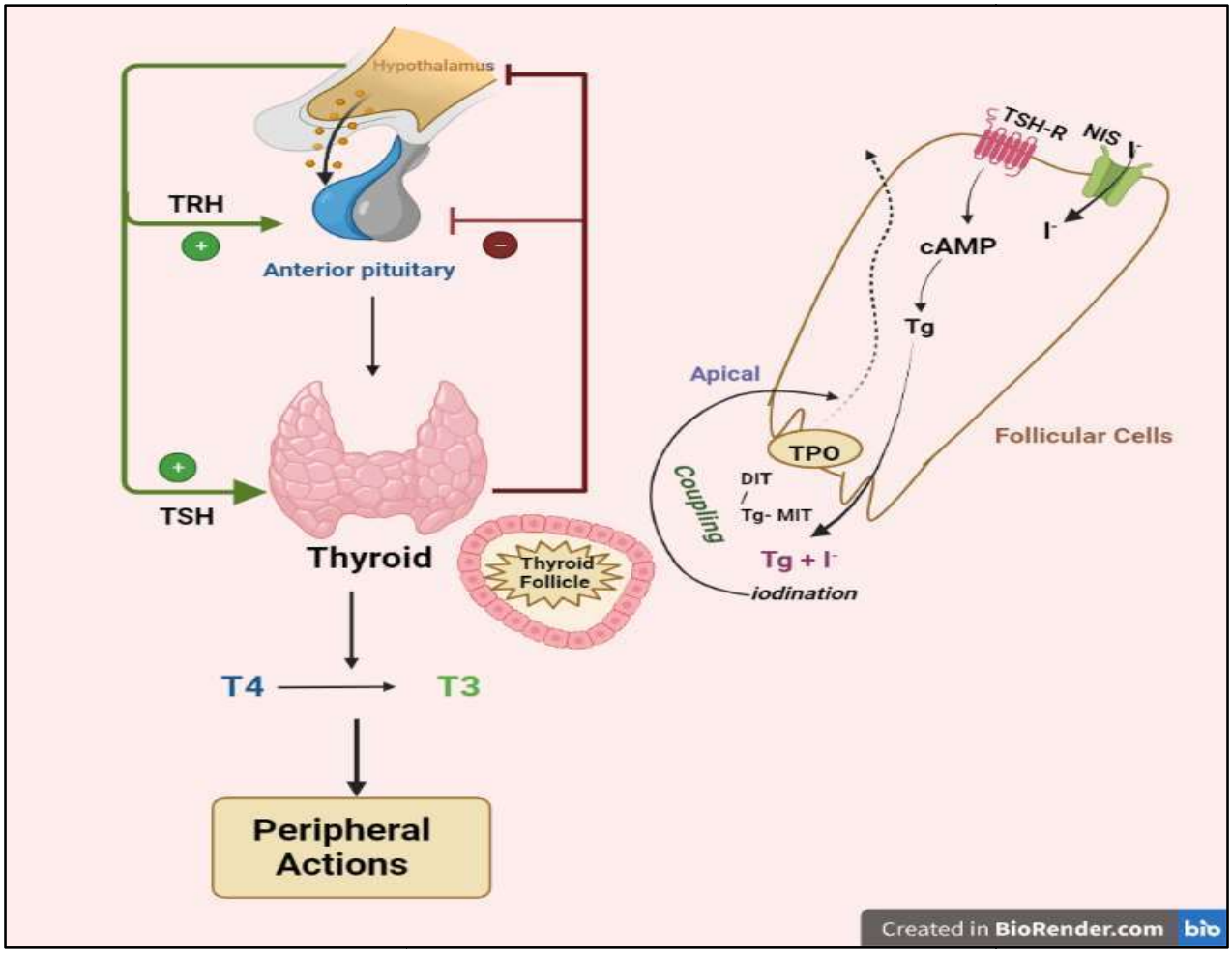


Figure 3- *Feedback Regulation of Thyroid hormone (Jameson J.L. et al., 2011).*

Thyroid receptors that have a greater sensitivity for T3 are affected by interactions between T3 and T4 hormones. T4 is hence essentially inactive. DIO1 and DIO2 activity causes T4 to become active T3 and vice versa. While T4 gets transformed by DIO3 activity into rT3

which is inactive form. Deiodinases are mainly three types which found in different cells of body. In the hepatic cells, both renal, skeletal muscles, and thyroid glands have DIO1 and DIO2 deiodinase enzyme respectively, type III Deiodinase are present in central nervous system and placental body (Brent, G. A.,2012).

Transporter of thyroid hormones in the blood

T3 and T4 bind to plasma carrier proteins as they are secreted into the bloodstream. Thyroxine-binding globulin (TBG), transthyretin, and plasma protein albumin are transporter proteins.TBG transports most of the T4 (2/3rd), while thyroxine and retinol are delivered by transthyretin.

About 70% of the circulating thyroid hormones are bound by thyroxin-binding globulin, 20% by prealbumin thyroxine binding and 10% by albumin. In healthy subjects, roughly 0.02 percent of thyroxine and 0.2 percent of T3 are free from the plasma. (Colin et al., 2015).

Hypothyroidism

Low levels of circulating thyroid hormones caused by structural and functional defects that limit thyroid hormone secretion characterize an illness known as hypothyroidism. (Salomon et al., 2016)

Hashimoto Thyroiditis is an especially prevalent cause of hypothyroidism in iodine-deficient locales.The thyroid gland is damaged as a result of the immunological process. cytotoxic T-cells are responsible for thyroid follicular death (Shahid, M. A. et al., 2018).In its last stages, atrophic thyroiditis, or autoimmune thyroiditis, there may be little or no thyroid tissue left over. because the autoimmune process ultimately diminishes thyroid function. A surge in TSH maintains normal thyroid hormone levels during the compensating period. Although some people may experience minimal symptoms, this condition is known as subclinical

hypothyroidism.Later, fT4 levels drop and TSH levels rise even more; at this point, overt hypothyroidism is known and symptoms are more obvious (usually TSH more than10 ml/L) (Jameson J.L et al., 2011).

Following factors can cause hypothyroidism

1. **Primary hypothyroidism:** This is caused by the thyroid gland producing inadequate thyroid hormone. This is the most widespread form of hypothyroidism.

Acquired - (a). Arises from Hashimoto's thyroiditis

(b). Deficiency of iodine (endemic goiter)

Congenital - (a)Iodide transport or utilization deficiency (NIS or pendrin mutations)

(b). Insufficiency of iodotyrosine dehalogenase

(c). Disorders of organification (TPO deficit or malfunction)

(d). Defects in the synthesis or processing of thyroglobulin.

2. Transient Hypothyroidism (Post-Thyroiditis)

3. Consumptive Hypothyroidism- Thyroid hormone is rapidly destroyed in large hemangiomas due to D3 expression.

4. Central Hypothyroidism:

Acquired- (a) Secondary pituitary origin.

(b) Tertiary hypothalamic disorders

Congenital- (a) TSH shortage or structural defect

(b) TSH receptor abnormality (Gregory A. et al., 2015).

Epidemiology

According to data from the National Health and Nutrition Examination Survey, 4.6%8 of Americans have hypothyroidism, which can be overt or subclinical (Brito, J. P. et al., 2021).

According to the NFHS IV (2015–2016), less than 1% of males and almost 2 percent of females between the ages of 15 and 49 reported having a thyroid disease or goitre, respectively. Additionally, the stated incidence in women (fifteen to nineteen years:0.7%; twenty to thirty four years: 1.8%; thirty five to fourty nine years: 3.4%) increased with age. A prevalent condition, hypothyroidism affects 1% of the overall population and 5% of people over the age of 60 (Banday T.H., 2016).

India is considered to have 'optimal' iodine dietary habits, with more than eighty-three percent of families in urban areas and 66.1% in rural region receiving appropriate iodized salt data given WHO report publish in 2004 (Unnikrishnan A.G. et al., 2013).

Signs and common symptoms associated with Hypothyroidism

Hypothyroidism typically takes long time to develop, and symptoms may not become apparent until much later. The most frequent symptoms, which are frequently vague, are fatigue, aversion to the cold, and constipation.The hallmark of diagnosis is the detection of elevated TSH and low fT4 levels. because there, a wide range of manifestations and the diagnosis of hypothyroidism is established with minimal specificity and high accuracy (i.e., hypothyroidism did not show unique symptoms). (Canaris, G. J. et al., 1997).

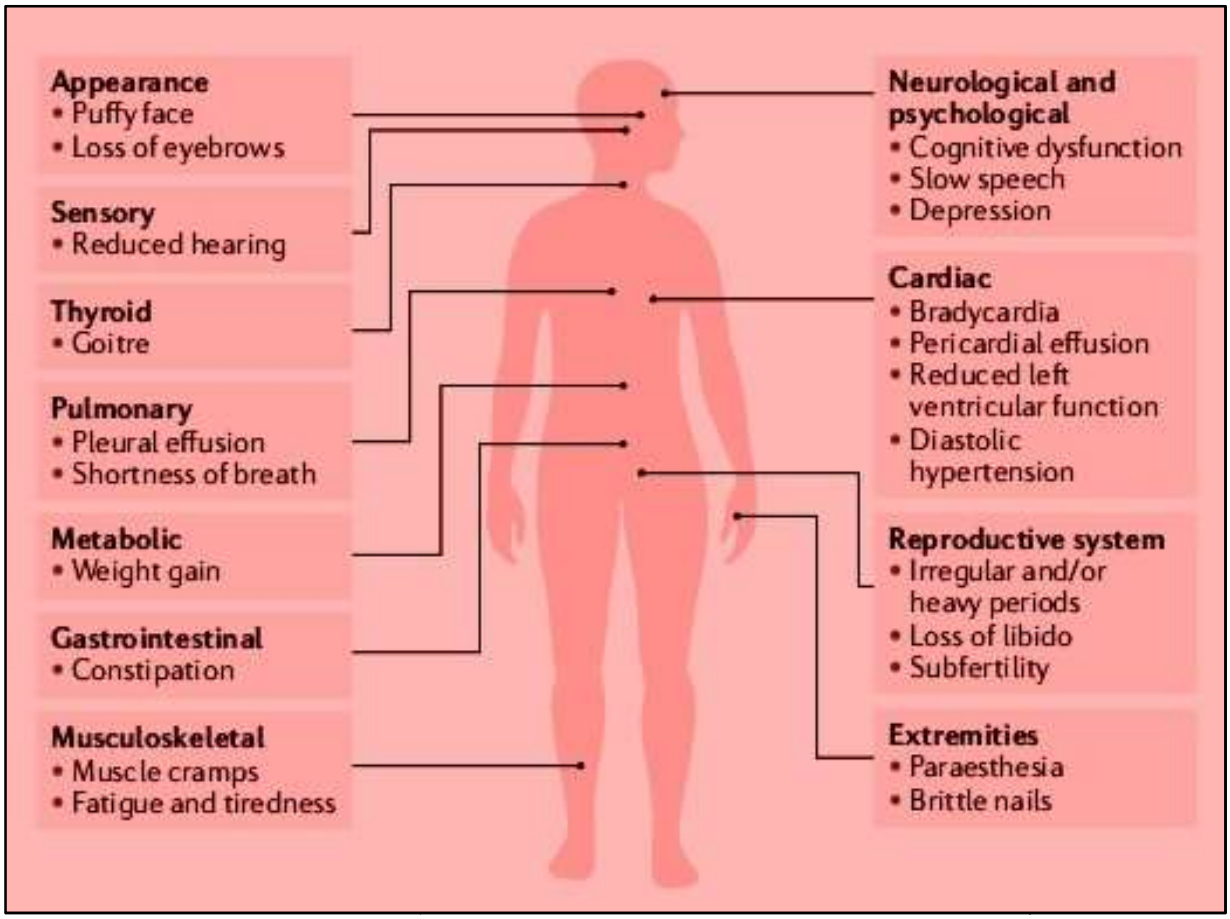


Figure 4- *Signs and Common symptoms associated with impaired thyroid hormone production (Hypothyroidism)(Chaker. L et al., 2022).*

Diagnosis of Hypothyroidism

The initial step in evaluating suspected primary hypothyroidism is to determine their blood TSH levels. To distinguish subclinical or overt hypothyroidism, thyroid hormone on periphery should be assessed if serum TSH levels are consistently raised. For patients with overt hypothyroidism, levothyroxine medication is required. Patients suffering subclinical hypothyroidism who have not begun LT4 medication should have their thyroid function checked on a regular basis (Chaker L. et al.,2022).

Along with a low amount of thyroid hormones and reduced TSH concentration enhances the possibility of central hypothyroidism, and the Activity of the pituitary should be evaluated (Chaker L. et al., 2022).

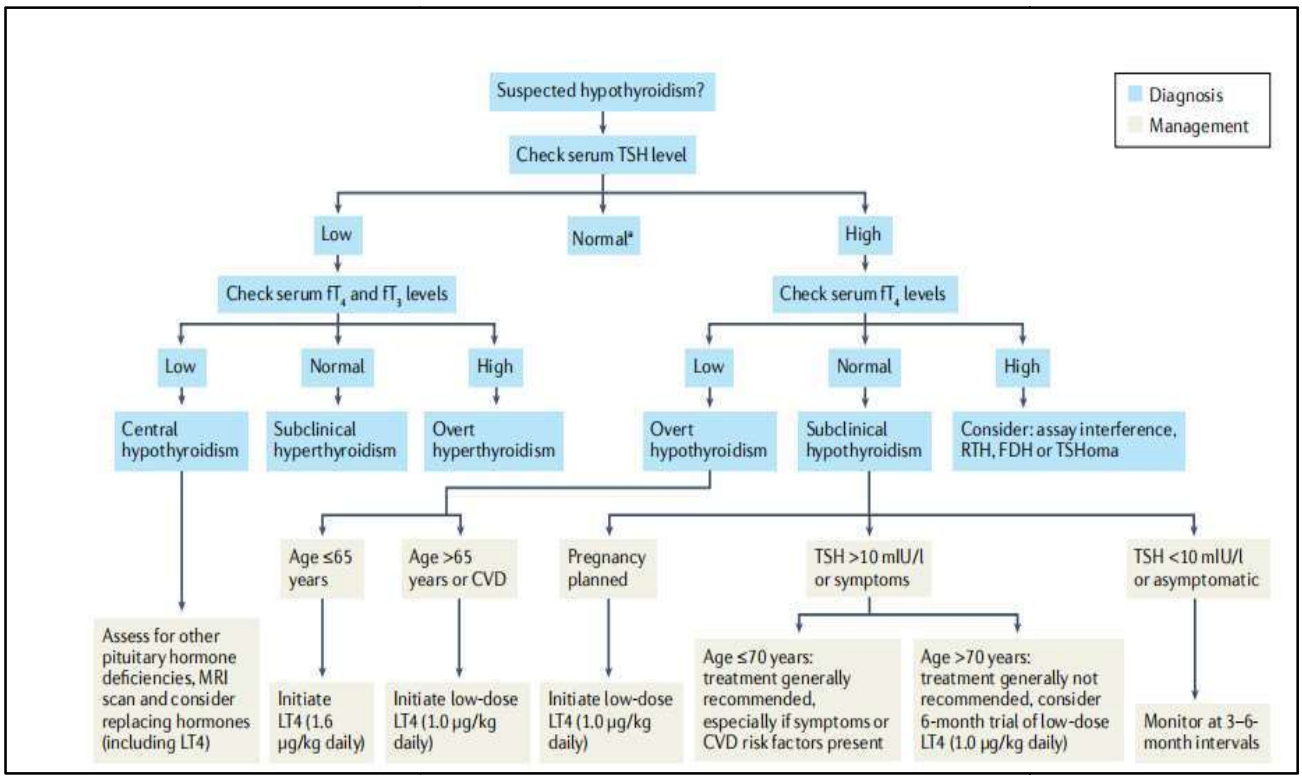


Figure 5- *Diagnosis and management of Hypothyroidism Patient (Chaker. L et al., 2022).*

Vitamin D synthesis and regulation

The Key suppliers of vitamin D, a fat-soluble molecule, are diet or synthesis in the skin (Holick MF, 2007). Ergocalciferol and cholecalciferol are the two most significant vitamin D derivatives. A sizeable proportion of vitamin D is transformed from 7-dehydrocholesterol to vitamin D3 after skin contact with ultraviolet B. Additionally, between ten and twenty percent of vitamin D—either D2 or D3—is acquired by diet. (Mithal A. et al., 2009). The predominant circulating form of vit. D that utilised to determine one's overall vitamin D conc. is 25-hydroxyvitamin D, which forms when vitamin D reaches in the hepatic cells and then hydroxylated(Dusso AS et al., 2005).

By activity of CYP27B1 in the kidney converts inactive vitamin D into functional vitamin D (Cythenia Aranow, 2011). IFN and other cytokines such as TNF, Interleukin-1, and 2 can stimulate circulating monocytes to produce 1, 25-dihydroxy vitamin D via the JAK/STAT, p38 MAPK, and NFB signalling pathways.PTH has not been demonstrated to inhibit macrophage CYP27B1 activity (Gyetko et al., 1993).

Ergocalciferol and cholecalciferol are two fat-soluble kinds of vit D. With identification of its receptors in many tissues, several novel biological roles for vitamin D have been found are becoming more apparent, and its significance in many human diseases such as cancer, diabetes, hypertension, cardiovascular disease, and immunological and dermatological diseases is being thoroughly investigated. The non-conventional activity of vitamin D includes regulating the growth of cells, differentiation, cell death, and both adaptive and innate immunity (Sutton AL, 2003).

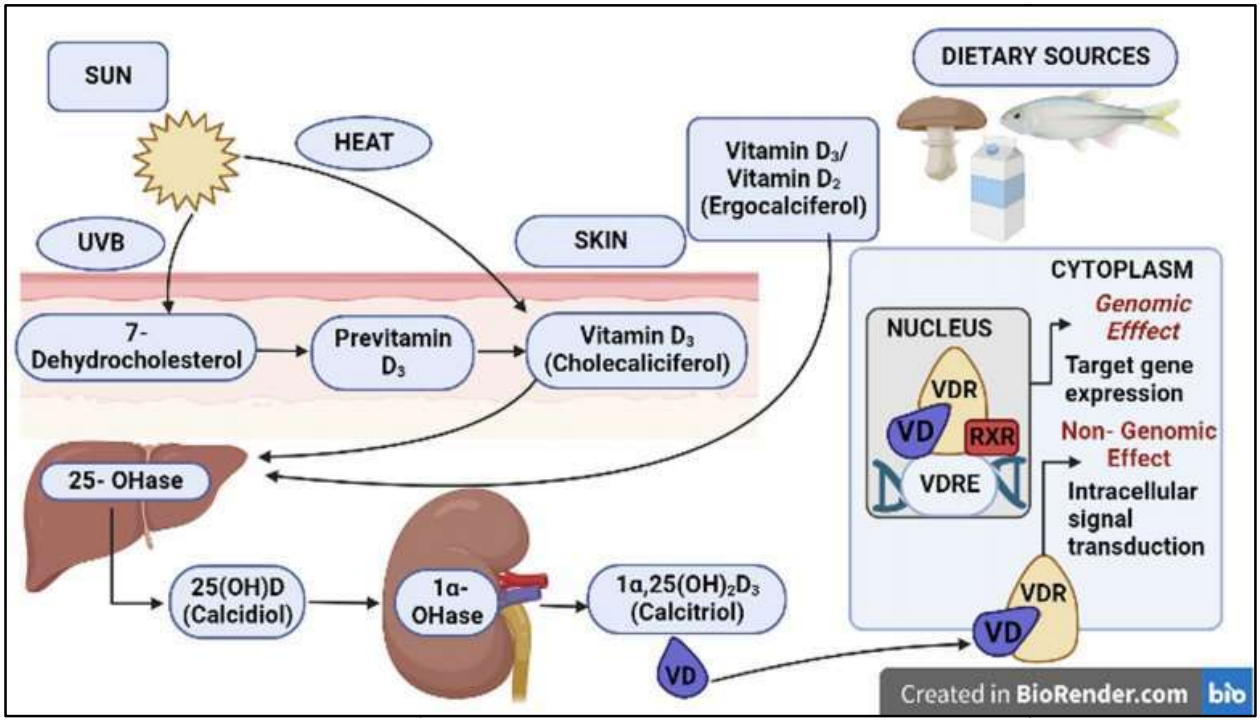


Figure 6- *Synthesis and mechanism of Vitamin D (Awasthi R. et al.,2023).*

Mechanism of action of vitamin D

Vitamin D act as steroidal hormone that interacts with VDR and induces genomic/non-genomic reactions for a wide range of cellular processes. VDR is additionally identified in various body cells, therefore it plays a significant function in vascular tone modulation, differentiation of cells induction, development of cells suppression, proliferation, and antioxidant benefits (Wiseman, 1993 and Bidar et al., 2015).VDR is found in numerous cells of the body, including some immune cells, demonstrating vitamin D and immune system share association (Sutton AL, 2003).

VDR has been identified in osteoblasts, the cells that make bone. 1, 25-dihydroxy vitamin D has been known to affect osteoblasts thus taking part in protein synthesis. The type of proteins formed are necessary for bone formation; they include collagen, alkaline phosphatase and osteocalcin. 1, 25-dihydroxy vitamin D also stimulates the production and function of osteoclasts

via inducing RANKL. As a result, 1, 25- dihydroxy vitamin D promotes both bone development and resorption of bone.

1, 25-dihydroxy vitamin D is actively involved alongside calcium. It greatly affects calcium entrance in the cell through cell membrane, its transportation and consequent elimination from the cell. A class of calcium binding proteins known as calbindins, govern calcium transport across the cell .The CaATPase which are ATP-requiring calcium pump and NCX1 (sodium/calcium exchange protein) perform this role (Bikle, D., 2015).

Non-classical role of vitamin D include cellular growth and differentiation modulation, hormone release regulation, and immunological regulation.

Vitamin D role in Adaptive immunity

Several number of immune cells also express VDR and 1-alpha hydroxylase. T and B cells release cytokines and immunoglobulins, which are involved in adaptive immunity. The cells known as T-helper cells are responsible for the synthesis of Interleukins-2 and INF- and for the proliferation of macrophages (Lemire et al., 1995).

Vitamin D reduces Th1 cytokines whereas increasing Th2 production of cytokines (Baeke F. et al.,2012) and elevating Intrleukins 4, 5 and 10, production in thyrocytes. Vitamin D3 stimulates the synthesis of IL-10 and the translation of the FoxP3 gene, which regulates the development of CD4+/CD25+ regulatory cells (Penna G & Adorni L, 2000).

Vitamin D role in innate immunity

TLRs are transmembrane pathogen recognition receptors found in polymorphonuclear cells, monocytes, epithelial cells, and macrophages that are triggered by an immune system response that is innate (Medzhitov R, 2007).

TLR-activated antimicrobial peptide Cathelicidin and reactive oxygen species help destroy the bacterium (Gombart AF et al., 2005). VDR and 1-alpha-hydroxylase have been identified in macrophages and epithelial cells, and they play a role in active vitamin D formation and the induction of the antimicrobial peptide cathelicidin.(Wang TT et al.,2004). Wang et al. revealed that 1,25dihydroxy vitamin D protects thyroid against apoptosis by enhancing Bcl-2 expression. Vitamin D modulates the immune system by stimulating the natural immune system while reduce function of adaptive immune system.

Vitamin D with hypothyroidism

The 1,25-dihydroxy vitamin D receptor (VDR) is a transactivator that controls gene expression that governs its biological function. Thyroid hormones and VDR both belong to nuclear receptor family, which also contains receptors for ACTH hormones and retinoids.

Prevelent rate of hypothyroidism has been link to variant form of VDR . Because thyroid hormone receptors are also members of the nuclear receptor superfamily, they have comparable mechanisms of action because they share two regions: cysteine-rich 70 and 62 amino acid sequence identified close to protein's carboxyl terminus (McDonnell DP et al., 1988). Thyroid and VDR produce a heterodimer complex with RXR and bind to DNA hormone response, either activating or suppressing transcription of the targeting gene (Christakos S., 2010).

The existence of VDR (Vitamin D receptors) in macrophages and other immune cells demonstrates that vitamin D and the immune system interact(Sutton AL & MacDonald P.

N., 2003). In autoimmune thyroid diseases and thyroid cancer vitamin D signaling is impaired been reported (Muscogiuri, et al., 2015). Wang et al. (2004), revealed that 1,25dihydroxy vitamin D protects thyroid against apoptosis by enhancing Bcl-2 expression. Vitamin D modulates the immune system by stimulating the natural immune system while reduce function of adaptive immune system. So There is a chance that vitamin D and hypothyroidism are linked.

Only a few research have found a link between vitamin D and hypothyroidism among Indians. As a result, the current study is designed to assess serum vitamin D levels in hypothyroid and control patients. If the findings are consistent with earlier research, they may aid in a better understanding of the pathophysiology and management of hypothyroidism.

AIM
&
OBJECTIVES

Aim

The aim of this study was to evaluate level of serum vitamin D level in diagnosed cases of hypothyroidism and Apparently Healthy control.

Objectives

1. To determine serum vitamin D levels in patients of hypothyroidism and Apparently Healthy control.

2. To correlate the serum vitamin D level between diagnosed cases of hypothyroidism and Apparently Healthy control.

MATERIALS AND METHODS

RESEARCH QUESTION

Is there any change in the level of vitamin D in diagnosed cases of hypothyroidism as compared to Apparently Healthy control?

Hypothesis

Null hypothesis H_o: There is no significant change in the level of vitamin D in diagnosed cases of hypothyroidism as compared to Apparently Healthy control.

Alternate hypothesis H_1: There is a significant change in the level of vitamin D in diagnosed cases of hypothyroidism as compared to Apparently Healthy control.

METHODOLOGY

Study design-Prospective

Type of study- Case-control study

SUBJECTS SELECTION

Selection of Controls

1. Apparently healthy individuals
2. Subjects between the age of 30 to 65 years
3. Individuals who have agreed to sign the consent form.

Selection of Cases

Inclusion Criteria

1. Diagnosed cases of Hypothyroidism.
2. Subjects between the age of 30 to 65 years
3. Patients who had agreed to sign the consent form.

Exclusion criteria

1. Pregnant and lactating females.
2. History of any chronic illness.

SAMPLE SIZE - The sample size is calculated using the formula

$$n = \left(\frac{r+1}{r}\right)\frac{\sigma^2 (Z_\beta + Z_{\alpha/2})^2}{(difference)^2}$$ Reference- (Charan J Biswas, 2013).

n = sample size

(r+1)/r = ratio of the case to control

σ = standard deviation (taken from previous studies)

Zβ = represent the desired power

Zα/2 = represent the desired level of statistical significance

Difference = effect size (the difference in means of the study group and comparison group taken from the previous studies)

Then,

For 80% power, Zβ = (0.84)

For 0.05 significance level, Zα/2=1.96

r = 1 (equal to a number of case and control)

σ = 1.4 (Lohokare R.,2016)

d= 0.72

$$n = 2\times \frac{(1.4)^2 \times (0.84 + 1.92)^2}{(0.72)^2}$$

Therefore, n = 30.01 ≈ 30

The study included **30** cases and **30** controls.

Place of study- Department of Biochemistry, Integral Institute of Medical Sciences and Research, Lucknow.

Collaborating department

Department of General Medicine, OPD at IIMS&R, Integral University, Lucknow.

Enrollment of participants

Cases were enrolled from the hypothyroidism attending the Integral Hospital.

Sampling Method-Non-probability, Purposive sampling

Collection of samples

1 ml of venous blood was collected from the subjects under aseptic conditions in a plain vial. The blood sample was allowed to clot at room temperature for 15 minutes. The sample was then centrifuged at 1000 rpm for 10 minutes to separate the serum (Henry J. B., 1979).

10 microliter serum was used for the estimation of 25-OH vitamin D.

Storage of samples

The serum samples for the estimation of 25-hydroxy vitamin D were stored at -20°C until testing in Central Clinical Laboratory, Department of Biochemistry, IIMS&R, Lucknow.

Laboratory Investigations

1. **Determination of serum 25-hydroxy vitamin D (Qualisa TM) by sandwich-based enzyme-linked immunosorbent assay method by using Thermo scientific multiskan FC.**

PRINCIPLE

The test employs a pair of monoclonal agglutinating sera, the first one is immobilized in the solid phase (Microwells) and the other monoclonal agglutinating sera is in the liquid phase. In the assay procedure, samples along with Calibrators are added to the coating.

Microwells & incubated together with the first & second agglutinating sera. The wells are then washed to remove the unbound components. The resulting Vitamin D-antibody immunocomplex is detected with third agglutinating sera conjugated with horseradish peroxidase (HRPO). After a short incubation, the wells are washed again and the bound enzyme is detected by adding substrate. The reaction is stopped after a specified time with a stop solution and absorbance is determined for each well using an ELISA reader Thermo scientific multiskan FC. The concentration of Vitamin D is directly proportional to the color intensity of the test sample.

PROCEDURE

Add 10 µl Calibrators/controls/samples into the respective Microwell

↓

Add 200 µl Sample diluent into each Microwell and gently shake the plate for 30 seconds

↓

Apply plate sealer and incubate for 20 minutes at 18-25°C

↓

Wash Microwells 5 times with 350 µl of diluted wash buffer

↓

Add 100 µl Enzyme conjugate in each well

↓

Apply plate sealer and incubate for 10 minutes. at 18-25°C

↓

Wash Microwells 5 times with 350 µl of diluted wash buffer

↓

Add 100 µl substrate in each well

↓

Incubate for 10 Minutes. at 18-25 c in dark

↓

Add 100 µl stop solution in each well

↓

Read results @450nm (Rf.600-700 nm) within 15 minutes (Qualisa TM)

ETHICS REVIEW

Permission from the Institutional Ethics Committee was taken (IEC/IIMSR&R/2023/67).

DATA COLLECTION

Details from the subjects were obtained using data collection proforma after taking written consent.

STATISTICAL ANALYSIS PLAN

Statistical analysis was performed using SPSS-16 version, GraphPad prism 6.0 and Microsoft office Excel, 2007. All the data were expressed as mean ± standard deviation. An unpaired t-test was performed to compare the study parameters between cases and controls. Karl Pearson's correlation analysis was employed to determine the relationship between variables. p-value <0.05 was considered statistically significant.

OBSERVATIONS
&
RESULTS

Vitamin D

The difference in the level of vitamin D between controls and cases was not statistically significant (p = 0.2415) (Table-1 & fig. 7)

Table 1- Mean and standard deviation of the study groups

25-hydroxy vitamin D (ng/ml)					
Groups	**n**	**Mean**	**Standard Deviation**	***p* - Value**	**Significance**
Controls	30	26.56	±15.11	**0.2415**	No Statistically Significant
Cases	30	21.63	±17.10		

N= Number of controls or cases, p<0.05 is considered statistically significant

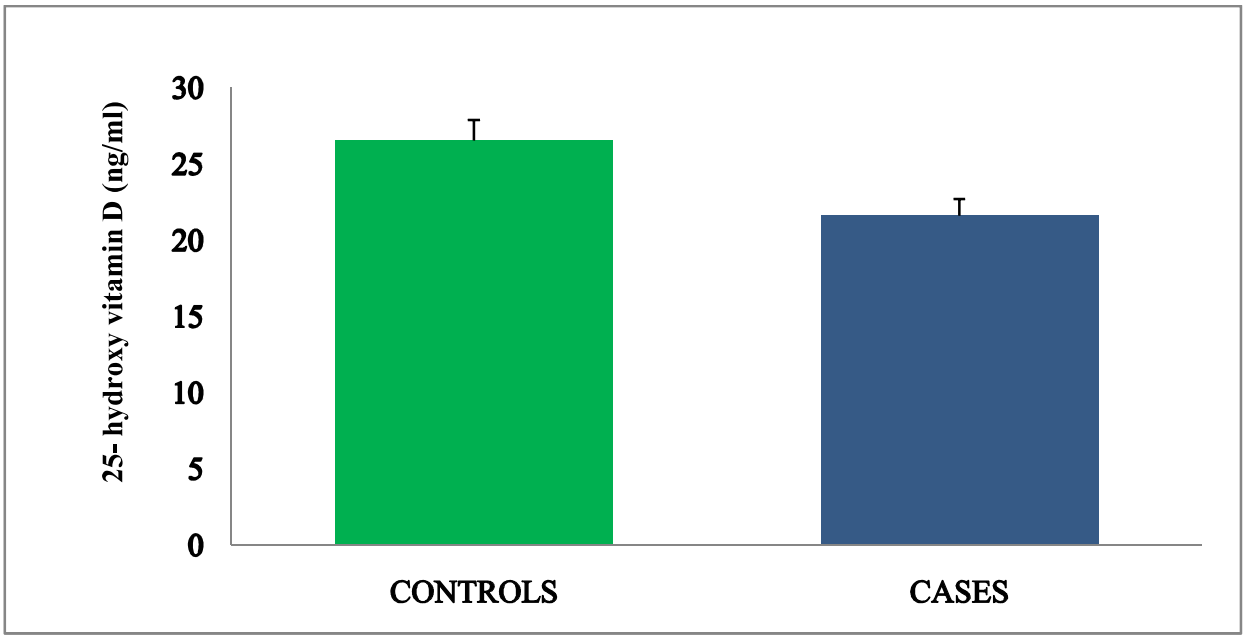


Figure 7- *Comparison of vitamin D levels between controls and cases.*

BODY MASS INDEX (BMI)

The difference in the BMI between cases and controls was statistically significant ($p < 0.001$) (Table- 2 & fig. 9)

Table -2 Mean and standard deviation of the study groups

Body Mass Index (kg/m^2)					
Groups	**n**	**Mean**	**Standard Deviation**	**p- value**	**Significance**
Controls	30	23.91	1.96	<0.001	Statistically Significant
Cases	30	28.4	1.90		

N= Number of cases or controls, p<0.05 is considered statistically significant

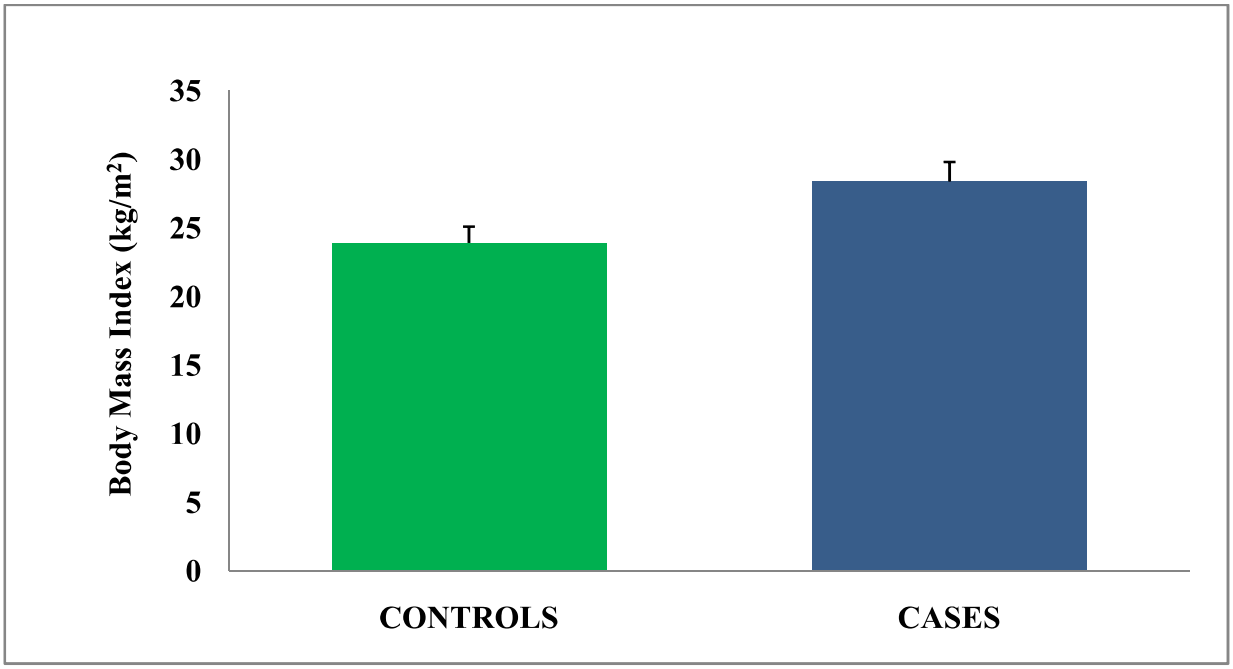


Figure 8- *Comparison of Body Mass Index (BMI) in cases and controls.*

Karl Pearson's coefficient of correlation between vitamin D and Body mass index in cases of hypothyroidism

There was no significant link between vitamin D and BMI in cases of hypothyroidism (p= 0.2).

Table 3- Karl Pearson's coefficient of correlation between cases

		Body Mass Index (kg/m^2)	25-hydroxy vitamin D (ng/ml)
Body Mass Index (Kg/m2)	Pearson Correlation	1	-.241
	Sig. (2-tailed)		.200
	n	30	30
25 hydroxy vitamin D ng/ml	Pearson Correlation	-.241	1
	Sig. (2-tailed)	.200	
	n	30	30

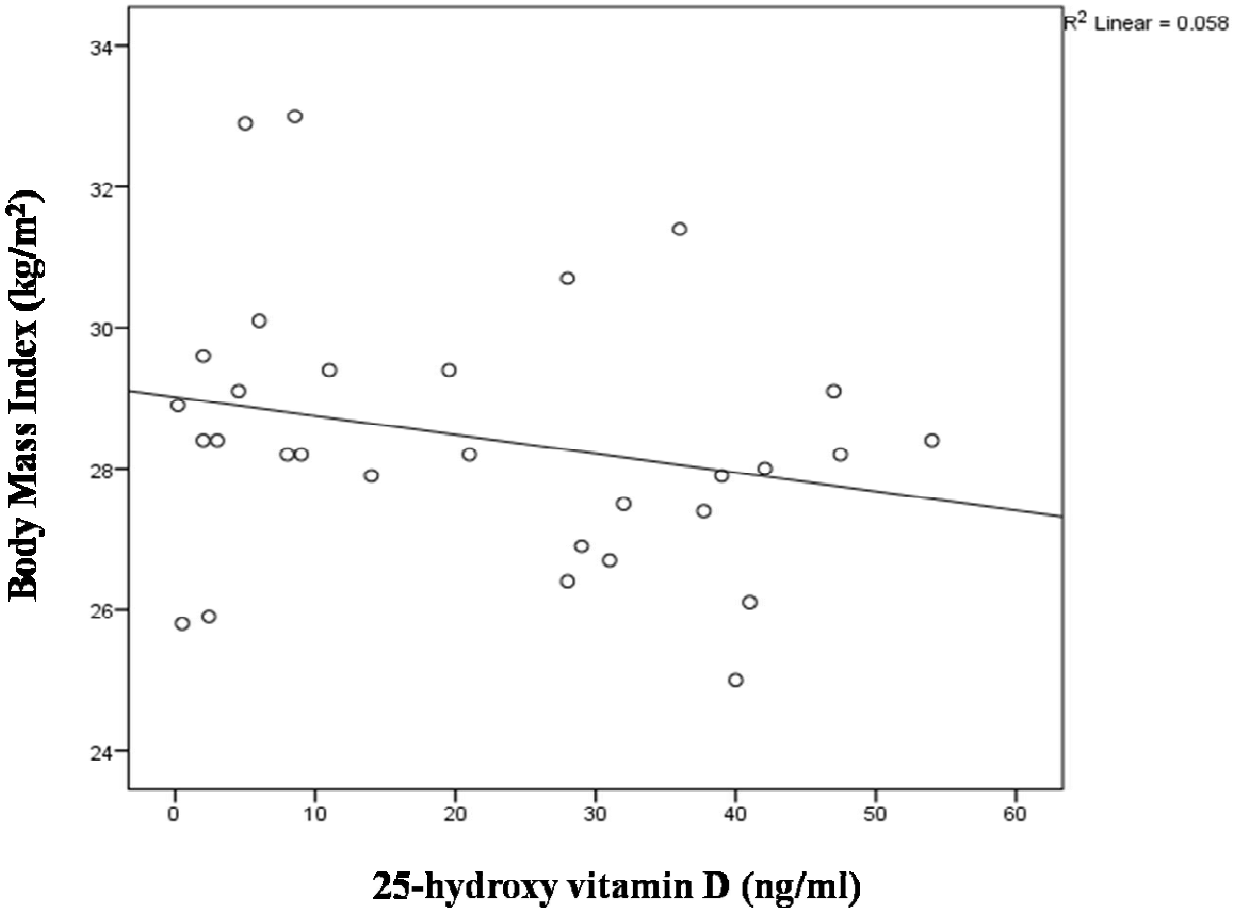


Figure 9- Scatter diagram showing a correlation between vitamin D and body mass index

DISCUSSION

Low levels of circulating thyroid hormones caused by structural and functional defects that limit thyroid hormone secretion characterize an illness known as hypothyroidism. (Salomon et al., 2016)

A few investigations with conflicting results have been carried out to find a causal relationship between vitamin D levels and hypothyroidism and to find out whether vitamin D deficiency is involved in the development of hypothyroidism or rather a result of the disease (Razvi et al., 2018).

Lohokare R. et al. (2016), suggested in their study that a substantial correlation was identified between serum TSH levels and serum 25-hydroxy vitamin D. Hoda A. et al. (2014), also reported similar findings in their study. This has been supported by the findings of Bozkurt et al. (2013), who observed that vitamin D conc. in Hypothyroidism were considerably low than those in controls. Although a causative association could not be proven, vitamin D have been participate in the pathogenesis of Hypothyroidism.

Our investigation showed, there was not found significant difference in vit.D (25-hydroxy vitamin D) levels between cases and controls ($p= 0.2415$). Effraimidis et al. (2012), also reported similar findings. Goswami et al. (2009), investigated the prevalence of inadequate vitamin D levels and its link with thyroid hormones. They also reported statistically insignificant relationship between thyroid hormones and vitamin D levels in Hypothyroidism.

Several studies conducted in India, has observed by Velayutham et al.(2015), and Unnikrishnan et al.(2013), found a high proportion of women suffer with hypothyroidism.

In our study, we observed that the BMI was considerably higher in cases as opposed to controls ($p<0.001$). Hypothyroidism has been linked to decreased production of heat and energy expenditure, as well as a higher BMI and a greater incidence of obesity (Danforth, E. et al., 1979). According to Antunes et al. (2006), leptin are induced by TSH which acts directly on

adipocytes, and increased leptin may explain the relationship between Hypothyroidism and Body mass index through the formation of insulin resistance. Furthermore, leptin can influence deiodinase activity in several tissues, influencing thyroid hormone levels (Cabanelas, A. et al., 2007).

In our study we also found there is no correlation between vitamin D and Body mass index in cases of Hypothyroidism (p=.-241). Obesity or increased fat deposition is another risk factor for low vitamin D levels. Vitamin D and other hormones are redistributed in fat tissue, which acts as a large reservoir (Carrelli A.et al., 2017). As a result, conc. of circulating vitamin D drops, resulting in vitamin D deficiency. Liel et al.(1988), discovered that obese people had lower levels of circulation vitamin D than non-obese people.

SUMMARY
AND
CONCLUSION

SUMMARY

The current study aimed to assess serum vitamin D levels in hypothyroid patients and apparently healthy controls.

The parameters estimated were:

- 25-hydroxy vitamin D

The observations in the study are as follows:

- The concentrations of vitamin D was between cases (21.63±17.10) and controls (26.56±15.11)which are not statistically significant (p= 0.2415)

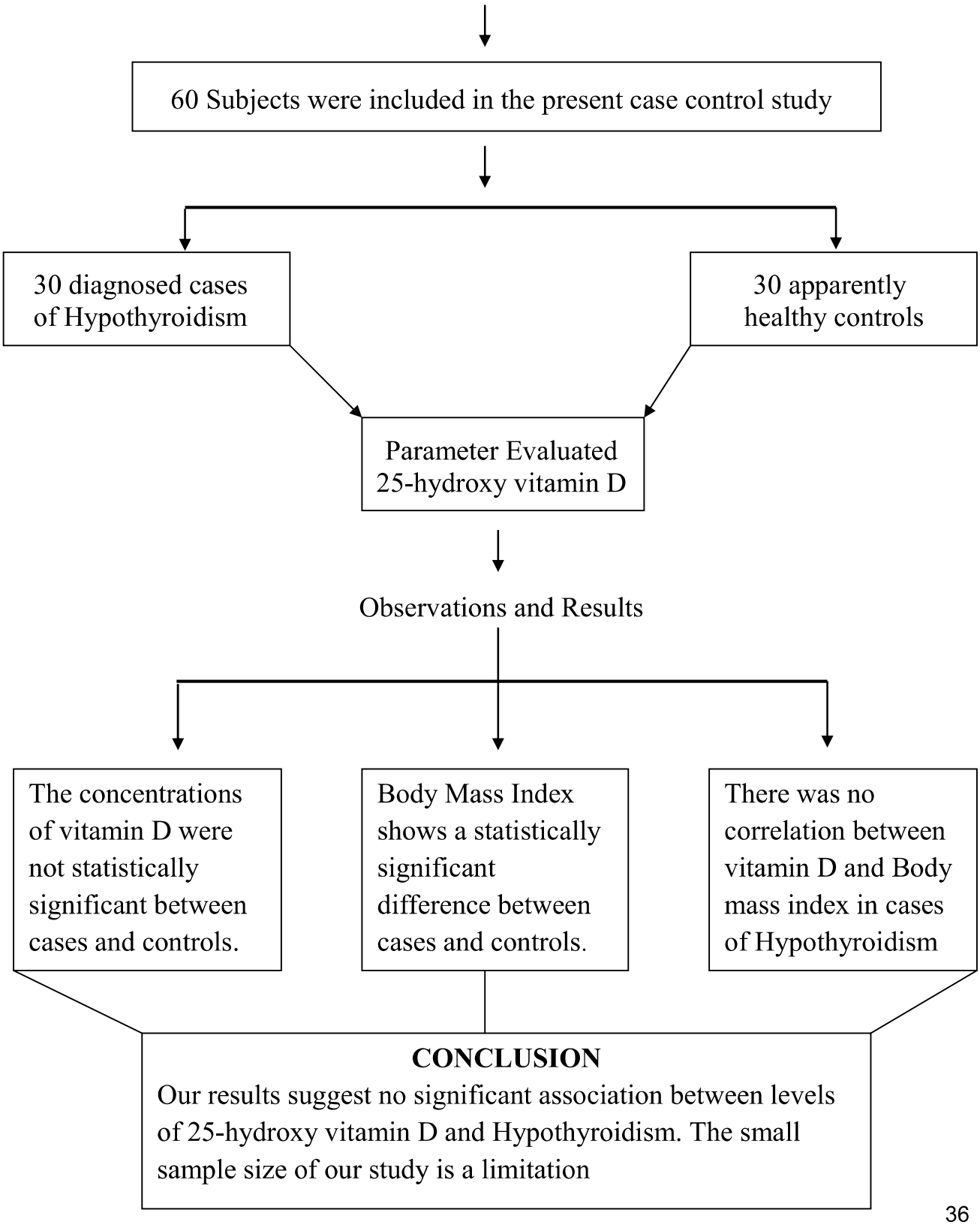
- p-value of BMI between cases(28.40±1.90) and controls (23.91±1.96)are <0.001which are statistically significant

- No correlation found between vitamin D and Body mass index in cases of Hypothyroidism (p= 0.200)

CONCLUSION

Our findings indicate that there is no link between 25-hydroxy vitamin D levels and hypothyroidism. Our sample size is a limitation. More research with a larger sample size will be required to determine whether low vitamin D levels are a cause of hypothyroidism or an effect of the disease.

FLOW CHART (RESEARCH WORK)



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ANNEXURES

Unique Identification No:

INTEGRAL INSTITUTE OF MEDICAL SCIENCES AND RESEARCH

LUCKNOW -226026

INCLUSION AND EXCLUSION CRITERIA -CASES

Inclusion Criteria

S.N.	Criteria	YES	NO
1	Diagnosed cases of hypothyroidism.		
2.	Subjects between the age of 30 to 65 years		

Exclusion Criteria

S.N.	Criteria	YES	NO
1.	Pregnant females and lactating females		
2.	History of any chronic illness		

Subjects are eligible for the study if all **INCLUSION** criteria are **YES** and all **EXCLUSION** criteria are **NO**.

INVESTIGATOR STATEMENT

I have verified the data entered in the case report form and have determined that it is complete, accurate, and compatible with the source documents

Investigator's name Investigator's signature Date

Unique Identification No:

IDENTIFIERS- FOR CASES

Registration No:

Contact No:

Name:

Father's Name /Husband's Name:

Address:

DEMOGRAPHICS- CASES

Age:

Sex: Male ☐ Female ☐

Place of Residence: Urban ☐ Rural ☐

Social / Economical Status: a) Upper b) Upper Middle c) Lower Middle
d) Upper Lower e) Lower

Education: a) Illiterate b) Primary c) Middle d) High School e) Intermediate
f) Graduation g) Post-graduation & above

ANTHROPOMETRIC PARAMETERS- CASES

Height (mts) ☐

Weight (kgs) ☐

Body Mass Index (kg/ m^2) ☐

Unique Identification No:

INTEGRAL INSTITUTE OF MEDICAL SCIENCES AND RESEARCH

LUCKNOW -226026

SELECTION OF CONTROLS

Inclusion Criteria

S.N.	Criteria	YES	NO
1.	Apparently healthy individuals		
2.	Subjects between the age of 30 to 65 years		

The subject is eligible for the study if all **INCLUSION** criteria are **YES** and all **EXCLUSION** criteria are **NO**.

INVESTIGATOR STATEMENT

I have verified the data entered in the control report form and have determined that it is complete, accurate, and compatible with the source documents

Investigator's name Investigator's signature Date

Unique Identification No:

IDENTIFIERS- CONTROL

Registration No:

Contact No:

Name:

Father's Name /Husband's Name:

Address:

DEMOGRAPHICS- CONTROL

Age: ☐

Sex: ☐ Male ☐ Female ☐

Place of Residence: Urban ☐ Rural ☐

Social / Economical Status:

Education: a) Illiterate b) Primary c) Middle d) High School e) Intermediate

f) Graduation g) Post-graduation & above

ANTHROPOMETRIC PARAMETERS- CONTROL

Height (mts.) ☐

Weight (kgs.) ☐

Body Mass Index (kg/ m^2) ☐

Unique Identification No:

INFORMED CONSENT FORM (FOR CASE)

Study title: Evaluation of vitamin D diagnosed cases of Hypothyroidism and control subjects

Subject's name…… Age…… Sex……

I confirm that I have read and understood/have been explained the information given by the researcher/moderator and I had an opportunity to ask questions. I understand that participation in the study is voluntary and I am free to withdraw at any time without giving any reason and without my medical care and legal rights being affected. I understand that my identity will not be revealed to any third party or in publication. I understand that the researchers/ regulatory authorities/ ethics committee will not need my permission to access my health records if necessary for the current study. I agree not to restrict the use of any data or results that arise from this study provided such use is only for the scientific purpose(s). I agree to take part in the above study.

Signature of the subject……..… Date……..

Name of the Investigator (printed) **Shanti Gupta**

Signature of the investigator…… Date……

Name and signature of the impartial witness with date if required

……

Unique Identification No:

INFORMED CONSENT FORM (FOR CONTROL)

title Study: Evaluation of vitamin D diagnosed cases of Hypothyroidism and control subjects

Subject's name…… Age…… Sex……

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Signature of the investigator…… Date……

Name and signature of the impartial witness with date if required

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विशिष्ट पहचान संख्या:

सूचित सहमति प्रपत्र (मामले के लिए)

अध्ययन शीर्षक: हाइपोथायरायडिज्म और नियंत्रण विषयों के मामलों में विटामिन डी के मूल्यांकन

विषय का नाम..... आयु ..... लिंग .....

मैं पुष्टि करता हूं कि मैंने शोधकर्ता/मॉडरेटर द्वारा दी गई जानकारी को पढ़ और समझ लिया है/समझा गया है और मुझे प्रश्न पूछने का अवसर मिला है। मैं समझती हूं कि अध्ययन में भाग लेना स्वैच्छिक है और मैं बिना कोई कारण बताए और अपनी चिकित्सा देखभाल और कानूनी अधिकारों को प्रभावित किए बिना किसी भी समय वापस लेने के लिए स्वतंत्र हूं। मैं समझती हूं कि मेरी पहचान किसी तीसरे पक्ष या प्रकाशन में प्रकट नहीं की जाएगी। मैं समझती हूं कि वर्तमान अध्ययन के लिए यदि आवश्यक हो तो शोधकर्ताओं/नियामक प्राधिकरणों/नैतिकता समिति को मेरे स्वास्थ्य रिकॉर्ड तक पहुंचने के लिए मेरी अनुमति की आवश्यकता नहीं होगी। मैं इस अध्ययन से उत्पन्न होने वाले किसी भी डेटा या परिणामों के उपयोग को प्रतिबंधित नहीं करने के लिए सहमत हूं, बशर्ते ऐसा उपयोग केवल वैज्ञानिक उद्देश्यों के लिए हो। मैं उपरोक्त अध्ययन में भाग लेने के लिए सहमत हूं।

विषय के हस्ताक्षर ..... तारीख .....

अन्वेषक का नाम (मुद्रित) शांति गुप्ता

अन्वेषक के हस्ताक्षर ..... तारीख .....

यदि आवश्यक हो तो तिथि के साथ निष्पक्ष गवाह का नाम और हस्ताक्षर

.....

विशिष्ट पहचान संख्याः

सूचित सहमति प्रपत्र ((नियंत्रण के लिए)

अध्ययन शीर्षकः हाइपोथायरायडिज्म और नियंत्रण विषयों के मामलों में विटामिन डी के मूल्यांकन

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विषय के हस्ताक्षर ..... तारीख .....

अन्वेषक का नाम (मुद्रित) शांति गुप्ता

अन्वेषक के हस्ताक्षर ..... तारीख .....

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.....

INSTITUTIONAL ETHICS COMMITTEE (IEC)

IIMS&R INTEGRAL UNIVERSITY, LUCKNOW

IEC/IIMS&R/2023/67

CERTIFICATE

This is to certify that research work entitled **"Evaluation of Vitamin D in Diagnosed Cases of Hypothyroidism and Control Subjects"** submitted by **Shanti Gupta, Dr. Priyanka Thapa Manger** for ethical approval before the Institutional Ethics Committee IIMS&R.

The above mentioned research work has been approved by Institutional Ethics Committee, IIMS&R with consensus in the meeting held on **30th December 2022**.

Dr.Q.S.Ahmed
(Member Secretary)
IRC/IEC
IIMS &R

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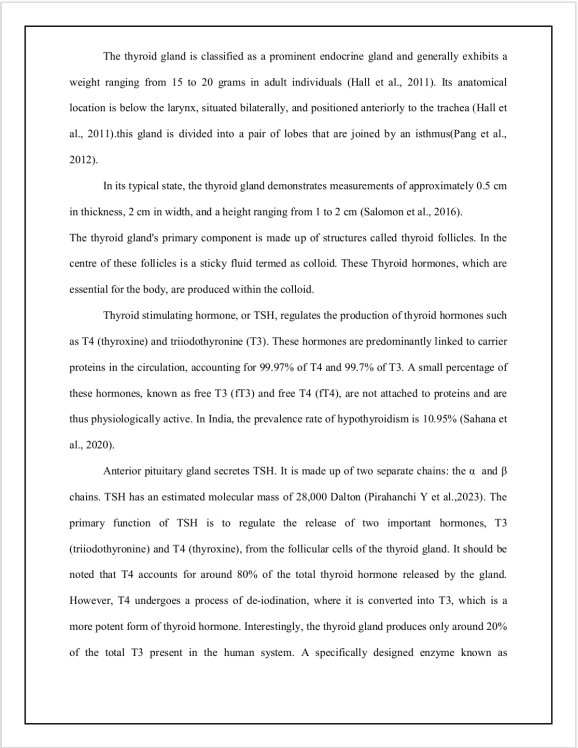

The thyroid gland is classified as a prominent endocrine gland and generally exhibits a weight ranging from 15 to 20 grams in adult individuals (Hall et al., 2011). Its anatomical location is below the larynx, situated bilaterally, and positioned anteriorly to the trachea (Hall et al., 2011).this gland is divided into a pair of lobes that are joined by an isthmus(Pang et al., 2012).

In its typical state, the thyroid gland demonstrates measurements of approximately 0.5 cm in thickness, 2 cm in width, and a height ranging from 1 to 2 cm (Salomon et al., 2016).

The thyroid gland's primary component is made up of structures called thyroid follicles. In the centre of these follicles is a sticky fluid termed as colloid. These Thyroid hormones, which are essential for the body, are produced within the colloid.

Thyroid stimulating hormone, or TSH, regulates the production of thyroid hormones such as T4 (thyroxine) and triiodothyronine (T3). These hormones are predominantly linked to carrier proteins in the circulation, accounting for 99.97% of T4 and 99.7% of T3. A small percentage of these hormones, known as free T3 (fT3) and free T4 (fT4), are not attached to proteins and are thus physiologically active. In India, the prevalence rate of hypothyroidism is 10.95% (Sahana et al., 2020).

Anterior pituitary gland secretes TSH. It is made up of two separate chains: the α and β chains. TSH has an estimated molecular mass of 28,000 Dalton (Pirahanchi Y et al.,2023). The primary function of TSH is to regulate the release of two important hormones, T3 (triiodothyronine) and T4 (thyroxine), from the follicular cells of the thyroid gland. It should be noted that T4 accounts for around 80% of the total thyroid hormone released by the gland. However, T4 undergoes a process of de-iodination, where it is converted into T3, which is a more potent form of thyroid hormone. Interestingly, the thyroid gland produces only around 20% of the total T3 present in the human system. A specifically designed enzyme known as

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deiodinase play role in peripheral conversion which release the remaining 80% of T3. (Delitala AP. et al.,2019).

TSH functions via a signalling route cAMP second messenger system. AMP is converted into cAMP in this mechanism. TSH also activates another signalling cascade known as the IP3 signalling cascade, which results in calcium being released from its stores. Both the cAMP and IP3/Ca2+ cascades have sequential effects that increase thyroid hormone synthesis and stimulate thyrocyte growth and development. These signalling pathways play an essential part in controlling the many physiological processes involved in production of T3 and T4. (Pirahanchi Y et.al., 2023).

Less than 1% of thyroid hormones are unbound in the bloodstream, while over 99% of them attach to thyroid-binding globulin, pre-albumin, and albumin (Delitala AP et. al.,2019). T4 and T3 absorption and outflow are mainly carried out by specialized transporters found on cell membrane. Only fT4 and fT3 can be transferred into thyroid hormone-target cells.

Iodine is necessary for thyroid production. Imbalance of iodine amount can also cause hypothyroidism; however, overt hypothyroidism is rare. T4 and T3 absorption and outflow are mainly carried out by specialized transporters found on cell membrane.

India is considered to have 'optimal' iodine dietary habits, with more than eighty-three percent of families in urban areas and 66.1% in rural region receiving appropriate iodized salt data given WHO report publish in 2004 (Unnikrishnan A.G. et al., 2013).

Hypothyroidism is a thyroid gland dysfunction characterized by decreased thyroid hormone T3 and T4 synthesis and release. Regardless of Thyroid hormone production, its action has been linked to clinical hypothyroidism. All organ systems, including the skin and appendages, cardiovascular, respiratory, gastrointestinal, central, and peripheral nervous systems,

skeletal (calcium and phosphorus metabolism), renal, pituitary, and energy metabolism, can be impacted by hypothyroidism. (Shlomon melmed et al., 2016).

The two basic kinds of hypothyroidism are primary and secondary (central).Iodine shortage is the most frequent cause of primary hypothyroidism, which occurs when the thyroid gland is unable to produce enough thyroid hormones. Impairment function of pituitary gland or hypothalamus is a root cause of central Hypothyroidism; however, thyroid gland is unaffected. Autoimmune thyroid diseases are the leading causes of hypothyroidism in the US and other iodine-sufficient nations. (Taylor, P. N. et al., 2018).

cholecalciferol or ergocalciferol, two forms of Vitamin D that primarily control the calcium and phosphate balance in the body, are induced under the effect of UVB light. Vitamin D3 is synthesized in the skin by a non-enzymatic way (Holick MF, 2007). The CYP27R1 enzyme converts vitamin D to 25-hydroxy vitamin D during hydroxylation in the liver. It is an inactive form with a 15- days half-life that has significance in measuring the serum level of vitamin D.

In the function of CYP27B1 enzyme, hydroxylation occurs in kidney to release the biologically active 1, 25-dihydroxy vitamin D which induces skin cells, osteoblasts, dendritic cells, and thyrocytes in target cells . A number of epithelial cells, immune system cells, and the parathyroid gland are examples of non-renal organs that also express CYP27B1 (Khammissa AG. et al., 2018). According to Bikle D. (2015), PTH stimulates 1,25-dihydroxy vitamin D production in the kidney while calcium, phosphate, and FGF23 suppress it.

Vitamin D act as a steroidal hormone that interacts with the vitamin D receptor (VDR) and triggers a variety of physiological reactions that can be genomic or non-genomic. Due to the fact that VDR is present in various bodily cells, it plays a significant part in the regulation of

vascular tone, activation of cell differentiation and proliferation, inhibition of cell growth, and also shows antioxidant effects. (Bidar et al., 2015; Wiseman, 1993).

It has been noted that vitamin D signaling is disturbed in autoimmune illnesses (Muscogiuri, et al., 2015).

- Vitamin D levels over 30 ng/ml are regarded as normal.
- Vitamin D level is insufficient at 20-29ng/ml (>50 nmol/L).
- Vitamin D levels below 10ng/ml (12.5nmol/L) indicate severe insufficiency. (2nd edition, William et al.).

Some studies found that High TSH levels found in hypothyroid patients are negatively correlated with vitamin D levels. (Zhang et al., 2014). Under normal circumstances, high TSH leads to increased thyroid hormone, which undergoes negative feedback regulation and favors the activity of the vitamin D receptor in thyrocytes, reducing iodine uptake. Thyrotropin-releasing hormone and thyroid-stimulating hormone secretion are regulated as a result of negative feedback (Dietrich et al., 2012). Therefore, it's possible that deficiencies in vitamin D raise the level of TSH in hypothyroidism.

Thyroid hormone, T3 and T4 plays an important role in enhancing cellular metabolic activity as well as the quantity and activity of mitochondria. Thyroid hormone promotes growth, particularly in children. Thyroid hormone stimulates ion transportation across cell membranes, cardiovascular function, the production of a vast variety of structural and transport proteins, and so on (Hall J.E. et al., 2012).

Insufficiency in synthesis and release of thyroid hormone manifest Hypothyroidism.

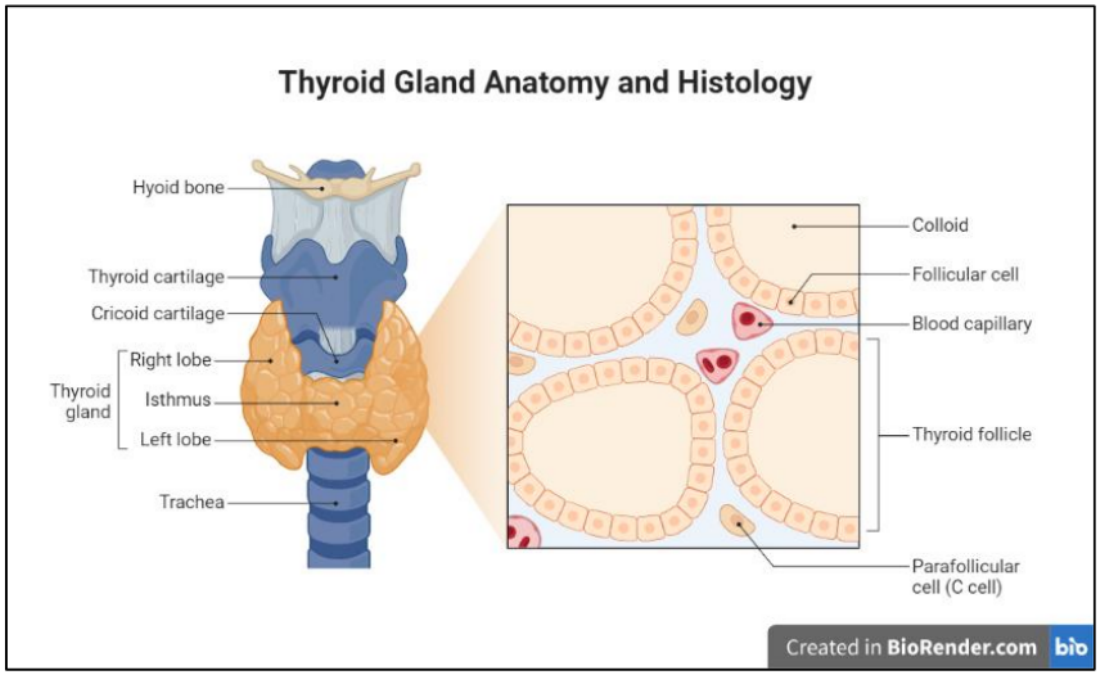

Figure 1- Structure of Thyroid Gland- *It is made up of small structures called follicles, which are surrounded by follicular cells and filled with a substance called colloid. These follicular cells play an important role in trapping iodine, which is then released into the follicle along with a protein called thyroglobulin (Boron W.F. et al., 2017).*

Hypothyroidism is a worldwide endocrine illness that affects about five percent of the population, with 99% of those affected suffering from primary hypothyroidism caused by iodine shortage in the thyrocytes, which causes Hashimoto's disease.

Thyroid hormones synthesis

1. **Synthesis of Thyroglobulin-** The confirmational essential for tyrosyl joining and Iodide organification in the formation of diamino acid thyroid hormones are supplied by a large precursor molecule term as, Thyroglobulin (Anthony P., 2012).
2. **Iodine uptake-** Triiodothyronine and Tetraiodothyronine need essential element iodine to be functional; they are synthesized as part of very big precursor molecules; and kept in a colloid; an intracellular reservoir (Chiovoto L et. al., 2019). Sodium-potassium ATPase are induces the function of Na+-I symporters have role to sent iodide in the thyrocytes which transportation regulated by phosphorylation of protein kinase A. Pendrin which is an iodide transporter helps to iodide reach to colloid mainly present on the apical side of cell . (Shahid, M. A. et al., 2018).
3. **Iodination of Tg -** Protein kinase A also phosphorylates and induces thyroid peroxidase, which performs the following roles: oxidation, organification, and coupling.
   (a) **Oxidation**- TPO uses hydrogen peroxide to oxidise iodide into I_2. H_2O_2 is generated by the apical enzyme NADPH-oxidase for thyroid peroxidase.
   (b) **Organification**- Thyroglobulin protein's tyrosine residues bind iodine through TPO. Monoiodotyrosine and diiodotyrosine are released as a result.Double residues of tyrosine are present on DIT,while MIT has only one tyrosine residue with iodine (Shahid, M. A. et al., 2018).Inefficient precursors, monoiodotyrosine (MIT) and diiodotyrosine (DIT), contribute to approximately 70% of the iodide in thyroglobulin.
4. **Coupling action**- thyroid peroxidase enzyme have role in combining of tyrosine residues with iodine to produce thyroid hormones. T4 gets formed when two DIT molecules interact, whereas T3 is synthesised when MIT and DIT both join together.

5. **Storage of thyroid hormone** – It is stored in lumen of follicle by binding to Tg**.**
6. **Release-** Thyrocytes release thyroid hormones in the manner described below into the interconnected system of capillaries:
   (a) Via endocytosis thyroglobulin iodinate form reaches to cell of thyroid gland.
   (b) Fusion occure between the lysosome and endosome that contains iodinated thyroglobulin.
   (c) Endolysosome proteolytic enzymes break thyroglobulin into T3, T4, MIT and DIT.
   (d) The MCT8 transporter transports 20% T3 and 80% of T4 into the thin capillaries (Schweizer, U. et al.,2013).
   (e) Deiodinase enzyme activity removes small amounts of iodine from diiodotyrosine and monoiodotyrosine. It is possible to recover iodine and add it to a reservoir of iodide inside the cell (Shahid, M. A. et al., 2018).

The basal metabolic rate (BMR) is increased by thyroid hormone. It enhances expression of Na+/K+ ATPase in several tissues, resulting in raised oxygen consumption, rate of respiration, and thermal regulation.

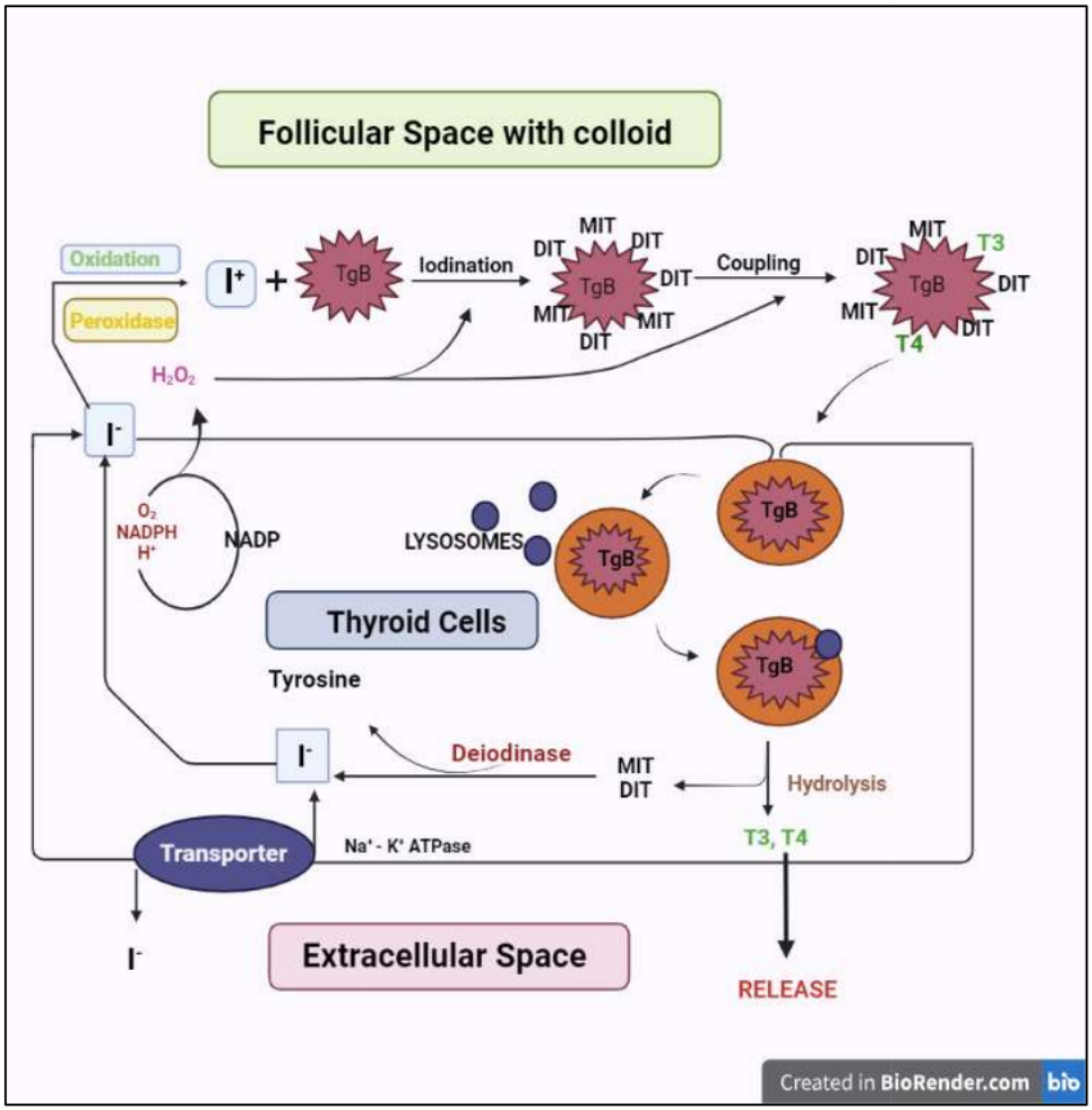

Figure 2- *Iodide mechanism in the production of Thyroid hormone (Anthony P., 2012).*

Regulation of thyroid hormones

TSH specifically regulates the release of T3 and T4 from the thyroid follicular cells. TSH promotes the amount of thyroid hormone secretion by increasing iodide absorption, thyroglobulin synthesis, and thyroperoxidase function. TSH additionally improves blood supply

to the thyroid gland and encourages hypertrophy as well as hyperplasia of thyroid follicular cells, causing the thyroid gland to enlarge (Rousset B. et. al., 2015).

Thyroid-releasing hormone, a hormone that triggers the rostral part of the pituitary thyrotrophs to produce Thyroid stimulating hormone, is secreted by axis of hypothalamus-pituitary which regulates the release of TSH. (Pirahanchi Y. et al.,2023). Thyroid- releasing hormone interacts to its receptors which present on the front part of pituitary gland, activating a sequential signal reaction controlled by serpentine Gpcr . Phospholipase C (PLC) activity is triggred when G alpha q protein is activated. PLC promotes PIP3 and DAG production , are second messengers, which promote intracellular calcium activity and stimulate protein kinase C, resulting in activation of genes and TSH production. TSH causes the follicular cells of the thyroid to secrete T4 about 80% T3 is approx 20%. However, Somatostatin is a hypothalamic hormone that suppresses the secretion of TSH from the anterior part of the pituitary. When T4 gets into circulation, it undergoes deiodination and is transformed into T3.

Mechanism of actions of Thyroid Hormones

T3 and T4 thyroid hormones inhibit hypothalamus synthesis of TRH and pituitary production of TSH. TSH promotes T3 and T4 synthesis. Thyroid epithelial cells wrap a proteinaceous colloid containing Thyroglobulin in order to form thyroid follicles.

Blood concentrations of T3 and T4 induce a compensatory feedback loop affecting the hypothalamus and pituitary gland. The negative feedback mechanism regulates the concentration of T3 and T4 hormone in the blood and helps the body achieve thyroid homeostasis.

TSH interacts with and activates the TSH receptor (TSHR) on the basolateral side of thyroid follicular cells, which is a G-protein coupled receptor (GPCR). TSHR is linked to both Gs and Gq G-proteins, triggering both the cAMP route (through G- stimulatory alpha) and the IP/Ca2+

(via Gq alpha) second messenger signaling cascades. Iodide absorption, thyroid hormone release, and gland growth and differentiation are all activated by the Gs pathway.

By increasing iodide organification, the Gq path limits Thyroid hormone production. Increase activity of TSH receptor and reduce activity of TSH receptor due to mutation, mainly cause Hyperthyroidism and hypothyroidism, respectively. (Pirahanchi Y et.al.,2023).

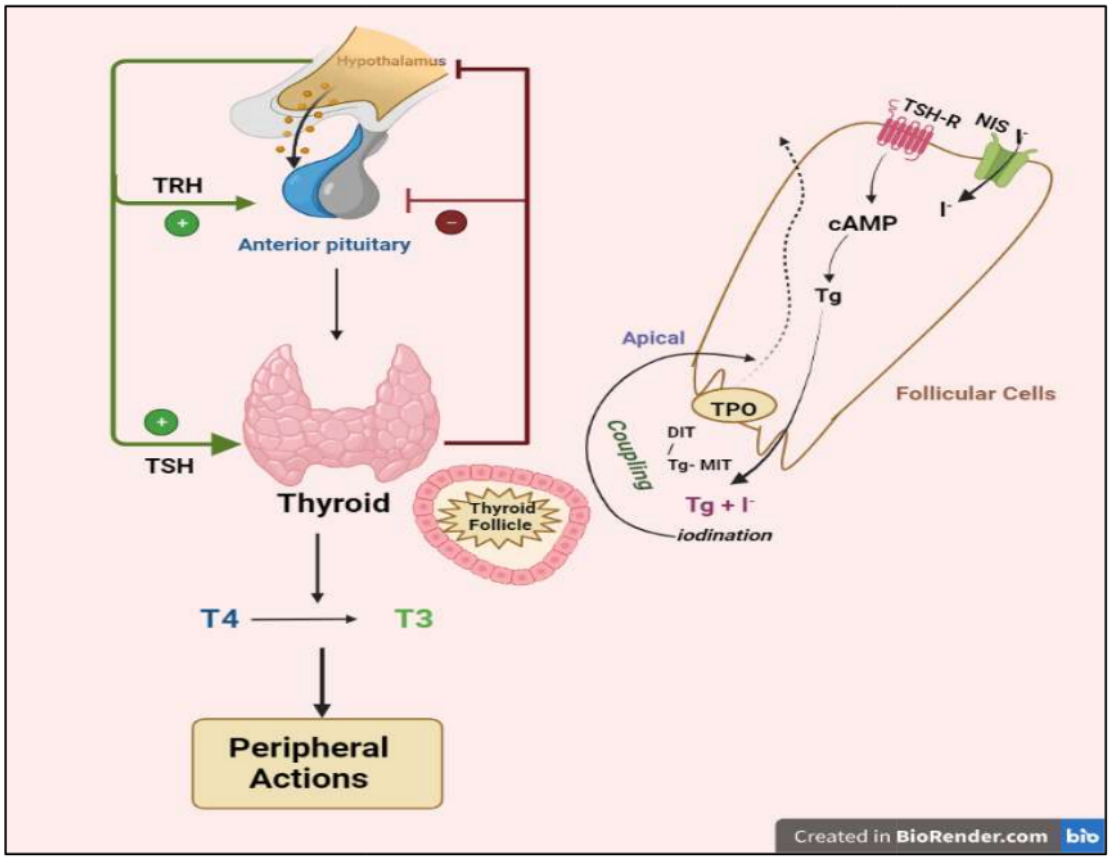

Figure 3- *Feedback Regulation of Thyroid hormone (Jameson J.L. et al., 2011).*

Thyroid receptors that have a greater sensitivity for T3 are affected by interactions between T3 and T4 hormones. **T**4 is hence essentially inactive. DIO1 and DIO2 activity causes T4 to become active T3 and vice versa. While T4 gets transformed by DIO3 activity into rT3

which is inactive form. Deiodinases are mainly three types which found in different cells of body. In the hepatic cells, both renal, skeletal muscles, and thyroid glands have DIO1 and DIO2 deiodinase enzyme respectively, type III Deiodinase are present in central nervous system and placental body (Brent, G. A.,2012).

Transporter of thyroid hormones in the blood

T3 and T4 bind to plasma carrier proteins as they are secreted into the bloodstream. Thyroxine-binding globulin (TBG), transthyretin, and plasma protein albumin are transporter proteins.TBG transports most of the T4 (2/3rd), while thyroxine and retinol are delivered by transthyretin.

About 70% of the circulating thyroid hormones are bound by thyroxin-binding globulin, 20% by prealbumin thyroxine binding and 10% by albumin. In healthy subjects, roughly 0.02 percent of thyroxine and 0.2 percent of T3 are free from the plasma. (Colin et al., 2015).

Hypothyroidism

Low levels of circulating thyroid hormones caused by structural and functional defects that limit thyroid hormone secretion characterize an illness known as hypothyroidism. (Salomon et al., 2016)

Hashimoto Thyroiditis is an especially prevalent cause of hypothyroidism in iodine-deficient locales.The thyroid gland is damaged as a result of the immunological process. cytotoxic T-cells are responsible for thyroid follicular death (Shahid, M. A. et al., 2018).In its last stages, atrophic thyroiditis, or autoimmune thyroiditis, there may be little or no thyroid tissue left over. because the autoimmune process ultimately diminishes thyroid function. A surge in TSH maintains normal thyroid hormone levels during the compensating period. Although some people may experience minimal symptoms, this condition is known as subclinical

hypothyroidism.Later, fT4 levels drop and TSH levels rise even more; at this point, overt hypothyroidism is known and symptoms are more obvious (usually TSH more than10 ml/L) (Jameson J.L et al., 2011).

Following factors can cause hypothyroidism

1. **Primary hypothyroidism:** This is caused by the thyroid gland producing inadequate thyroid hormone. This is the most widespread form of hypothyroidism.

Acquired - (a). Arises from Hashimoto's thyroiditis

(b). Deficiency of iodine (endemic goiter)

Congenital - (a)Iodide transport or utilization deficiency (NIS or pendrin mutations)

(b). Insufficiency of iodotyrosine dehalogenase

(c). Disorders of organification (TPO deficit or malfunction)

(d). Defects in the synthesis or processing of thyroglobulin.

2. Transient Hypothyroidism (Post-Thyroiditis)

3. Consumptive Hypothyroidism- Thyroid hormone is rapidly destroyed in large hemangiomas due to D3 expression.

4. Central Hypothyroidism:

Acquired- (a) Secondary pituitary origin.

(b) Tertiary hypothalamic disorders

Congenital- (a) TSH shortage or structural defect

(b) TSH receptor abnormality (Gregory A. et al., 2015).

Epidemiology

According to data from the National Health and Nutrition Examination Survey, 4.6%8 of Americans have hypothyroidism, which can be overt or subclinical (Brito, J. P. et al., 2021).

According to the NFHS IV (2015–2016), less than 1% of males and almost 2 percent of females between the ages of 15 and 49 reported having a thyroid disease or goitre, respectively. Additionally, the stated incidence in women (fifteen to nineteen years:0.7%; twenty to thirty four years: 1.8%; thirty five to fourty nine years: 3.4%) increased with age. A prevalent condition, hypothyroidism affects 1% of the overall population and 5% of people over the age of 60 (Banday T.H., 2016).

India is considered to have 'optimal' iodine dietary habits, with more than eighty-three percent of families in urban areas and 66.1% in rural region receiving appropriate iodized salt data given WHO report publish in 2004 (Unnikrishnan A.G. et al., 2013).

Signs and common symptoms associated with Hypothyroidism

Hypothyroidism typically takes long time to develop, and symptoms may not become apparent until much later. The most frequent symptoms, which are frequently vague, are fatigue, aversion to the cold, and constipation.The hallmark of diagnosis is the detection of elevated TSH and low fT4 levels. because there, a wide range of manifestations and the diagnosis of hypothyroidism is established with minimal specificity and high accuracy (i.e., hypothyroidism did not show unique symptoms). (Canaris, G. J. et al., 1997).

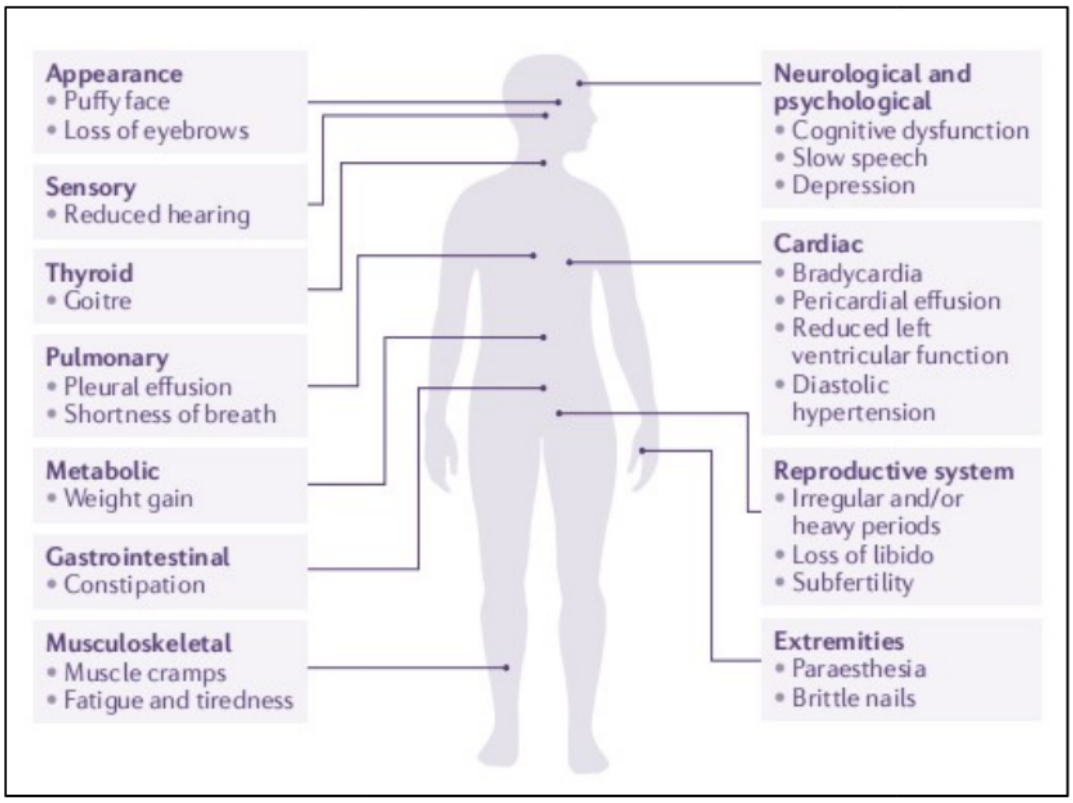

Figure 4- *Signs and Common symptoms associated with impaired thyroid hormone production (Hypothyroidism)(Chaker. L et al., 2022).*

Diagnosis of Hypothyroidism

The initial step in evaluating suspected primary hypothyroidism is to determine their blood TSH levels. To distinguish subclinical or overt hypothyroidism, thyroid hormone on periphery should be assessed if serum TSH levels are consistently raised. For patients with overt hypothyroidism, levothyroxine medication is required. Patients suffering subclinical hypothyroidism who have not begun LT4 medication should have their thyroid function checked on a regular basis (Chaker L. et al.,2022).

Along with a low amount of thyroid hormones and reduced TSH concentration enhances the possibility of central hypothyroidism, and the Activity of the pituitary should be evaluated (Chaker L. et al., 2022).

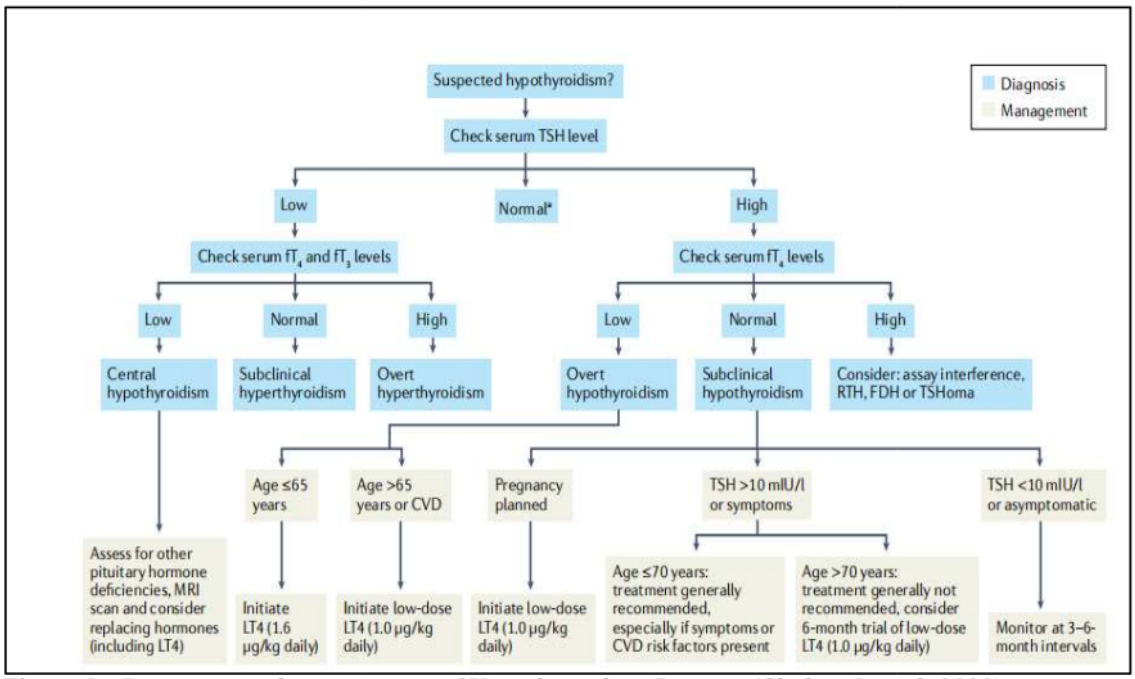

Figure 5- *Diagnosis and management of Hypothyroidism Patient (Chaker. L et al.,2022).*

Vitamin D synthesis and regulation

The Key suppliers of vitamin D, a fat-soluble molecule, are diet or synthesis in the skin (Holick MF, 2007). Ergocalciferol and cholecalciferol are the two most significant vitamin D derivatives. A sizeable proportion of vitamin D is transformed from 7-dehydrocholesterol to vitamin D3 after skin contact with ultraviolet B. Additionally, between ten and twenty percent of vitamin D—either D2 or D3—is acquired by diet. (Mithal A. et al., 2009). The predominant circulating form of vit. D that utilised to determine one's overall vitamin D conc. is 25-hydroxyvitamin D, which forms when vitamin D reaches in the hepatic cells and then hydroxylated(Dusso AS et al., 2005).

By activity of CYP27B1 in the kidney converts inactive vitamin D into functional vitamin D (Cythenia Aranow, 2011). IFN and other cytokines such as TNF, Interleukin-1, and 2 can stimulate circulating monocytes to produce 1, 25-dihydroxy vitamin D via the JAK/STAT, p38 MAPK, and NFB signalling pathways.PTH has not been demonstrated to inhibit macrophage CYP27B1 activity (Gyetko et al., 1993).

Ergocalciferol and cholecalciferol are two fat-soluble kinds of vit D. With identification of its receptors in many tissues, several novel biological roles for vitamin D have been found are becoming more apparent, and its significance in many human diseases such as cancer, diabetes, hypertension, cardiovascular disease, and immunological and dermatological diseases is being thoroughly investigated. The non-conventional activity of vitamin D includes regulating the growth of cells, differentiation, cell death, and both adaptive and innate immunity (Sutton AL, 2003).

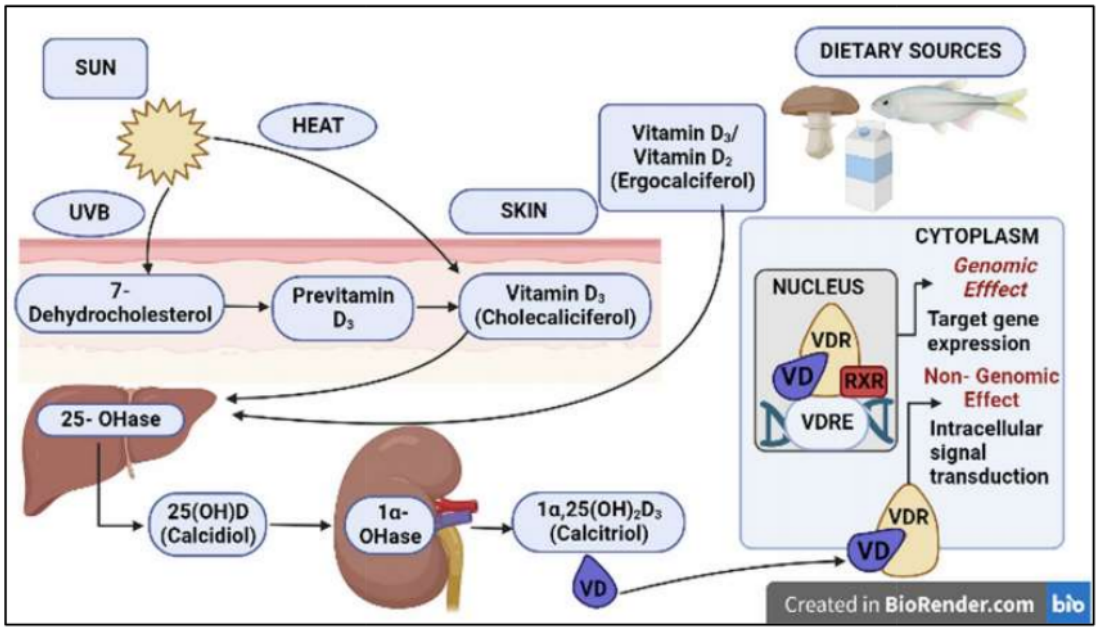

Figure 6- *Synthesis and mechanism of Vitamin D (Awasthi R. et al.,2023).*

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Mechanism of action of vitamin D

Vitamin D act as steroidal hormone that interacts with VDR and induces genomic/non-genomic reactions for a wide range of cellular processes. VDR is additionally identified in various body cells, therefore it plays a significant function in vascular tone modulation, differentiation of cells induction, development of cells suppression, proliferation, and antioxidant benefits (Wiseman, 1993 and Bidar et al., 2015).VDR is found in numerous cells of the body, including some immune cells, demonstrating vitamin D and immune system share association (Sutton AL, 2003).

VDR has been identified in osteoblasts, the cells that make bone. 1, 25-dihydroxy vitamin D has been known to affect osteoblasts thus taking part in protein synthesis. The type of proteins formed are necessary for bone formation; they include collagen, alkaline phosphatase and osteocalcin. 1, 25-dihydroxy vitamin D also stimulates the production and function of osteoclasts

via inducing RANKL. As a result, 1, 25- dihydroxy vitamin D promotes both bone development and resorption of bone.

1, 25-dihydroxy vitamin D is actively involved alongside calcium. It greatly affects calcium entrance in the cell through cell membrane, its transportation and consequent elimination from the cell. A class of calcium binding proteins known as calbindins, govern calcium transport across the cell .The CaATPase which are ATP-requiring calcium pump and NCX1 (sodium/calcium exchange protein) perform this role (Bikle, D., 2015).

Non-classical role of vitamin D include cellular growth and differentiation modulation, hormone release regulation, and immunological regulation.

Vitamin D role in Adaptive immunity

Several number of immune cells also express VDR and 1-alpha hydroxylase. T and B cells release cytokines and immunoglobulins, which are involved in adaptive immunity. The cells known as T-helper cells are responsible for the synthesis of Interleukins-2 and INF- and for the proliferation of macrophages (Lemire et al., 1995).

Vitamin D reduces Th1 cytokines whereas increasing Th2 production of cytokines (Baeke F. et al.,2012) and elevating Intrleukins 4, 5 and 10, production in thyrocytes. Vitamin D3 stimulates the synthesis of IL-10 and the translation of the FoxP3 gene, which regulates the development of CD4+/CD25+ regulatory cells (Penna G & Adorni L, 2000).

Vitamin D role in innate immunity

TLRs are transmembrane pathogen recognition receptors found in polymorphonuclear cells, monocytes, epithelial cells, and macrophages that are triggered by an immune system response that is innate (Medzhitov R, 2007).

TLR-activated antimicrobial peptide Cathelicidin and reactive oxygen species help destroy the bacterium (Gombart AF et al., 2005). VDR and 1-alpha-hydroxylase have been identified in macrophages and epithelial cells, and they play a role in active vitamin D formation and the induction of the antimicrobial peptide cathelicidin.(Wang TT et al.,2004). Wang et al. revealed that 1,25dihydroxy vitamin D protects thyroid against apoptosis by enhancing Bcl-2 expression. Vitamin D modulates the immune system by stimulating the natural immune system while reduce function of adaptive immune system.

Vitamin D with hypothyroidism

The 1,25-dihydroxy vitamin D receptor (VDR) is a transactivator that controls gene expression that governs its biological function. Thyroid hormones and VDR both belong to nuclear receptor family, which also contains receptors for ACTH hormones and retinoids.

Prevelent rate of hypothyroidism has been link to variant form of VDR . Because thyroid hormone receptors are also members of the nuclear receptor superfamily, they have comparable mechanisms of action because they share two regions: cysteine-rich 70 and 62 amino acid sequence identified close to protein's carboxyl terminus (McDonnell DP et al., 1988). Thyroid and VDR produce a heterodimer complex with RXR and bind to DNA hormone response, either activating or suppressing transcription of the targeting gene (Christakos S., 2010).

The existence of VDR (Vitamin D receptors) in macrophages and other immune cells demonstrates that vitamin D and the immune system interact(Sutton AL & MacDonald P.

N., 2003). In autoimmune thyroid diseases and thyroid cancer vitamin D signaling is impaired been reported (Muscogiuri, et al., 2015). Wang et al. (2004), revealed that 1,25dihydroxy vitamin D protects thyroid against apoptosis by enhancing Bcl-2 expression. Vitamin D modulates the immune system by stimulating the natural immune system while reduce function of adaptive immune system. So There is a chance that vitamin D and hypothyroidism are linked.

Only a few research have found a link between vitamin D and hypothyroidism among Indians. As a result, the current study is designed to assess serum vitamin D levels in hypothyroid and control patients. If the findings are consistent with earlier research, they may aid in a better understanding of the pathophysiology and management of hypothyroidism.

Aim

The aim of this study was to evaluate level of serum vitamin D level in diagnosed cases of hypothyroidism and Apparently Healthy control.

Objectives

1. To determine serum vitamin D levels in patients of hypothyroidism and Apparently Healthy control.

2. To correlate the serum vitamin D level between diagnosed cases of hypothyroidism and Apparently Healthy control.

Vitamin D

The difference in the level of vitamin D between controls and cases was not statistically significant ($p = 0.2415$) (Table-1 & fig. 7)

Table 1- Mean and standard deviation of the study groups

25-hydroxy vitamin D (ng/ml)					
Groups	**n**	**Mean**	**Standard Deviation**	***p* - Value**	**Significance**
Controls	30	26.56	±15.11	**0.2415**	No Statistically Significant
Cases	30	21.63	±17.10		

N= Number of controls or cases, p<0.05 is considered statistically significant

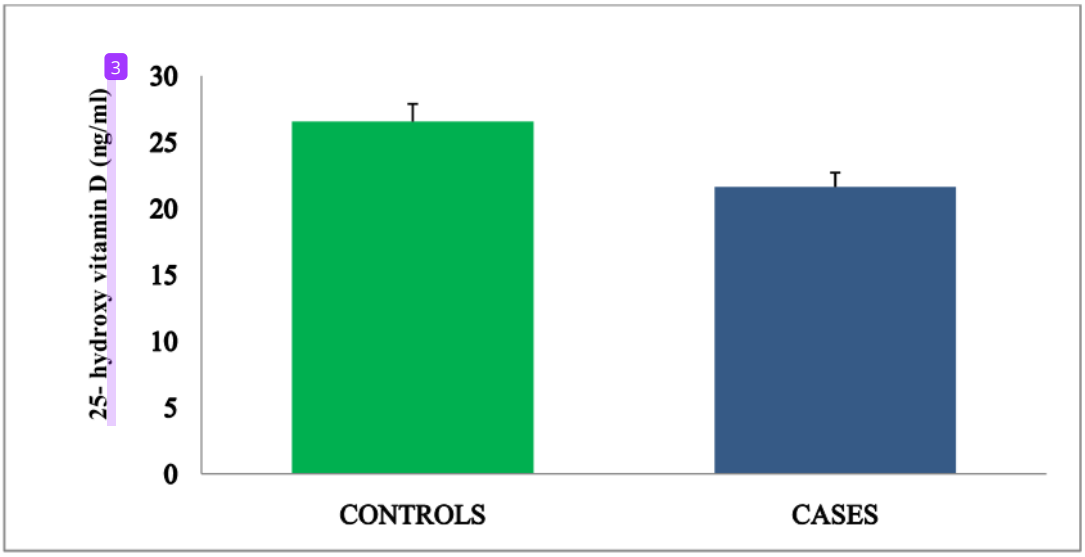

Figure 7- *Comparison of vitamin D levels between controls and cases.*

BODY MASS INDEX (BMI)

The difference in the BMI between cases and controls was statistically significant ($p < 0.001$) (Table- 2 & fig. 9)

Table -2 Mean and standard deviation of the study groups

Body Mass Index (kg/m^2)					
Groups	**n**	**Mean**	**Standard Deviation**	**p- value**	**Significance**
Controls	30	23.91	1.96	<0.001	Statistically Significant
Cases	30	28.4	1.90		

N= Number of cases or controls, $p<0.05$ is considered statistically significant

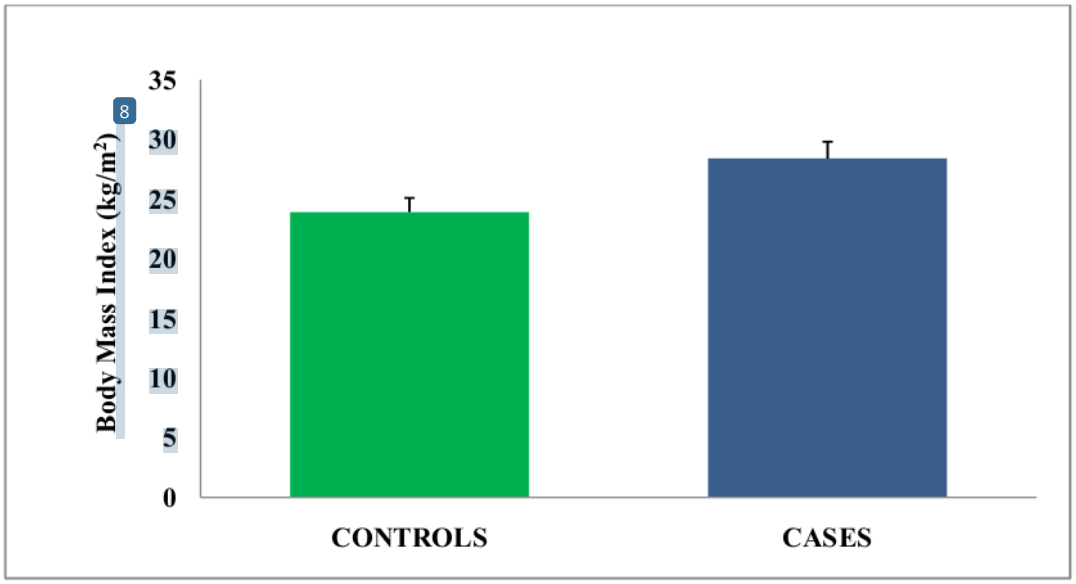

Figure 8- *Comparison of Body Mass Index (BMI) in cases and controls.*

Karl Pearson's coefficient of correlation between vitamin D and Body mass index in cases of hypothyroidism

There was no significant link between vitamin D and BMI in cases of hypothyroidism (p= 0.2).

Table 3- Karl Pearson's coefficient of correlation between cases

		Body Mass Index (kg/m^2)	25-hydroxy vitamin D (ng/ml)
Body Mass Index (Kg/m2)	Pearson Correlation	1	-.241
	Sig. (2-tailed)		.200
	n	30	30
25 hydroxy vitamin D ng/ml	Pearson Correlation	-.241	1
	Sig. (2-tailed)	.200	
	n	30	30

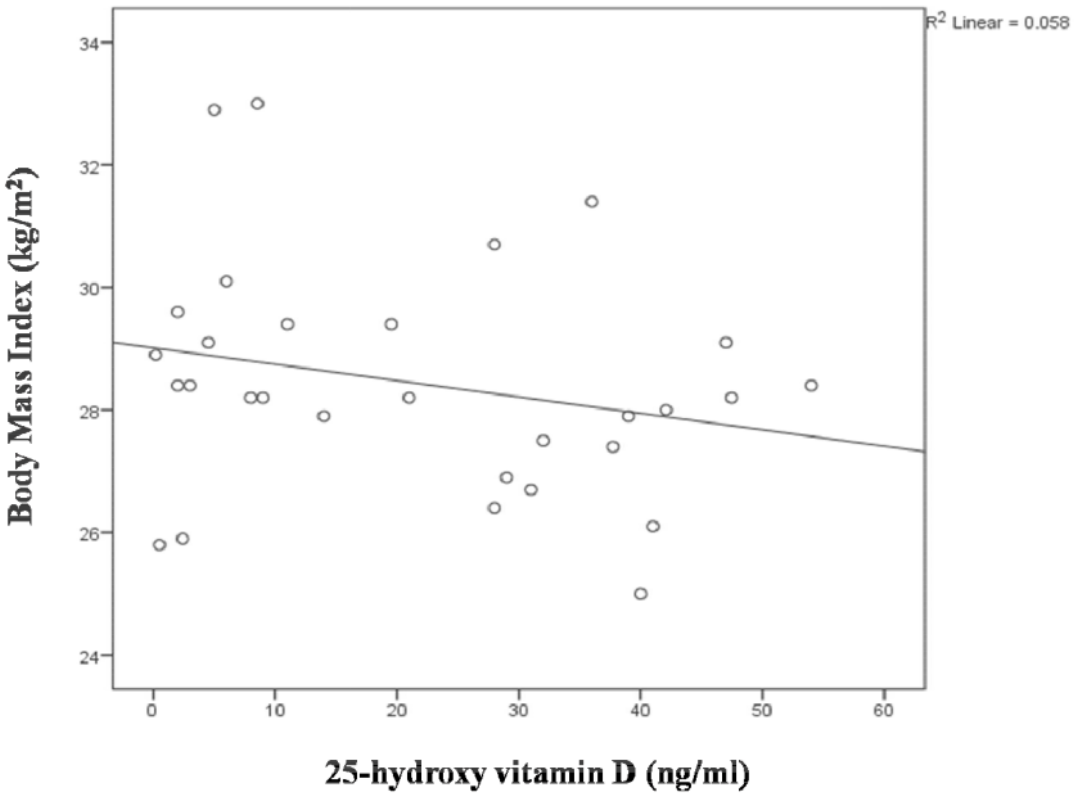

Figure 9- Scatter diagram showing a correlation between vitamin D and body mass index

Low levels of circulating thyroid hormones caused by structural and functional defects that limit thyroid hormone secretion characterize an illness known as hypothyroidism. (Salomon et al., 2016)

A few investigations with conflicting results have been carried out to find a causal relationship between vitamin D levels and hypothyroidism and to find out whether vitamin D deficiency is involved in the development of hypothyroidism or rather a result of the disease (Razvi et al., 2018).

Lohokare R. et al. (2016), suggested in their study that a substantial correlation was identified between serum TSH levels and serum 25-hydroxy vitamin D. Hoda A. et al. (2014), also reported similar findings in their study. This has been supported by the findings of Bozkurt et al. (2013), who observed that vitamin D conc. in Hypothyroidism were considerably low than those in controls. Although a causative association could not be proven, vitamin D have been participate in the pathogenesis of Hypothyroidism.

Our investigation showed, there was not found significant difference in vit.D (25-hydroxy vitamin D) levels between cases and controls (p= 0.2415). Effraimidis et al. (2012), also reported similar findings. Goswami et al. (2009), investigated the prevalence of inadequate vitamin D levels and its link with thyroid hormones. They also reported statistically insignificant relationship between thyroid hormones and vitamin D levels in Hypothyroidism.

Several studies conducted in India, has observed by Velayutham et al.(2015), and Unnikrishnan et al.(2013), found a high proportion of women suffer with hypothyroidism.

In our study, we observed that the BMI was considerably higher in cases as opposed to controls ($p<0.001$). Hypothyroidism has been linked to decreased production of heat and energy expenditure, as well as a higher BMI and a greater incidence of obesity (Danforth, E. et al., 1979). According to Antunes et al. (2006), leptin are induced by TSH which acts directly on

adipocytes, and increased leptin may explain the relationship between Hypothyroidism and Body mass index through the formation of insulin resistance. Furthermore, leptin can influence deiodinase activity in several tissues, influencing thyroid hormone levels (Cabanelas, A. et al., 2007).

In our study we also found there is no correlation between vitamin D and Body mass index in cases of Hypothyroidism (p=.-241). Obesity or increased fat deposition is another risk factor for low vitamin D levels. Vitamin D and other hormones are redistributed in fat tissue, which acts as a large reservoir (Carrelli A.et al., 2017). As a result, conc. of circulating vitamin D drops, resulting in vitamin D deficiency. Liel et al.(1988), discovered that obese people had lower levels of circulation vitamin D than non-obese people.

SUMMARY

The current study aimed to assess serum vitamin D levels in hypothyroid patients and apparently healthy controls.

The parameters estimated were:

- 25-hydroxy vitamin D

The observations in the study are as follows:

- The concentrations of vitamin D was between cases (21.63±17.10) and controls (26.56±15.11)which are not statistically significant (p= 0.2415)
- p-value of BMI between cases(28.40±1.90) and controls (23.91±1.96)are <0.001which are statistically significant
- No correlation found between vitamin D and Body mass index in cases of Hypothyroidism (p= 0.200)

CONCLUSION

Our findings indicate that there is no link between 25-hydroxy vitamin D levels and hypothyroidism. Our sample size is a limitation. More research with a larger sample size will be required to determine whether low vitamin D levels are a cause of hypothyroidism or an effect of the disease.

SHANTI GUPTA

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