DISSERTATION SUBMITTED FOR THE MASTER'S DEGREE IN MEDICAL BIOCHEMISTRY

TITLE

URIC ACID AND TOTAL BILIRUBIN IN HYPOTHYROIDISM PATIENTS AND CONTROL SUBJECTS

SUBMITTED

BY

SMITA SINGH CHAUHAN

2023

DEPARTMENT OF BIOCHEMISTRY

INTEGRAL INSTITUTE OF MEDICAL SCIENCES AND RESEARCH

FACULTY OF HEALTH AND MEDICAL SCIENCES

INTEGRAL UNIVERSITY

LUCKNOW-226026, U.P

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In partial fulfillment of the requirement for the award of degree of
Master of Science
In
Medical Biochemistry

By

SMITA SINGH CHAUHAN

Enrollment No: 2000100622

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CERTIFICATE

This is to certify that **Miss Smita Singh Chauhan,** a student of **M.Sc. Medical Biochemistry**, Integral University has completed her dissertation titled **"Uric Acid and Total Bilirubin in Hypothyroidism Patients and Control Subjects"** successfully. She has completed this work from 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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Declaration by the candidate

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.

I will publish the research paper related to my dissertation only with the consent of my guide.

Date: **Smita Singh Chauhan**

`

Place: Lucknow

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Date: ***Smita Singh Chauhan***

Place: Lucknow, Uttar Pradesh, India.

CONTENTS

LIST OF ABBREVIATIONS

UA	Uric Acid
TB	Total Bilirubin
T3	Triiodothyronine
T4	Thyroxin
fT3	Free Triiodothyronine
fT4	Free Thyroxine
TSH	Thyroid Stimulating Hormone
TRα	Thyroid hormone receptor alpha
TRβ	Thyroid hormone receptor beta
TRE	T3 Response Element
RXR	Retinoid X Receptor

INTRODUCTION

The thyroid gland is an essential endocrine gland that resembles a butterfly in shape. It is located in the neck, positioned around the trachea, just below the larynx. The thyroid gland is composed of two lobes, namely the right lobe and the left lobe, which are connected by a middle structure known as the isthmus.((Chaudhary et al., 2013).

The lobes are around 25 g in weight. It stretches from the first thoracic vertebra to the fifth cervical vertebra. The lobes reach all the way to the fifth tracheal ring from the centre of the thyroid cartilage. The isthmus, which connects the second and third tracheal rings, is 1.2 × 1.2 cm in size (Esen et al., 2018).

One of the major endocrine glands, the thyroid weighs 25 g in adults and 2-3 g in newborns. It grows throughout pregnancy. Each lobe is roughly 5 * 3 * 2 cm in length, width, and thickness. In general, women have larger thyroid glands joined by the isthmus. The isthmus measures about 1.25*1.25 cm in breadth and width and present in front of the 2nd and 3rd ring of trachea. There may be variations in its size and situation (Hall et al., 2011).

TSH (Thyroid Stimulating Hormone) controls the thyroid gland's secretion of the hormones triiodothyronine (T3) and thyroxine (T4). The negative feedback system regulates the hormones' production and secretion. In the bloodstream, carrier proteins are reversibly attached to 99.9% of T4 and 99.7% of T3. Thus, only a small part of these hormones are free in circulation. (Sahana et al., 2020)

Hypothyroidism, which occurs when the thyroid gland is underactive, often presents with symptoms such as bradycardia (slow heart rate), intolerance to cold, constipation, fatigue, and weight gain. On the other hand, hyperthyroidism, resulting from an overactive thyroid gland, is characterized by symptoms such as weight loss, intolerance to heat, diarrhea, fine tremors, and muscle weakness (Malani P.N., 2012).

Iodine is a crucial trace element that is absorbed in the small intestine and plays an integral role in the formation of T3 and T4 hormones. It can be obtained from various sources, including iodized table salt, seafood, seaweed, and certain vegetables. Insufficient intake of iodine can lead to iodine deficiency, resulting in decreased synthesis of thyroid hormones. This deficiency can give rise to conditions such as cretinism, goiter, myxedema coma, and hypothyroidism (Anton et al., 2020).

Hypothyroidism is a prevalent medical condition characterized by an inadequate production of thyroid hormones. If left untreated, it can result in significant detrimental effects on health and, in severe cases, can be fatal. Subclinical hypothyroidism, often considered an indication of early thyroid complication, is defined by elevated TSH levels, while free thyroxine levels remain within ranges considered normal (Cooper et.al.,2012).

Insufficient levels of environmental iodine are the leading cause of thyroid disorders globally, including hypothyroidism (Vanderpump et.al., 2011). In regions with inadequate iodine supply, hypothyroidism occurs as a result of autoimmune thyroiditis; this is known as Hashimoto's disease (Carle et.al. 2006).

Uric acid biosynthesis occurs within the entero-hepatic tissues. It is the byproduct of purine synthesis derived mainly from dietary animal proteins, comprising an exogenous pool. Moreover, uric acid is formed as a result of the degradation of nucleic acids in living and dying cells (Chaudhary et.at., 2013).

Normally, the majority of uric acid elimination takes place through the kidneys. However, humans lack the uricase enzyme necessary for the oxidation of uric acid into the more soluble compound called allantoin. Uricase, also known as urate oxidase, has the ability to metabolize uric acid into a highly soluble form known as 5-hydroxyisourate. Unfortunately, due to the absence of uricase in humans, uric acid remains in its less soluble form, contributing to the propensity for hyperuricemia and the formation of urate crystals in conditions such as gout (Chang et.al. 2014)

Bilirubin is a significant byproduct of heme (ferriprotoporphyrin IX) metabolism, which is a complex that plays a crucial role in iron coordination within different proteins. It is a substance that can potentially be harmful. Nevertheless, the body has evolved mechanisms to safely process and eliminate it. Bilirubin, along with its metabolites, is responsible for giving bile and stool their characteristic yellow color and, to a lesser extent, influencing the color of urine. (Chen et.al, 2016)

Bilirubin originates from two primary sources. Approximately 80% of bilirubin is generated through the process of biodegrading old red blood cells (Dosch et.al., 2019). The remaining portion

arises as a result of the diverse heme-containing proteins present in various tissues being degraded. On estimate, about 4mg of bilirubin is produced per kilogram of weight.

REVIEW

OF

LITERATURE

Hypothyroidism refers to a slight elevation in TSH levels. In contrast, clinical hypothyroidism, or overt hypothyroidism, occurs when TSH levels are higher and free T4 levels are low, resulting in more noticeable symptoms.

Epidemiology of Hypothyroidism

Evidently, hypothyroidism occurs in the general population ranging from 0-3 % in the United States and from 3 to 7% in Europe. In Europe, the prevalence is typically between 0 to 2%, although it can reach up to 5 to 8% in certain regions (Asvold et al., 2013).

Hypothyroidism is more commonly found in females, older individuals (over the age of 65), and among people of Caucasian descent. However, there is limited available data on the variations in prevalence based on ethnicity (Aoki et al., 2007). Occurence is higher among individuals with autoimmune conditions, including type 1 diabetes, autoimmune gastric atrophy, and intestinal diseases. Individuals with Down or Turner syndrome are more at risk. On the other hand, cigarette smokers and alcohol consumers have been found to be at a lower risk of developing the disease. (Carle et al., 2012).

The heritability of serum TSH concentrations is estimated to be around 65%. Similarly, about 23% – 65% of free thyroxine hormone is inherited from parent to offspring.(Panicker et al., 2008).

In India, 11% of people have hypothyroidism. This is more than the 2% occurring in the UK and 46% in the Americas.

. Compared to coastal cities, cities located inland have a higher prevalence (Bagcchi S., 2014).

A higher percentage of females (15.86%) compared to males (5.02%; $P < 0.0001$) were observed to have hypothyroidism. Furthermore, females were more likely to be diagnosed with hypothyroidism than males (Unnikrishnan et al., 2013).

The incidence of subclinical hypothyroidism, which refers to a milder form of hypothyroidism with subtle or no symptoms, tends to increase with age. Data from the Netherlands and the USA indicate that up to 10% of women over the age of 60 years may have subclinical hypothyroidism.

Thyroid hormones

The primary hormones synthesized in the thyroid gland are thyroxine (T4) and triiodothyronine (T3). The hormones play crucial roles in regulating various metabolic processes in the body. The

hypothalamus is involved in releasing thyrotropin-releasing hormone (TRH), that acts to prompt release of thyroid-stimulating hormone (TSH) from the anterior pituitary gland. It is the TSH that exerts its effect on the thyroid gland releasing T4 into the bloodstream. T4 is converted into the more active T3 in target tissues. This intricate interplay between TRH, TSH, T4, and T3 ensures a feedback mechanism and maintains homeostasis within the body (Shahid et al., 2018).

Synthesis in the biosynthesis of thyroid hormones:-

Step 1: Uptake of iodine

The thyroid gland has been known to absorb and concentrate iodine. However, this process can be blocked by thiocyanate and perchlorate, as they compete with iodine for the carrier mechanism. On the other hand, the uptake and concentration of iodine are stimulated by thyroid-stimulating hormone (TSH). In cases of congenital iodine trapping defect, where this process is impaired, large doses of iodine can be administered as a treatment.

Step 2: Oxidation of iodine

The iodide that is absorbed by the thyroid cell undergoes oxidation, converting it into active iodine. . It is crucial to note that this only occurs in the thyroid gland as it is the sole organ capable of carrying out the oxidation step. This process is facilitated by the enzyme thyroperoxidase. To catalyse the reaction, hydrogen peroxide is required, which is produced through a reaction dependent on NADPH.

Step 3: Iodination

Iodination occurs on the thyroglobulin (Tgb) molecule within the follicular space. This process involves the addition of iodine to specific tyrosine residues present in the intact Tgb molecule. As a result, two iodinated products are formed: 3-monoiodotyrosine (MIT) and 3,5-di-iodotyrosine (DIT).

Step 4: Coupling

When two molecules of 3,5-di-iodotyrosine (DIT) combine, they form one molecule of tetraiodothyronine (T4), also known as thyroxine. Triiodothyronine (T3) can be formed from T4 through the deiodination of the outer ring by the enzyme 5'-deiodinase.

Step 5: Storage

The thyroid gland possesses a distinctive characteristic as it is the only endocrine gland that stores significant quantities of hormones. Within the thyroid gland, the hormone is stored in the form of a colloid, which consists of thyroglobulin. Each thyroglobulin molecule contains approximately 8 residues of thyroxine (T4).

Step 6: Utilization

When the need arises, thyroglobulin is internalized from the colloid within the thyroid acini into the cells through a process called endocytosis.

Step 7: Hydrolysis

Tetraiodothyronine (T4) is released through hydrolysis mediated by specific proteases. These protease enzyme are affected by TSH. However, the hydrolysis process is inhibited by iodide, and as a result, potassium iodide (KI) is employed as an adjunctive treatment in hyperthyroidism.

Specific proteases hydrolyze the T4 to release it. TSH notably improves this action.

Step 8: Release

The generated T4 is subsequently released into the bloodstream. T3, on the other hand, is formed through deiodination at the 5' position, which occurs either within the thyroid cell itself or in the peripheral tissues.

Step 9: Salvaging of Iodine

Any unused monoiodotyrosine (MIT) and diiodotyrosine (DIT) molecules are subjected to deiodination and then recycled within the cell for further utilization

Step 10: Transport of Thyroid Hormones

Proteins in the plasma serve as carriers for thyroid hormones during transportation. While the bound form of thyroid hormones is biologically inactive, they can be quickly released when needed. The total protein-bound iodine (PBI) concentration is approximately 10 mg/dL, with T4 accounting for 8 mg/dL of this total.

Step 11: Catabolism of Thyroid Hormones

T4 functions as a prohormone and undergoes deiodination to form T3. This deiodination process occurs in the peripheral tissues and is facilitated by a specific enzyme known as deiodinase, which contains selenium. Some of the T3 and T4 hormones are conjugated with glucuronic acid and eliminated from the body through bile, while a smaller amount is excreted in urine (Bieberman et al., 2020).

Mechanism of Action of Thyroid Hormone

There are two distinct genes, TRα and TRβ, on different chromosomes (3 and 17, respectively, in humans) that are responsible for encoding thyroid receptors. Multiple thyroid receptor isoforms are produced by alternative splicing from each gene, including TRα2, TRα1, and TRα3 from the TRα gene and TRβ1 and TRβ2 from the TRβ gene. (Zhang et al., 2000)

Through DNA response elements, a TR's primary role is to control the expression of its target genes therefore acting as a transcription factor. The repeating DNA sequences in various configurations make up the T3 response element (TRE). Although thyroid hormone receptors (TRs) can be bound to TREs as homodimers or monomers, the predominant TR-TRE heterodimer is the retinoid X receptor (RXR). The target gene's transcription is activated by the TR's conformational change in response to ligand interaction. The molecular processes of thyroid hormone activity have been significantly elucidated thanks to advances in our understanding of the structure, dynamics, and interactions of thyroid hormone receptors (TRs) and the proteins that interact with them. (Zhang et al., 2000)

THYROID HORMONE'S BIOLOGICAL ACTIVITIES:

The thyroid hormones have several purposes;

1. Thyroid hormone regulation of calorigenesis and basal metabolic rate.
2. Increasing the metabolism of mitochondria.
3. Promoting healthy neural development and growth.
4. Improvement of sexual maturation and growth.
5. Myocardial contractions and an increased heart rate are also associated with adrenal stimulation.

6. Increasing the production and breakdown of triglycerides and cholesterol.
7. Increasing vitamin needs.
8. Enhancing calcium and phosphorus metabolism.
9. Making catecholamine more sensitive to adrenergic receptors. In 2016 (Salomon et al.)

ETIOLOGY OF HYPOTHYROIDISM

Hypothyroidism is classified into two primary categories: primary hypothyroidism and secondary (or central) hypothyroidism.

Primary hypothyroidism: - It is characterized by decreased levels of thyroid hormone in the bloodstream, primarily caused by the destruction of the thyroid gland. This destruction can occur as a result of autoimmune disorders, when the body's immunity fails to recognize self and launches an immunes attack on the thyroid gland. Additionally, interventions such as surgery, radioiodine therapy, or radiation treatments can also lead to the destruction of the thyroid gland, resulting in primary hypothyroidism (Patil et al., 2021).

When diagnosed based on distinctive features, primary hypothyroidism is identified by elevated levels of thyroid-stimulating hormone (TSH) in the blood and decreased levels of thyroxine (T4). Subclinical hypothyroidism, on the other hand, is diagnosed when serum TSH levels are elevated, but serum T4 levels remain within the normal range and there are no apparent symptoms of thyroid dysfunction.

Secondary hypothyroidism: - It occurs as a result of a dysfunction in the pituitary gland hypothalamus. This dysfunction affects the release of TSH from the anterior pituitary that has a downstream effect on the thyroid hormones. Secondary hypothyroidism occurs when the dysfunction cause originate from the pituitary gland and tertiary hypothyroidism when the causes arise from the hypothalamus.

Primary and Secondary Hypothyroidism (Patil et al., 2021).

A. Primary Hypothyroidism Causes;

- Hashimoto's disease
- Iodine deficiency
- Surgical removal of thyroid gland
- External radiotherapy
- Drugs
- Thyroid agenesis or dysgenesis
- Therapy with radioactive iodine

B. Secondary Hypothyroidism Causes;

a. Pituitary

1. Non-cancerous tumor
2. Radiotherapy
3. Head trauma
4. Pituitary apoplexy

b. Hypothalamus

1- Hypothalamic tumor

2- Radiotherapy

SYMPTOMS:-

Typical symptoms & signs include:-

- Tiredness
- Inactivity
- Obesity
- Stomach upsets
- Pitch variation
- Rough skin

Diagnosis of hypothyroidism:

TSH concentration values exceeding the normal range and free thyroxine hormone concentrations falling short of the known normal value characterize primary hypothyroidism.. (Chaker et al., 2017) For diagnosis of hypothyroidism the standard test done for screening hypothyroidism is the estimation of serum TSH and also estimation of T3 and T4 will diagnose the type of hypothyroidism.

Thyroid hormone therapy is used to treat hypothyroidism. The medication of choice is levothyroxine. It is an artificial type of 'T4' created in a lab that is identical to the T4 the thyroid naturally produces. Not all medications for thyroid hormones are similar. You ought to stick with the same brand if at all possible. The requirement for thyroid-related hormone replacement is typically permanent. TSH blood tests should be repeated if the medication needs to be altered for any reason. Your TSH testing will be used to adjust your dose. Thyroid hormone levels that are too high in the long run can cause bone loss, irregular heart rhythms, and improper cardiac function. If your dose is insufficient, the signs could not get better. Dose modification may. If your dose is too low, your symptoms could not get better. Adjusting the dosage may be required throughout the pregnancy and at other times as well; this can be discussed with your doctor during your routine checkups.

Hypothyroidism can have wide-ranging effects on various organ systems in the body, including the skin and its appendages, cardiovascular, respiratory, central and peripheral nervous systems, skeletal system (in terms of calcium and phosphorus metabolism), renal system (in terms of water and electrolyte metabolism), pituitary glands, and energy metabolism (Shlomon Melmed et al. in 2016)

Bilirubin is a byproduct of heme breakdown in the body and can be neurotoxic in infants (Ostrow J.D et al., 2004). However, bilirubin also possesses beneficial properties in different diseases, including antioxidative, anti-inflammatory, and immunosuppressive functions (Kinderlerer, A. R. et al., 2009).

Normal healthy individuals produce 110nmol of T4 and 10nmol of T3 per day (Larsen, P. R et al.,1975).

The metabolism of thyroid hormones is regulated by three groups of enzymes: Type 1 (D1), Type 2 (D2), and Type 3 (D3). These enzymes play different roles in the conversion and regulation of thyroid hormones. They form part of the iodothyronine seleno-deiodinase enzyme system. Type 1 deiodinase is primarily present in the hepatocytes and renal tissues (Sanders, J. P. et al., 1997). Its main role is to activate T4 to T3, inactivate T4 to reverse T3 (rT3), and also forms part of the conversion of rT3 and T3 to T2. Type 1 deiodinase forms majority of the T3 produced outside of the thyroid gland; about 30%.

While both the D1 and D2 enzyme systems have the capability to inactivate thyroxine and triiodothyronine hormones, the principle enzyme involved is the type 3 deiodinase (Tu, H. M et al., 1999).

The liver synthesizes many plasma proteins that bind to the lipophilic thyroid hormones creating a pool of active hormones. These plasma proteins, including thyroxine-binding globulin and albumin, bind to over 99% of the thyroid hormones in the bloodstream. However, the concentration of free hormones in various tissues varies based on the specific transport mechanisms and deiodinase activity within each tissue. (Bianco, A. C et al., 2002).

Total Bilirubin and Hypothyroidism

There is evidence suggesting that hypothyroidism can greatly affect the liver. Experimental studies have shown that in hypothyroidism, the activity of the enzyme involved in the conjugation of bilirubin, is reduced. This reduction in enzyme activity leads to a decrease in the excretion of bilirubin, potentially causing disturbances in bilirubin metabolism (Van Steenbergen et al., 1989).

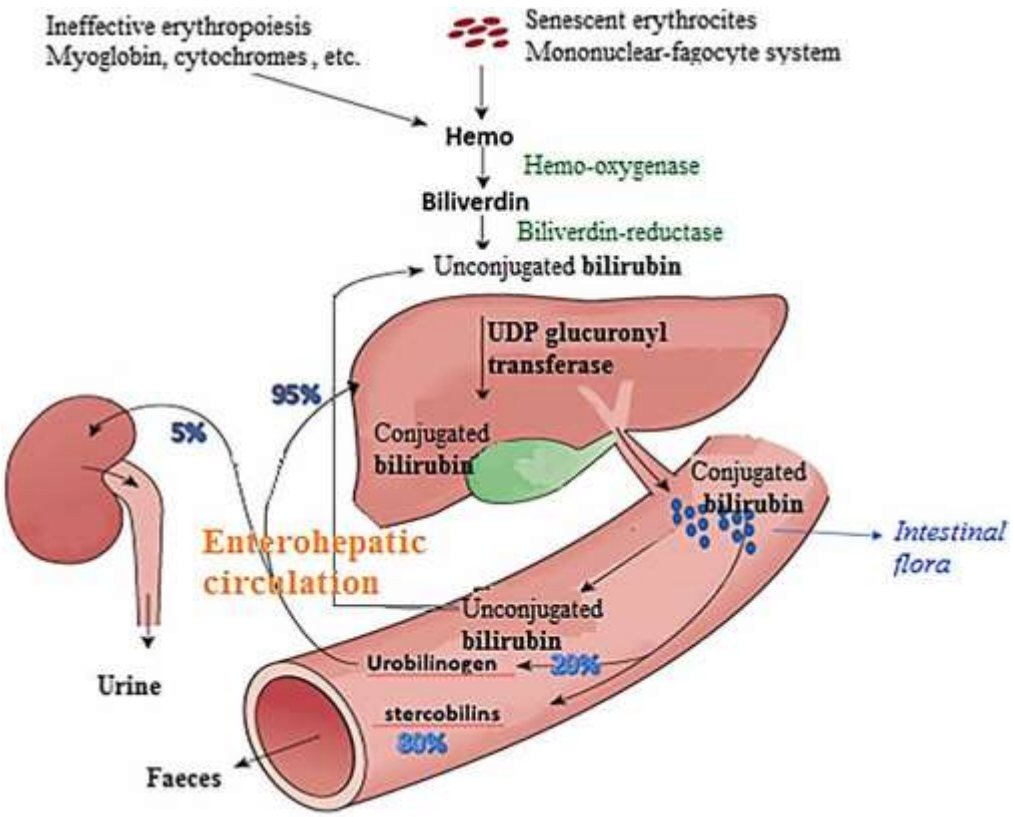


Fig-1 Formation of Bilirubin(Van Steenbergen et al., 1989).

Uric Acid and Hypothyroidism

Uric acid, an antioxidant that is primarily synthesized by the liver (Becker & B. F., 1993), is water-soluble. It plays a role in inhibiting the damaging effects of free radicals and provides protection to cell membranes and DNA (Spitsin, S. V.et al., 2000). The metabolic rate, stimulated by thyroid hormones, is increased by enhancing ATP production for various metabolic processes and by uncoupling oxidative phosphorylation in the mitochondria (Hafner, R. P. et al., 1988).

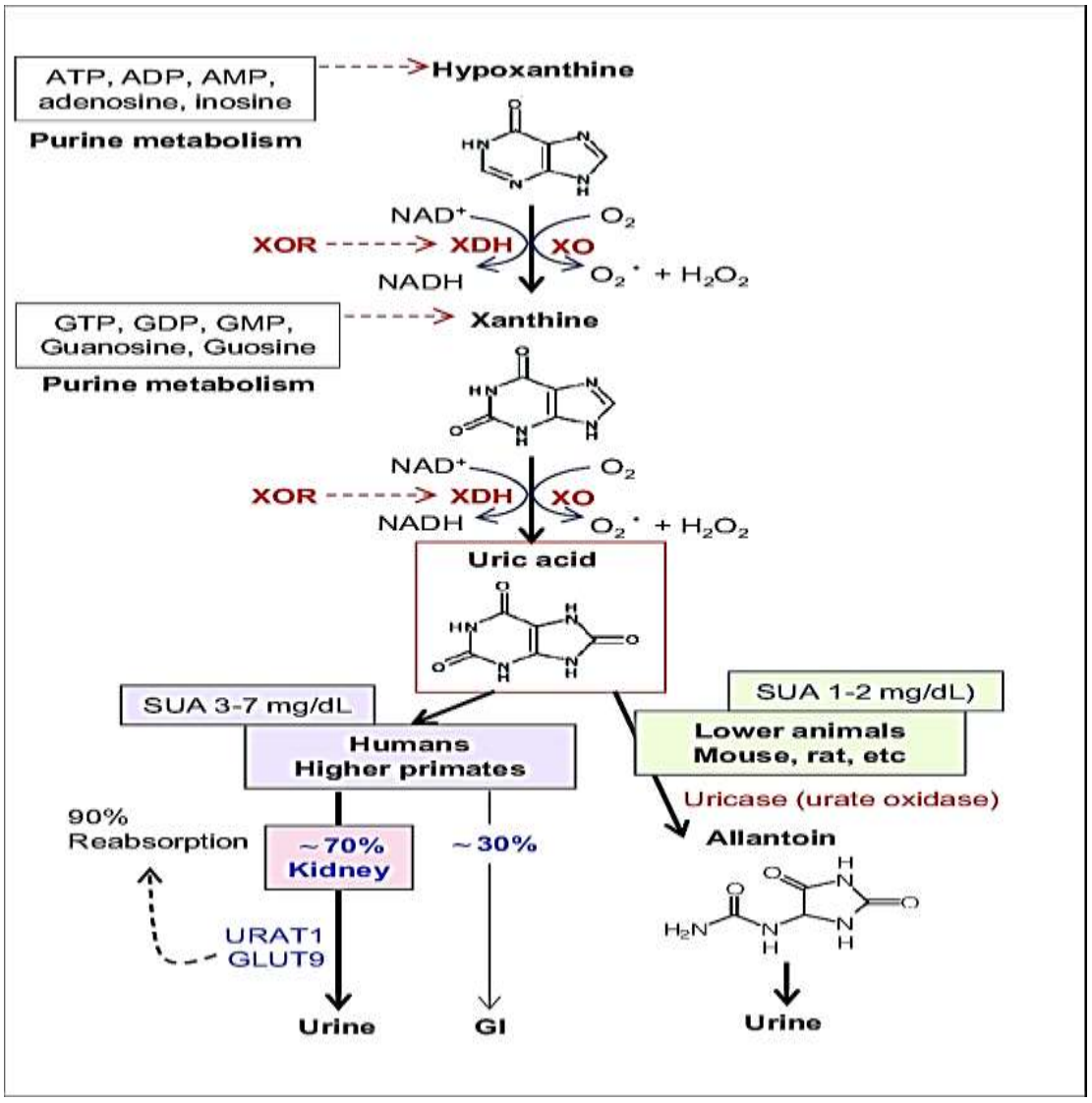


Fig-2 Synthesis of Uric Acid(Spitsin, S. V.et al., 2000).

Among the significant biochemical parameters, uric acid functions as an antioxidant that impacts thyroid function (Chatterjee M & Shinde R et al., 2012). Furthermore, thyroid dysfunction can influence purine metabolism, potentially leading to increased concentrations of uric acid (Rodrigues, Sérgio Lamego et al., 2012). So, there may be the possibility of a link between serum uric acid, & total bilirubin in hypothyroidism.

However, numerous studies reported an association between uric acid, total bilirubin & hypothyroidism within the Indian population. Therefore, this study was designed to investigate the association between hypothyroidism and serum total bilirubin and serum uric acid levels, if any. A significant association would add to a better diagnosis and management of hypothyroidism.

AIM

AND

OBJECTIVES

Aim

To evaluate the levels of serum total bilirubin and serum uric acid in diagnosed cases of hypothyroidism and Apparently Healthy control subjects.

Objectives:-

1. To determine the concentration of serum total bilirubin in diagnosed hypothyroidism patients and Apparently Healthy control subjects.
2. To determine the concentration of serum uric acid in hypothyroidism patients and Apparently Healthy control subjects.
3. To correlate the association between hypothyroidism and serum total bilirubin and serum uric acid in hypothyroidism.

MATERIALS

AND

METHODS

Research Question

Is there any significant association between the levels of uric acid & total bilirubin in diagnosed cases of Hypothyroidism as compared to Apparently Healthy control subjects?

Hypothesis

Null hypothesis Ho: There is no significant association between the levels of uric acid & total bilirubin in diagnosed patients of hypothyroidism as compared to Apparently Healthy control subjects.

Alternate hypothesis H_1: There is a significant association between the levels of serum uric acid and total bilirubin in diagnosed patients of hypothyroidism as compared to Apparently Healthy control subjects.

METHODOLOGY

Type of Study: - Case-control study

Study design: - Prospective

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

Collaborating department:-

Department of Medicine, OPD at IIMSR, Integral University, Lucknow.

Enrollment of participants:-

Cases were enrolled from the hypothyroidism attending the Integral Hospital.

Sampling Method:-Non-probability, Purposive sampling

SUBJECTS SELECTION:-

Selection of Controls

1. Apparently healthy individuals.
2. Subjects between the age of 30 to 60 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 60 year
3. Patients who have agreed to sign the consent form.

Exclusion criteria

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

Collection of samples:-

2 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 be centrifuged at 1000 rpm for 10 minutes to separate the serum (Henry J. B et al., 1979).

25 microliter serum was used for the estimation of total bilirubin and 20 microliter serum was used for the estimation of uric acid.

Storage of samples:-

The serum samples for the estimation of uric acid and total bilirubin was stored at -20°C until testing in Central Clinical Laboratory, Department of Biochemistry, IIMS&R, Lucknow.

Sample size

$$n = \left(\frac{r+1}{r}\right)\frac{\sigma^2(Z_\beta + Z_{\alpha/2})^2}{(d\text{ifference})^2}$$

The sample size is calculated using the formula

Reference :(Charan J Biswas, 2013)

n= sample size

r = ratio of the case to control

σ = standard deviation (taken from previous studies)

$\mathbf{Z_\beta}$ = represents the desired power of statistical

$\boldsymbol{Z\alpha_{/2}}$ = represents 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, $Z\alpha_{/2}$=1.96

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

σ=1.4 (Arora.S,et.al.,2009)

$$n = 2 \times \frac{(1.49)^2}{(0.77)^2} \times (0.842+1.96)^2$$

Therefore, n = 58.8 ≈ 60

The study will include **30** cases and **30** controls

Laboratory Investigations

Determine of serum Total bilirubin by Diazo method of Peadman & Lee:

Principle for Bilirubin:-

Bilirubin reacts with diazotized sulfanilic acid in an acidic medium to form pink-coloured azobilirubin with absorbance directly proportional to bilirubin concentration. Direct bilirubin, being water soluble, directly reacts in an acidic medium. However indirect or unconjugated bilirubin is solubilized using a surfactant and then it reacts similarly to direct Bilirubin (Henry R.J et al., 1974).

Procedure: - **For bilirubin estimation:-**

Pipette into tubes marked	Blank (B)	Standard (S)	Test (T)
Working Reagents	500µl	500µl	500µl
Distilled Water	25µl	–	–
Standard	–	25µl	–
Test	–	–	25µl

Mix well & incubate for 5 minutes at 37^0C for total bilirubin and direct bilirubin.

Read Absorbance at 546/630 nm against reagent blank.

Determine the serum uric acid by a modified Trinder peroxidase method using Tribromo-3 hydroxybenzoic acid:

Principle for Serum uric acid:-C

$$\text{Uric Acid} + O_2 + H_2O \xrightarrow{\text{Uricase}} \text{Allantoin} + CO_2 + H_2O_2$$

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

4-AAP: 4- Amino antipyrine

TBHB: 2,4,6-Tribromo-3 hydroxybenzoic acid (Henry R.J, 1974).

The intensity of chromogen (Quinoneimine) formed is proportional to the uric acid concentration in the sample when measured at 505 nm (500-540 nm).

For Serum Uric Acid estimation:-

Pipette into tubes marked	**Blank**	**Standard**	**Test**
Working Reagent	1000µl	1000µl	1000µl
Distilled Water	20µl	--	--
Standard	--	20µl	--
Test	--	--	20µl

Mix and incubate for 5 minutes at 37^0C. Read the absorbance of the standard and each test at 505 nm (500-540 nm) or 505/670 nm on a bichromatic analyzer against the reagent blank.

ETHICS REVIEW

Permission from the Institutional Ethics Committee was taken (**IEC/IIMS&R/2023/70**)

DATA COLLECTION

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

STATISTICAL ANALYSIS

Statistical analysis was performed using Graph Pad and Microsoft –Excel (Version 2010). All the data was expressed as mean and 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

TOTAL BILIRUBIN

The difference in concentration of Total Bilirubin was not statistically significant (p=0.54) between cases and controls (Table.1 & Fig.3)

TABLE 1: TOTAL BILIRUBIN

Total bilirubin (mg/dl)					
Groups	**N**	**Mean**	**Standard Deviation**	**p- value**	**Significance**
Controls	30	0.50	±0.26	0.54	Not Significant
Cases	30	0.56	±0.47		

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

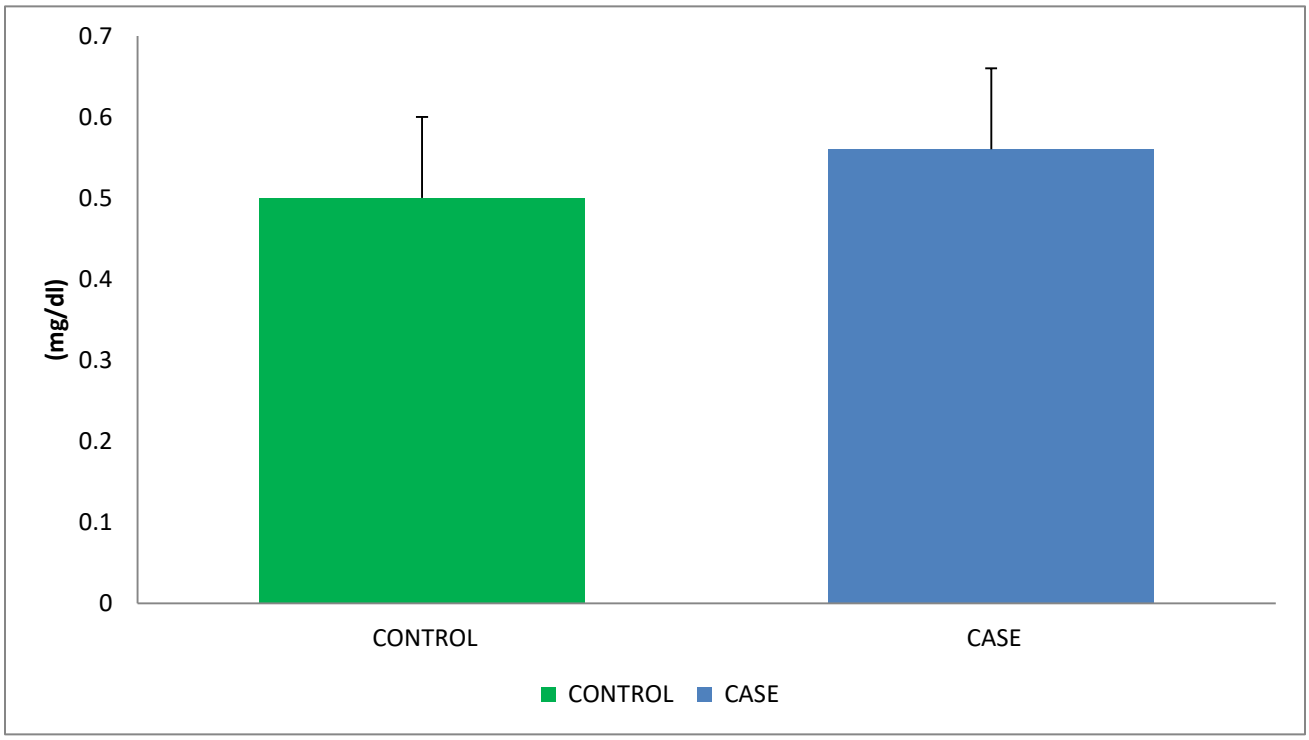


Fig 3.The Mean of Total Bilirubin between Controls and Cases.

URIC ACID

The difference in concentration of Uric Acid was not statistically significant (p=0.5137) between cases and controls (Table.2 & Fig.4)

TABLE 2: URIC ACID

URIC ACID (mg/dl)					
Groups	**N**	**Mean**	**Standard Deviation**	**p-value**	**Significance**
Controls	30	5.59	±3.02	0.5137	Not significant
Cases	30	6.0	±1.60		

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

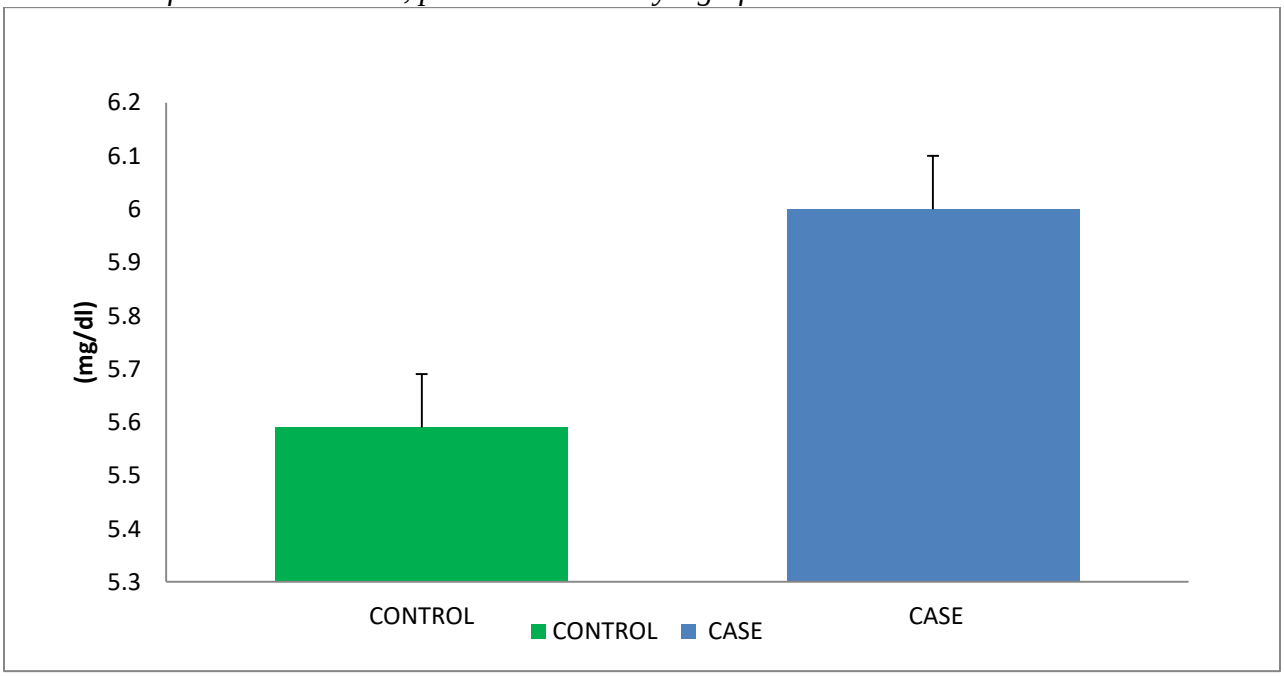


Fig 4.The Mean of Uric acid between Controls and Cases

BODY MASS INDEX (BMI)

Body Mass Index showed statistically significant difference (p=0.0001) between cases and controls (Table.3 & Fig.5)

Table 3: Mean and standard deviation of the study groups

Body Mass Index (kg/m^2)					
Groups	**N**	**Mean**	**Standard Deviation**	**p-value**	**Significance**
Controls	30	24.12	±2.04	0.0001	Significant
Cases	30	28.56	±2.55		

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

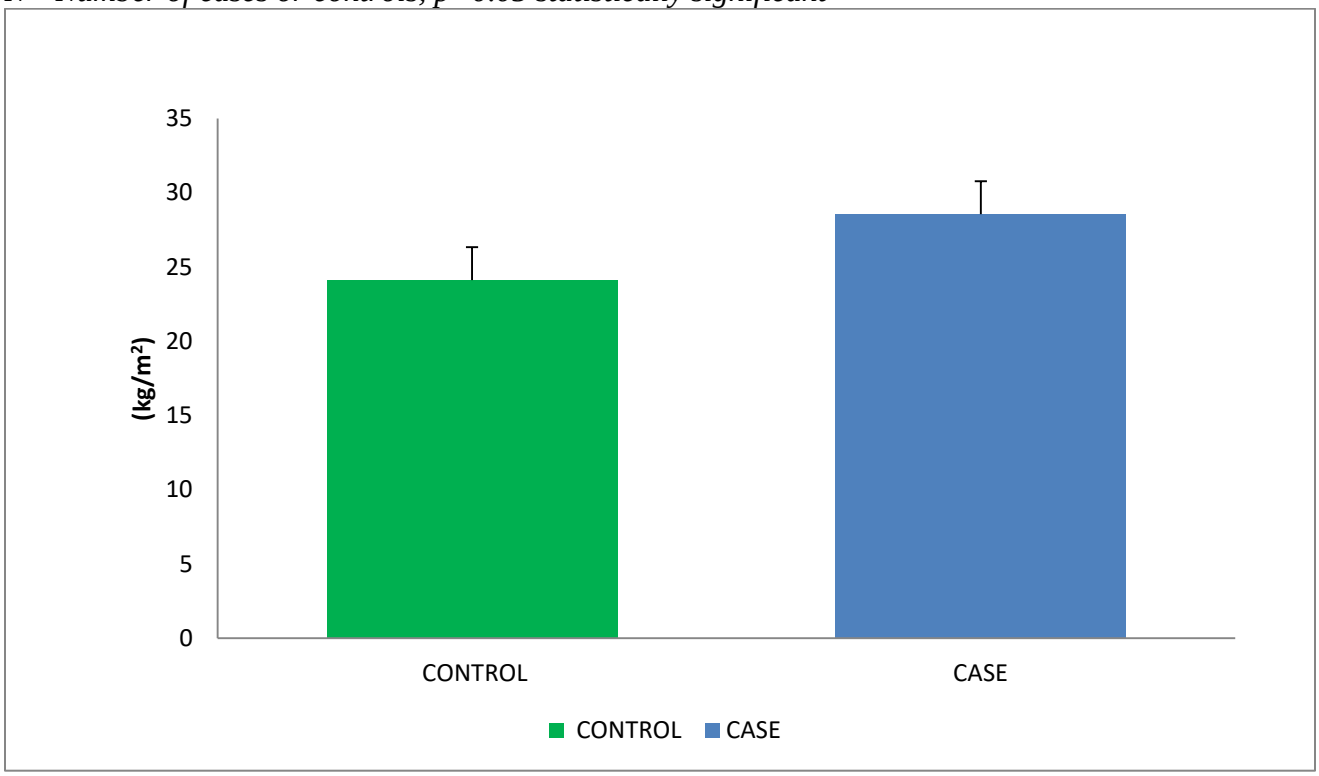


Fig 5.BMI mean in Controls and Cases Groups

KARL PEARSON'S CORRELATION OF COEFFICIENT AMONG THE STUDY PARAMETERS IN CASES.

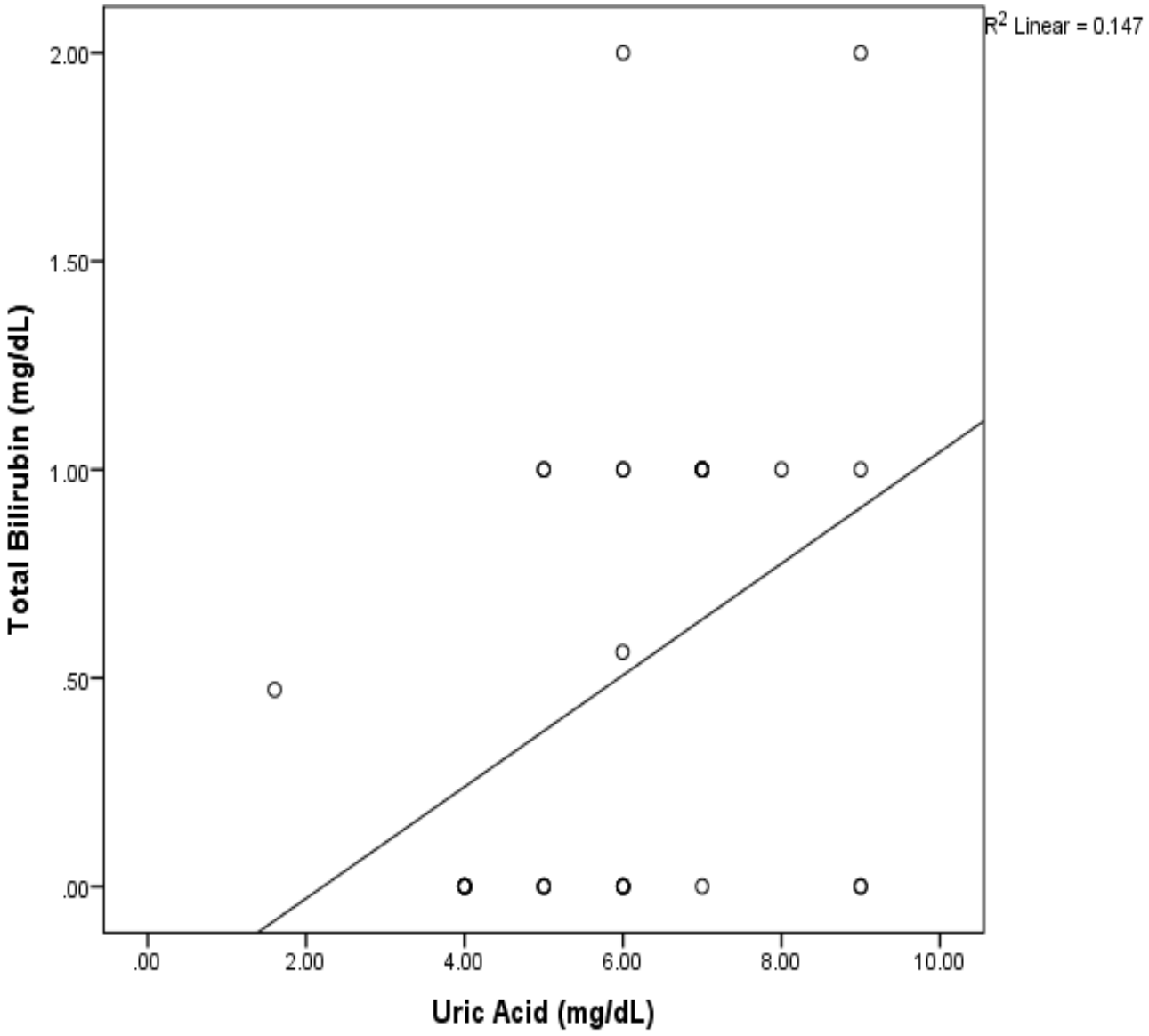
There was no correlation between Uric Acid and Total Bilirubin in Hypothyroidism cases.

Correlations

		Total Bilirubin (mg/dL)	Uric Acid (mg/dL)
Total Bilirubin (mg/dL)	Pearson Correlation	1	**.383***
	Sig. (2-tailed)		.030
	N	32	32
Uric Acid (mg/dL)	Pearson Correlation	**.383***	1
	Sig. (2-tailed)	.030	
	N	32	32

*. Correlation is significant at the 0.05 level (2-tailed). N=Number of cases

A scatter plot between levels of Total bilirubin and Uric acid



DISCUSSION

Clinical hypothyroidism, or overt hypothyroidism, occurs when TSH levels are higher and free T4 levels are low, resulting in more noticeable symptoms (Jameson et al., 2018). Studies conducted in India as reported by Velayutham et al.,2017 and Unnikrishnan et al., 2013 have shown a higher prevalence of hypothyroidism among female patients.

Few studies have been done on serum uric acid & bilirubin in diagnosed cases of hypothyroidism, with varying outcomes. These studies showed the relationship between TSH, serum uric acid (SUA) & bilirubin levels were insignificant (Abebe et al., 2016). In a previous research, there were elevated levels of serum uric acid concentrations in patients diagnosed with clinical hypothyroidism. This is the result of low thyroid hormone secretion affecting proper functioning of the liver, thus increasing the liver metabolic rate. More purine is synthesized and uric acid, the by-product is released more (Khan et al., 2010).

Similar to our findings, Rafat, et al., (2019) found that uric acid level was elevated in hypothyroidism patients. In his study, he notes that increased Uric acid levels coincided with abnormalities in the parameters tested for chronic kidney disease. Thus, it can be deduced that the lack of efficiency of the renal system to excrete uric acid was the leading pathogenetic condition elevated in hypothyroidism. Khan et al., (2010) & Giordano et al., (2001) also found higher uric acid in hypothyroidism.

In a study done by Soliman, A. T et al, (2013), there was no significant correlation between bilirubin levels and thyroid disorders. This is contrary to our study findings. We found an insignificant increase in serum bilirubin levels in diagnosed hypothyroid patients ($p=>0.05$) with a mean concentration of 0.56 ± 0.47 in cases compared to 0.50 ± 0.26 in control groups.

A similar study on children age 2 to 14 years showed a slight increase in serum uric acid concentration in subclinical hypothyroidism compare to control though the difference was statistically negligible (Sayari et al., 2018). In another study, it was discovered that hyperuricemia in females with hypothyroid status is not related. (Satyajit, K et al., 2017).

We enrolled hypothyroidism patients along with apparently healthy controls to reduce the effect of confounding factors. In our study we did not find any statistically significant difference between cases and controls with respect to the levels of uric acid (p=0.5137) correlation and total bilirubin (p= 0.54).

There are increased levels of uric acid & bilirubin in hypothyroidism cases that show that uric acid and bilirubin could both be affected by thyroid gland disorders in one way or the other.

In our investigation, we did not find any correlation between uric acid and total bilirubin in cases of hypothyroidism. However, we found that the BMI of patients with hypothyroidism was significantly raised as compared to controls (p=0.0001).

Further research needs to be done specifically on the role hypothyroidism plays in bilirubin biosynthesis to determine association, if any.

SUMMARY AND

CONCLUSION

Summary-

In the study designed to evaluate serum Uric acid and Total Bilirubin in diagnosed patients of hypothyroidism and apparently healthy controls, the parameters estimated were:

- Serum Uric acid
- Total Bilirubin

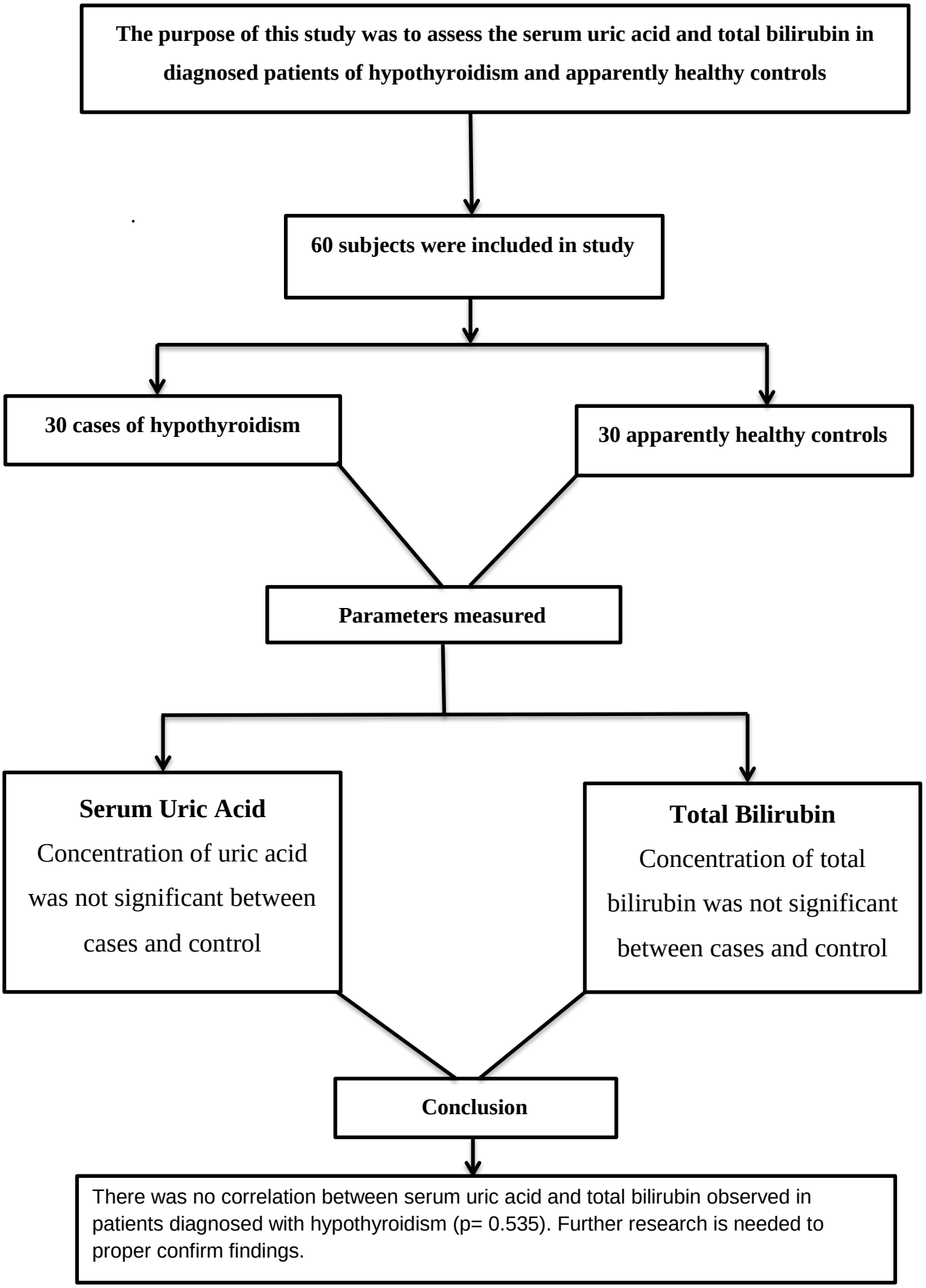
From this, the following were observed:

- The concentration of serum Uric acid was not statistically significant in cases($p=0.5137$) compared to controls.
- The level of Total Bilirubin was not significant though raised in cases ($p=0.54$) when compare to controls.
- There was no correlation between serum Uric acid and Total Bilirubin($r=0.383, p=>0.05$) among cases.

Conclusion-

There was no significant difference between cases and controls with regard to the levels of Uric Acid and Total Bilirubin. We also observed no correlation between Uric Acid and Total Bilirubin in hypothyroidism. This may be attributed to the small sample size undertaken in this project. There is a necessity for further research to confirm findings but on a larger sample size

FLOWCHART OF RESEARCH PROJECT



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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.	Subjects between the age of 30 to 60 years		
2.	Diagnosed cases of hypothyroidism.		

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- 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

INCLUSION AND EXCLUSION CRITERIA -CONTROLS

Inclusion Criteria

S.N.	Criteria	YES	NO
1.	Apparently healthy individuals		
2.	Subjects between the age of 30 to 60 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- CONTROLS

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:

INFORMED CONSENT FORM (FOR CASE)

Study Title: Uric acid and total bilirubin in hypothyroidism patients 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 scientific purposes (s). I agree to take part in the above study.

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

Name of the Investigator (printed)……

Signature of the investigator…… Date……

Name and signature of the impartial witness with date if required……

Unique Identification No:

INFORMED CONSENT FORM (FOR CASE)

Study Title: Uric acid and total bilirubin in hypothyroidism patients and control subjects

Subject's name…… Age…… Sex……

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

Name of the Investigator (printed)……

Signature of the investigator…… Date……

Name and signature of the impartial witness with date if required……

Unique Identification No:

INFORMED CONSENT FORM (FOR CONTROLS)

Study Title: Uric acid and total bilirubin in hypothyroidism patients 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 scientific purposes (s). I agree to take part in the above study.

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

Name of the Investigator (printed)……

Signature of the investigator…… Date……

Name and signature of the impartial witness with date if required……

विशिष्ट पहचान संख्या:

सूचित सहमति प्रपत्र (FOR CASES)

अध्ययन का शीर्षक: हाइपोथायरायडिज्म रोगियों और नियंत्रण विषयों में यूरिक एसिड और कुल बिलीरुबिन

विषय का नाम..... **आयु** ..... **लिंग** .....

मैं पुष्टि करता हूं कि मैंने शोधकर्ता/मॉडरेटर द्वारा दी गई जानकारी को पढ़ और समझ लिया है/मुझे समझा दिया गया है और मुझे प्रश्न पूछने का अवसर मिला है। मैं समझता हूं कि अध्ययन में भागीदारी स्वैच्छिक है और मैं बिना कोई कारण बताए और अपनी चिकित्सा देखभाल और कानूनी अधिकारों को प्रभावित किए बिना किसी भी समय इससे हटने के लिए स्वतंत्र हूं। मैं समझता हूं कि मेरी पहचान किसी तीसरे पक्ष या प्रकाशन में उजागर नहीं की जाएगी। मैं समझता हूं कि यदि वर्तमान अध्ययन के लिए 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 परिणाम के उपयोग को प्रतिबंधित नहीं करने पर सहमत हूं, बशर्ते कि ऐसा उपयोग केवल वैज्ञानिक उद्देश्यों के लिए हो। मैं उपरोक्त अध्ययन में भाग लेने के लिए सहमत हूं।

विषय के हस्ताक्षर..... **दिनांक**.....

अन्वेषक का नाम (मुद्रित).....

अन्वेषक के हस्ताक्षर..... दिनांक.....

यदि आवश्यक हो तो तारीख सहित निष्पक्ष गवाह का नाम और हस्ताक्षर.....

विशिष्ट पहचान संख्या:

सूचित सहमति प्रपत्र (FOR CONTROLS)

अध्ययन का शीर्षक: हाइपोथायरायडिज्म रोगियों और नियंत्रण विषयों में यूरिक एसिड और कुल बिलीरुबिन

विषय का नाम..... **आयु** ..... **लिंग** .....

मैं पुष्टि करता हूं कि मैंने शोधकर्ता/मॉडरेटर द्वारा दी गई जानकारी को पढ़ और समझ लिया है/मुझे समझा दिया गया है और मुझे प्रश्न पूछने का अवसर मिला है। मैं समझता हूं कि अध्ययन में भागीदारी स्वैच्छिक है और मैं बिना कोई कारण बताए और अपनी चिकित्सा देखभाल और कानूनी अधिकारों को प्रभावित किए बिना किसी भी समय इससे हटने के लिए स्वतंत्र हूं। मैं समझता हूं कि मेरी पहचान किसी तीसरे पक्ष या प्रकाशन में उजागर नहीं की जाएगी। मैं समझता हूं कि यदि वर्तमान अध्ययन के लिए 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 परिणाम के उपयोग को प्रतिबंधित नहीं करने पर सहमत हूं, बशर्ते कि ऐसा उपयोग केवल वैज्ञानिक उद्देश्यों के लिए हो। मैं उपरोक्त अध्ययन में भाग लेने के लिए सहमत हूं।

विषय के हस्ताक्षर..... **दिनांक**.....

अन्वेषक का नाम (मुद्रित).....

अन्वेषक के हस्ताक्षर..... **दिनांक.....**

यदि आवश्यक हो तो तारीख सहित निष्पक्ष गवाह का नाम और हस्ताक्षर.....

INSTITUTIONAL ETHICS COMMITTEE (IEC)

IIMS&R INTEGRAL UNIVERSITY, LUCKNOW

IEC/IIMS&R/2023/470

CERTIFICATE

This is to certify that research work entitled **"Uric Acid and Total Bilirubin in Hypothyroidism Patients and Control Subjects"** submitted by **Smita Singh Chauhan, 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**.

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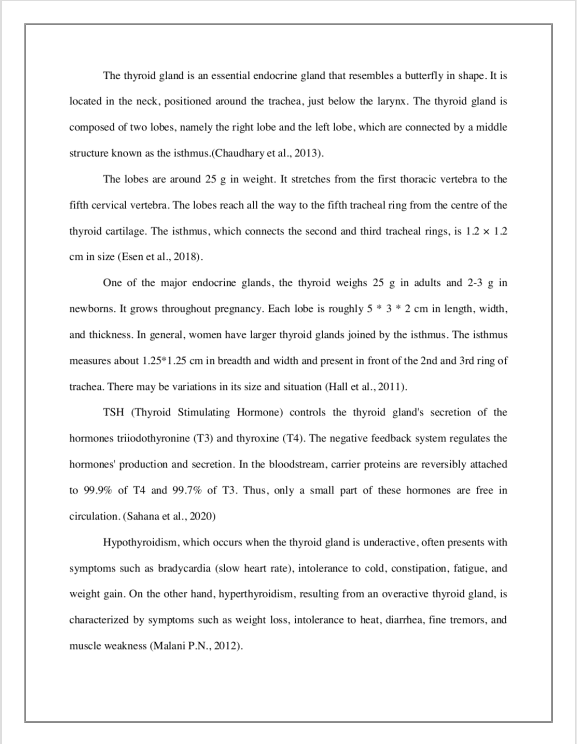

The thyroid gland is an essential endocrine gland that resembles a butterfly in shape. It is located in the neck, positioned around the trachea, just below the larynx. The thyroid gland is composed of two lobes, namely the right lobe and the left lobe, which are connected by a middle structure known as the isthmus.(Chaudhary et al., 2013).

The lobes are around 25 g in weight. It stretches from the first thoracic vertebra to the fifth cervical vertebra. The lobes reach all the way to the fifth tracheal ring from the centre of the thyroid cartilage. The isthmus, which connects the second and third tracheal rings, is 1.2 × 1.2 cm in size (Esen et al., 2018).

One of the major endocrine glands, the thyroid weighs 25 g in adults and 2-3 g in newborns. It grows throughout pregnancy. Each lobe is roughly 5 * 3 * 2 cm in length, width, and thickness. In general, women have larger thyroid glands joined by the isthmus. The isthmus measures about 1.25*1.25 cm in breadth and width and present in front of the 2nd and 3rd ring of trachea. There may be variations in its size and situation (Hall et al., 2011).

TSH (Thyroid Stimulating Hormone) controls the thyroid gland's secretion of the hormones triiodothyronine (T3) and thyroxine (T4). The negative feedback system regulates the hormones' production and secretion. In the bloodstream, carrier proteins are reversibly attached to 99.9% of T4 and 99.7% of T3. Thus, only a small part of these hormones are free in circulation. (Sahana et al., 2020)

Hypothyroidism, which occurs when the thyroid gland is underactive, often presents with symptoms such as bradycardia (slow heart rate), intolerance to cold, constipation, fatigue, and weight gain. On the other hand, hyperthyroidism, resulting from an overactive thyroid gland, is characterized by symptoms such as weight loss, intolerance to heat, diarrhea, fine tremors, and muscle weakness (Malani P.N., 2012).

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Iodine is a crucial trace element that is absorbed in the small intestine and plays an integral role in the formation of T3 and T4 hormones. It can be obtained from various sources, including iodized table salt, seafood, seaweed, and certain vegetables. Insufficient intake of iodine can lead to iodine deficiency, resulting in decreased synthesis of thyroid hormones. This deficiency can give rise to conditions such as cretinism, goiter, myxedema coma, and hypothyroidism (Anton et al., 2020).

Hypothyroidism is a prevalent medical condition characterized by an inadequate production of thyroid hormones. If left untreated, it can result in significant detrimental effects on health and, in severe cases, can be fatal. Subclinical hypothyroidism, often considered an indication of early thyroid complication, is defined by elevated TSH levels, while free thyroxine levels remain within ranges considered normal (Cooper et.al.,2012).

Insufficient levels of environmental iodine are the leading cause of thyroid disorders globally, including hypothyroidism (Vanderpump et.al., 2011). In regions with inadequate iodine supply, hypothyroidism occurs as a result of autoimmune thyroiditis; this is known as Hashimoto's disease (Carle et.al. 2006).

Uric acid biosynthesis occurs within the entero-hepatic tissues. It is the byproduct of purine synthesis derived mainly from dietary animal proteins, comprising an exogenous pool. Moreover, uric acid is formed as a result of the degradation of nucleic acids in living and dying cells (Chaudhary et.at., 2013).

Normally, the majority of uric acid elimination takes place through the kidneys. However, humans lack the uricase enzyme necessary for the oxidation of uric acid into the more soluble compound called allantoin. Uricase, also known as urate oxidase, has the ability to metabolize uric acid into a highly soluble form known as 5-hydroxyisourate. Unfortunately, due

to the absence of uricase in humans, uric acid remains in its less soluble form, contributing to the propensity for hyperuricemia and the formation of urate crystals in conditions such as gout (Chang et.al. 2014)

Bilirubin is a significant byproduct of heme (ferriprotoporphyrin IX) metabolism, which is a complex that plays a crucial role in iron coordination within different proteins. It is a substance that can potentially be harmful. Nevertheless, the body has evolved mechanisms to safely process and eliminate it. Bilirubin, along with its metabolites, is responsible for giving bile and stool their characteristic yellow color and, to a lesser extent, influencing the color of urine. (Chen et.al, 2016)

Bilirubin originates from two primary sources. Approximately 80% of bilirubin is generated through the process of biodegrading old red blood cells (Dosch et.al., 2019). The remaining portion arises as a result of the diverse heme-containing proteins present in various tissues being degraded. On estimate, about 4mg of bilirubin is produced per kilogram of weight.

Hypothyroidism refers to a slight elevation in TSH levels. In contrast, clinical hypothyroidism, or overt hypothyroidism, occurs when TSH levels are higher and free T4 levels are low, resulting in more noticeable symptoms.

Epidemiology of Hypothyroidism

Evidently, hypothyroidism occurs in the general population ranging from 0-3 % in the United States and from 3 to 7% in Europe. In Europe, the prevalence is typically between 0 to 2%, although it can reach up to 5 to 8% in certain regions (Asvold et al., 2013).

Hypothyroidism is more commonly found in females, older individuals (over the age of 65), and among people of Caucasian descent. However, there is limited available data on the variations in prevalence based on ethnicity (Aoki et al., 2007). Occurence is higher among individuals with autoimmune conditions, including type 1 diabetes, autoimmune gastric atrophy, and intestinal diseases. Individuals with Down or Turner syndrome are more at risk. On the other hand, cigarette smokers and alcohol consumers have been found to be at a lower risk of developing the disease. (Carle et al., 2012).

The heritability of serum TSH concentrations is estimated to be around 65%. Similarly, about 23% – 65% of free thyroxine hormone is inherited from parent to offspring.(Panicker et al., 2008).

In India, 11% of people have hypothyroidism. This is more than the 2% occurring in the UK and 46% in the Americas.

. Compared to coastal cities, cities located inland have a higher prevalence (Bagcchi S., 2014).

A higher percentage of females (15.86%) compared to males (5.02%; P < 0.0001) were observed to have hypothyroidism. Furthermore, females were more likely to be diagnosed with hypothyroidism than males (Unnikrishnan et al., 2013).

The incidence of subclinical hypothyroidism, which refers to a milder form of hypothyroidism with subtle or no symptoms, tends to increase with age. Data from the Netherlands and the USA indicate that up to 10% of women over the age of 60 years may have subclinical hypothyroidism.

Thyroid hormones

The primary hormones synthesized in the thyroid gland are thyroxine (T4) and triiodothyronine (T3). The hormones play crucial roles in regulating various metabolic processes in the body. The hypothalamus is involved in releasing thyrotropin-releasing hormone (TRH), that acts to prompt release of thyroid-stimulating hormone (TSH) from the anterior pituitary gland. It is the TSH that exerts its effect on the thyroid gland releasing T4 into the bloodstream. T4 is converted into the more active T3 in target tissues. This intricate interplay between TRH, TSH, T4, and T3 ensures a feedback mechanism and maintains homeostasis within the body (Shahid et al., 2018).

Steps in the biosynthesis of thyroid hormones:-

Step 1: Uptake of Iodine

The thyroid gland has been known to absorb and concentrate iodine. However, this process can be blocked by thiocyanate and perchlorate, as they compete with iodine for the carrier mechanism. On the other hand, the uptake and concentration of iodine are stimulated by thyroid-stimulating hormone (TSH). In cases of congenital iodine trapping defect, where this process is impaired, large doses of iodine can be administered as a treatment.

Step 2: Oxidation of iodine

The iodide that is absorbed by the thyroid cell undergoes oxidation, converting it into active iodine. It is crucial to note that this only occurs in the thyroid gland as it is the sole organ capable of carrying out the oxidation step. This process is facilitated by the enzyme thyroperoxidase. To catalyse the reaction, hydrogen peroxide is required, which is produced through a reaction dependent on NADPH.

Step 3: Iodination

Iodination occurs on the thyroglobulin (Tgb) molecule within the follicular space. This process involves the addition of iodine to specific tyrosine residues present in the intact Tgb molecule. As a result, two iodinated products are formed: 3-monoiodotyrosine (MIT) and 3,5-di-iodotyrosine (DIT).

Step 4: Coupling

Combining two molecules of 3,5-di-iodotyrosine (DIT) form one molecule of tetraiodothyronine (T4), also known as thyroxine. Triiodothyronine (T3) can be formed from T4 through the deiodination of the outer ring by the enzyme 5'-deiodinase.

Step 5: Storage

The thyroid gland possesses a distinctive characteristic as it is the only endocrine gland that stores significant quantities of hormones. Within the thyroid gland, the hormone is stored in colloidal form, which consists of thyroglobulin. Each thyroglobulin molecule contains approximately 8 residues of thyroxine (T4).

Step 6: Utilization

When the need arises, thyroglobulin is internalized from the colloid within the thyroid acini into the cells through a process called endocytosis.

Step 7: Hydrolysis

Tetraiodothyronine (T4) is released through hydrolysis mediated by specific proteases. These protease enzyme are affected by TSH. However, the hydrolysis process is inhibited by iodide, and as a result, potassium iodide (KI) is employed as an adjunctive treatment in hyperthyroidism.

Specific proteases hydrolyze the T4 to release it. TSH notably improves this action.

Step 8: Release

The generated T4 is subsequently released into the bloodstream. T3, on the other hand, is formed through deiodination at the 5' position, which occurs either within the thyroid cell itself or in the peripheral tissues.

Step 9: Salvaging of Iodine

Any unused monoiodotyrosine (MIT) and diiodotyrosine (DIT) molecules are subjected to deiodination and then recycled within the cell for further utilization

Step 10: Transport of Thyroid Hormones

Proteins in the plasma serve as carriers for thyroid hormones during transportation. While the bound form of thyroid hormones is biologically inactive, they can be quickly released when needed. The total protein-bound iodine (PBI) concentration is approximately 10 mg/dL, with T4 accounting for 8 mg/dL of this total.

Step 11: Catabolism of Thyroid Hormones

T4 functions as a prohormone and undergoes deiodination to form T3. This deiodination process occurs in the peripheral tissues and is facilitated by a specific enzyme known as deiodinase, which contains selenium. Some of the T3 and T4 hormones are conjugated with

glucuronic acid and eliminated from the body through bile, while a smaller amount is excreted in urine (Bieberman et al., 2020).

Mechanism of Action of Thyroid Hormone

There are two distinct genes, TRα and TRβ, on different chromosomes (3 and 17, respectively, in humans) that are responsible for encoding thyroid receptors. Multiple thyroid receptor isoforms are produced by alternative splicing from each gene, including TRα2, TRα1, and TRα3 from the TRα gene and TRβ1 and TRβ2 from the TRβ gene. (Zhang et al., 2000)

Through DNA response elements, a TR's primary role is to control the expression of its target genes therefore acting as a transcription factor. The repeating DNA sequences in various configurations make up the T3 response element (TRE). Although thyroid hormone receptors (TRs) can be bound to TREs as homodimers or monomers, the predominant TR-TRE heterodimer is the retinoid X receptor (RXR). The target gene's transcription is activated by the TR's conformational change in response to ligand interaction. The molecular processes of thyroid hormone activity have been significantly elucidated thanks to advances in our understanding of the structure, dynamics, and interactions of thyroid hormone receptors (TRs) and the proteins that interact with them. (Zhang et al., 2000)

THYROID HORMONE'S BIOLOGICAL ACTIVITIES:

The thyroid hormones have several purposes;

1. Thyroid hormone regulation of calorigenesis and basal metabolic rate.
2. Increasing the metabolism of mitochondria.
3. Promoting healthy neural development and growth.

4. Improvement of sexual maturation and growth.
5. Myocardial contractions and an increased heart rate are also associated with adrenal stimulation.
6. Increasing the production and breakdown of triglycerides and cholesterol.
7. Increasing vitamin needs.
8. Enhancing calcium and phosphorus metabolism.
9. Making catecholamine more sensitive to adrenergic receptors. In 2016 (Salomon et al.)

ETIOLOGY OF HYPOTHYROIDISM

Hypothyroidism is classified into two primary categories: primary hypothyroidism and secondary (or central) hypothyroidism.

Primary hypothyroidism: - It is characterized by decreased levels of thyroid hormone in the bloodstream, primarily caused by the destruction of the thyroid gland. This destruction can occur as a result of autoimmune disorders, when the body's immunity fails to recognize self and launches an immune attack on the thyroid gland. Additionally, interventions such as surgery, radioiodine therapy, or radiation treatments can also lead to the destruction of the thyroid gland, resulting in primary hypothyroidism (Patil et al., 2021).

When diagnosed based on distinctive features, primary hypothyroidism is identified by elevated levels of thyroid-stimulating hormone (TSH) in the blood and decreased levels of thyroxine (T4). Subclinical hypothyroidism, on the other hand, is diagnosed when serum TSH levels are elevated, but serum T4 levels remain within the normal range and there are no apparent symptoms of thyroid dysfunction.

Secondary hypothyroidism: - It occurs as a result of a dysfunction in the pituitary gland hypothalamus. This dysfunction affects the release of TSH from the anterior pituitary that has a downstream effect on the thyroid hormones. Secondary hypothyroidism occurs when the dysfunction cause originate from the pituitary gland and tertiary hypothyroidism when the causes arise from the hypothalamus.

Primary and Secondary Hypothyroidism (Patil et al., 2021).

A. Primary Hypothyroidism Causes;

- Hashimoto's disease
- Iodine deficiency
- Surgical removal of thyroid gland
- External radiotherapy
- Drugs
- Thyroid agenesis or dysgenesis
- Therapy with radioactive iodine

B. Secondary Hypothyroidism Causes;

a. Pituitary

1. Non-cancerous tumor
2. Radiotherapy
3. Head trauma
4. Pituitary apoplexy

b. Hypothalamus

1- Hypothalamic tumor

2- Radiotherapy

SYMPTOMS:-

Typical symptoms & signs include:-

- Tiredness
- Inactivity
- Obesity
- Stomach upsets
- Pitch variation
- Rough skin

Diagnosis of hypothyroidism:

TSH concentration values exceeding the normal range and free thyroxine hormone concentrations falling short of the known normal value characterize primary hypothyroidism. (Chaker et al., 2017) For diagnosis of hypothyroidism the standard test done for screening hypothyroidism is the estimation of serum TSH and also estimation of T3 and T4 will diagnose the type of hypothyroidism.

Thyroid hormone therapy is used to treat hypothyroidism. The medication of choice is levothyroxine. It is an artificial type of 'T4' created in a lab that is identical to the T4 the thyroid naturally produces. Not all medications for thyroid hormones are similar. You ought to stick with the same brand if at all possible. The requirement for thyroid-related hormone replacement is typically permanent. TSH blood tests should be repeated if the medication needs to be altered for any reason. Your TSH testing will be used to adjust your dose. Thyroid hormone levels that are too high in the long run can cause bone loss, irregular heart rhythms, and improper cardiac function. If your dose is insufficient, the signs could not get better. Dose modification may. If your dose is too low, your symptoms could not get better. Adjusting the dosage may be required throughout the pregnancy and at other times as well; this can be discussed with your doctor during your routine checkups.

Hypothyroidism can have wide-ranging effects on various organ systems in the body, including the skin and its appendages, cardiovascular, respiratory, central and peripheral nervous systems, skeletal system (in terms of calcium and phosphorus metabolism), renal system (in terms of water and electrolyte metabolism), pituitary glands, and energy metabolism. (Shlomon Melmed et al. in 2016)

Bilirubin is a byproduct of heme breakdown in the body and can be neurotoxic in infants (Ostrow J.D et al., 2004). However, bilirubin also possesses beneficial properties in different diseases, including antioxidative, anti-inflammatory, and immunosuppressive functions (Kinderlerer, A. R. et al., 2009).

Normal healthy individuals produce 110nmol of T4 and 10nmol of T3 per day (Larsen, P. R et al.,1975).

The metabolism of thyroid hormones is regulated by three groups of enzymes: Type 1 (D1), Type 2 (D2), and Type 3 (D3). These enzymes play different roles in the conversion and regulation of thyroid hormones. They form part of the iodothyronine seleno-deiodinase enzyme system. Type 1 deiodinase is primarily present in the hepatocytes and renal tissues (Sanders, J. P. et al., 1997). Its main role is to activate T4 to T3, inactivate T4 to reverse T3 (rT3), and also forms part of the conversion of rT3 and T3 to T2. Type 1 deiodinase forms majority of the T3 produced outside of the thyroid gland; about 30%.

While both the D1 and D2 enzyme systems have the capability to inactivate thyroxine and triiodothyronine hormones, the principle enzyme involved is the type 3 deiodinase (Tu, H. M et al., 1999).

The liver synthesizes many plasma proteins that bind to the lipophilic thyroid hormones creating a pool of active hormones. These plasma proteins, including thyroxine-binding globulin and albumin, bind to over 99% of the thyroid hormones in the bloodstream. However, the concentration of free hormones in various tissues varies based on the specific transport mechanisms and deiodinase activity within each tissue. (Bianco, A. C et al., 2002).

Total Bilirubin and Hypothyroidism

There is evidence suggesting that hypothyroidism can greatly affect the liver. Experimental studies have shown that in hypothyroidism, the activity of the enzyme involved in the conjugation of bilirubin, is reduced. This reduction in enzyme activity leads to a decrease in the excretion of bilirubin, potentially causing disturbances in bilirubin metabolism (Van Steenbergen et al., 1989).

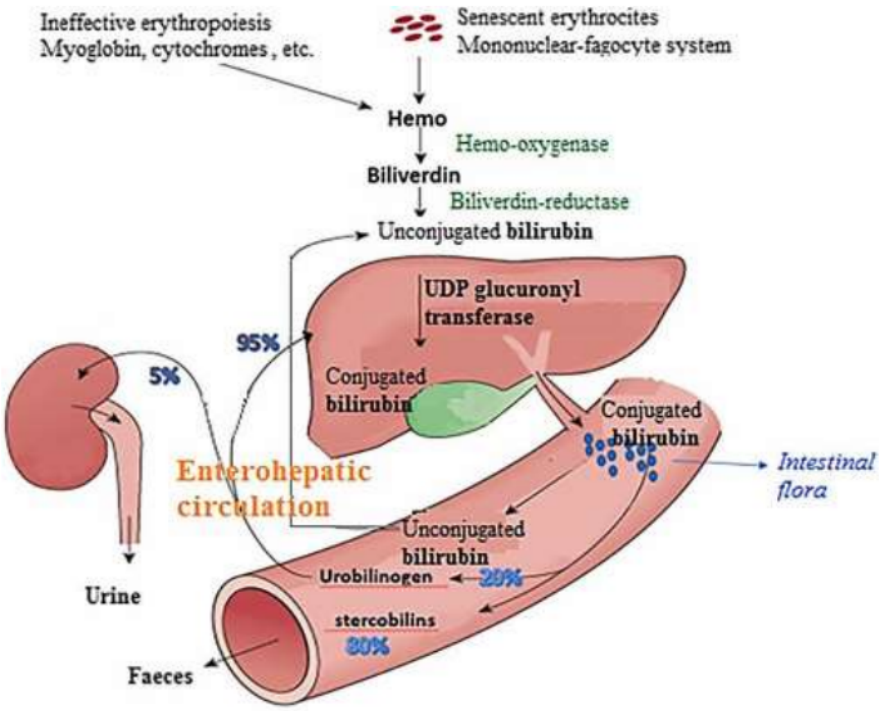


Fig-1 Formation of Bilirubin(Van Steenbergen et al., 1989).

Uric Acid and Hypothyroidism

Uric acid, an antioxidant that is primarily synthesized by the liver (Becker & B. F., 1993), is water-soluble. It plays a role in inhibiting the damaging effects of free radicals and provides protection to cell membranes and DNA (Spitsin, S. V.et al., 2000). The metabolic rate, stimulated by thyroid hormones, is increased by enhancing ATP production for various

metabolic processes and by uncoupling oxidative phosphorylation in the mitochondria (Hafner, R. P. et al., 1988).

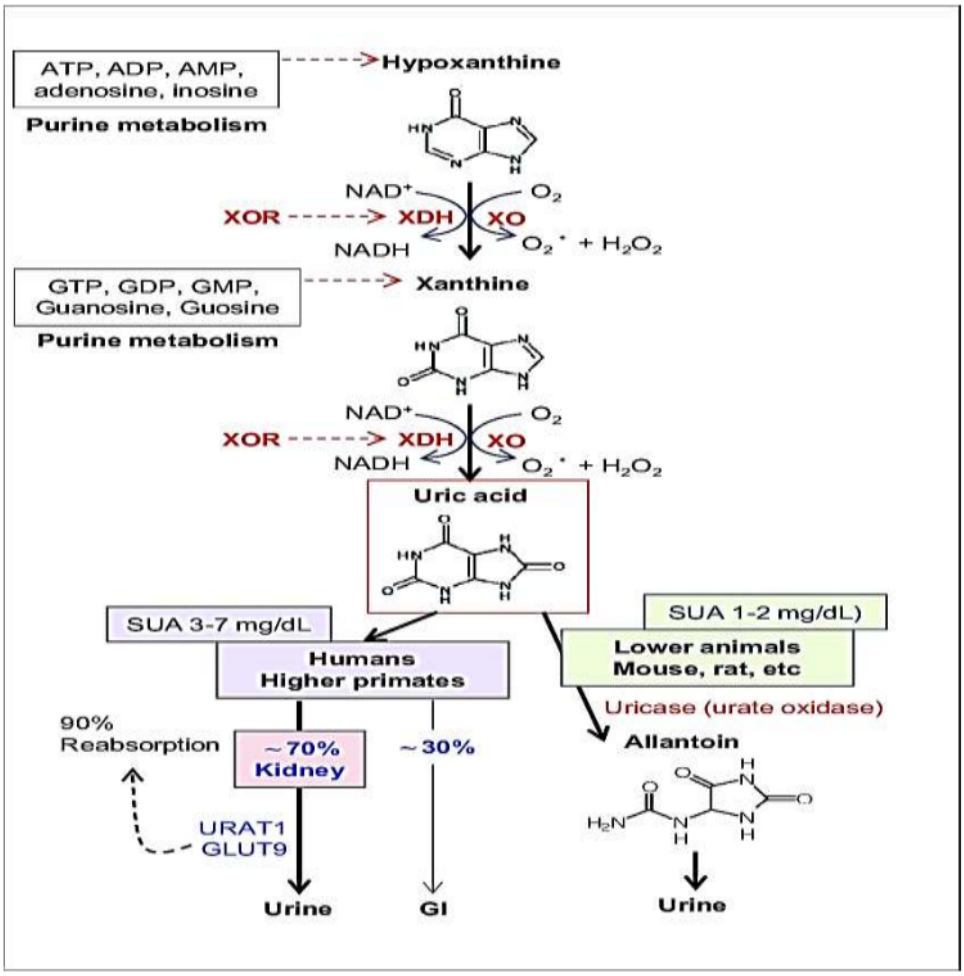


Fig-2 Synthesis of Uric Acid(Spitsin, S. V.et al., 2000).

Among the significant biochemical parameters, uric acid functions as an antioxidant that impacts thyroid function (Chatterjee M & Shinde R et al., 2012). Furthermore, thyroid dysfunction can influence purine metabolism, potentially leading to increased concentrations of uric acid (Rodrigues, Sérgio Lamego et al., 2012). So, there may be the possibility of a link between serum uric acid, & total bilirubin in hypothyroidism.

However, numerous studies reported an association between uric acid, total bilirubin & hypothyroidism within the Indian population. Therefore, this study was designed to investigate the association between hypothyroidism and serum total bilirubin and serum uric acid levels, if any. A significant association would add to a better diagnosis and management of hypothyroidism.

Aim

To evaluate the levels of serum total bilirubin and serum uric acid in diagnosed cases of hypothyroidism and Apparently Healthy control subjects.

Objectives:-

1. To determine the concentration of serum total bilirubin in diagnosed hypothyroidism patients and Apparently Healthy control subjects.
2. To determine the concentration of serum uric acid in hypothyroidism patients and Apparently Healthy control subjects.
3. To correlate the association between hypothyroidism and serum total bilirubin and serum uric acid in hypothyroidism.

TOTAL BILIRUBIN

The difference in concentration of Total Bilirubin was not statistically significant (p=0.54) between cases and controls (Table.2 & Fig.4)

TABLE 2: TOTAL BILIRUBIN

Total bilirubin (mg/dl)				
Groups		**Mean**	**Standard Deviation**	**p-value**
Controls	30	0.50	±0.26	0.54
Cases	30	0.56	±0.47	

N= Number of cases or controls, p<0.05 significance

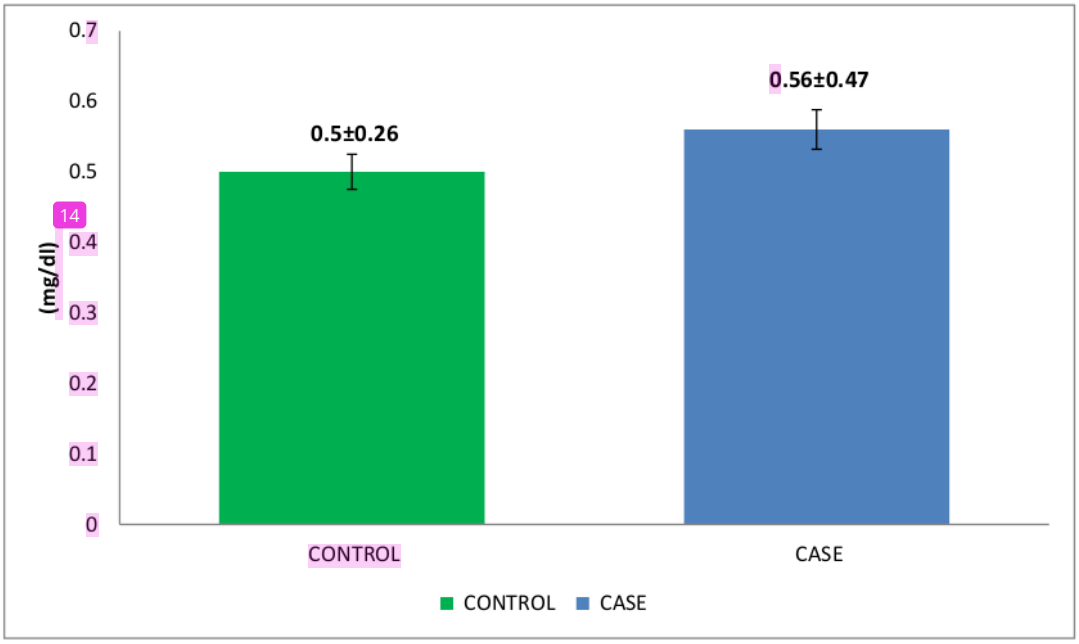


Fig 4.The Mean of Total Bilirubin between Controls and Cases.

URIC ACID

The difference in concentration of Uric Acid was not statistically significant (p=0.5137) between cases and controls (Table.3 & Fig.4)

TABLE 2: URIC ACID

URIC ACID (mg/dl)				
Groups	**N**	**Mean**	**Standard Deviation**	**p-value**
Controls	30	5.59	±3.02	0.5137
Cases	30	6.0	±1.60	

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

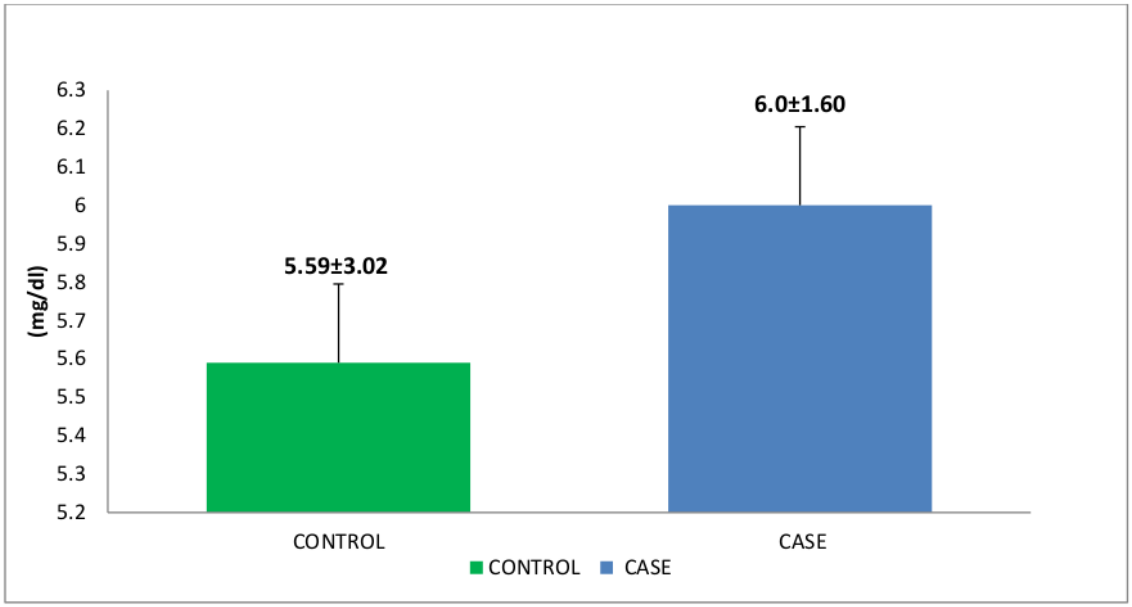


Fig 4.The Mean of uric acid between Controls and Cases

BODY MASS INDEX (BMI)

Table-1: Mean and standard deviation of the study groups

Body Mass Index (kg/m^2)				
Groups	**N**	**Mean**	**Standard Deviation**	**p-value**
Controls	30	24.12	±2.04	0.0001
Cases	30	28.56	±2.55	

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

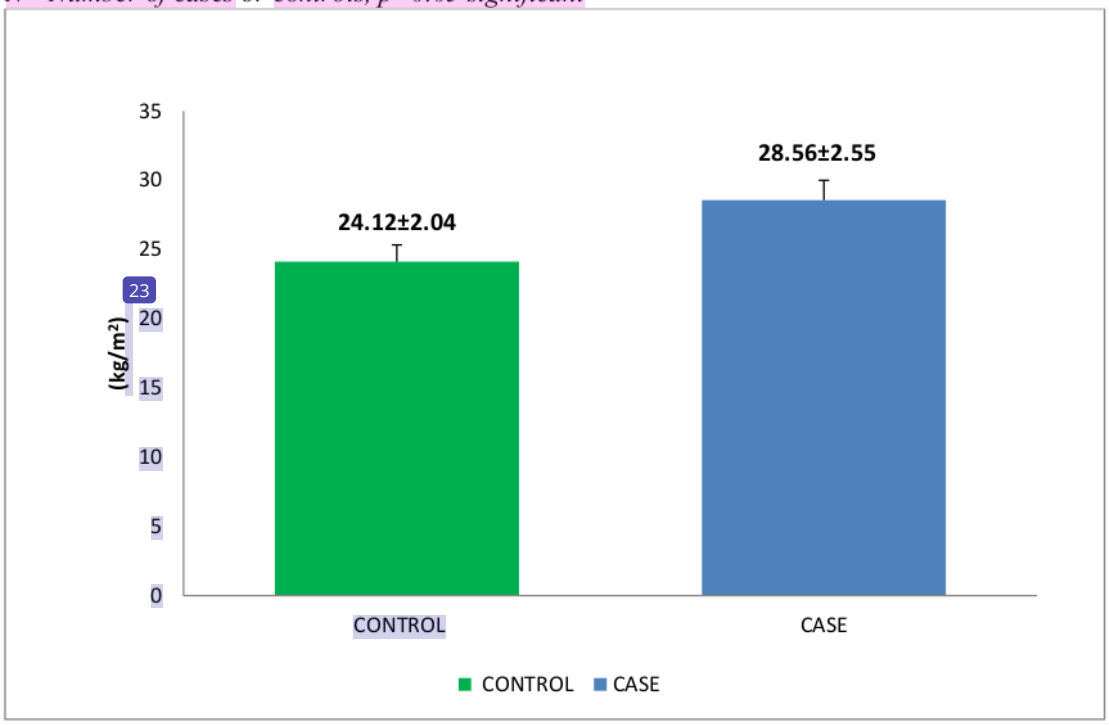


Fig. 3. BMI mean in Controls and Cases Groups.

KARL PEARSON'S CORRELATION OF COEFFICIENT AMONG THE STUDY PARAMETERS IN CASES.

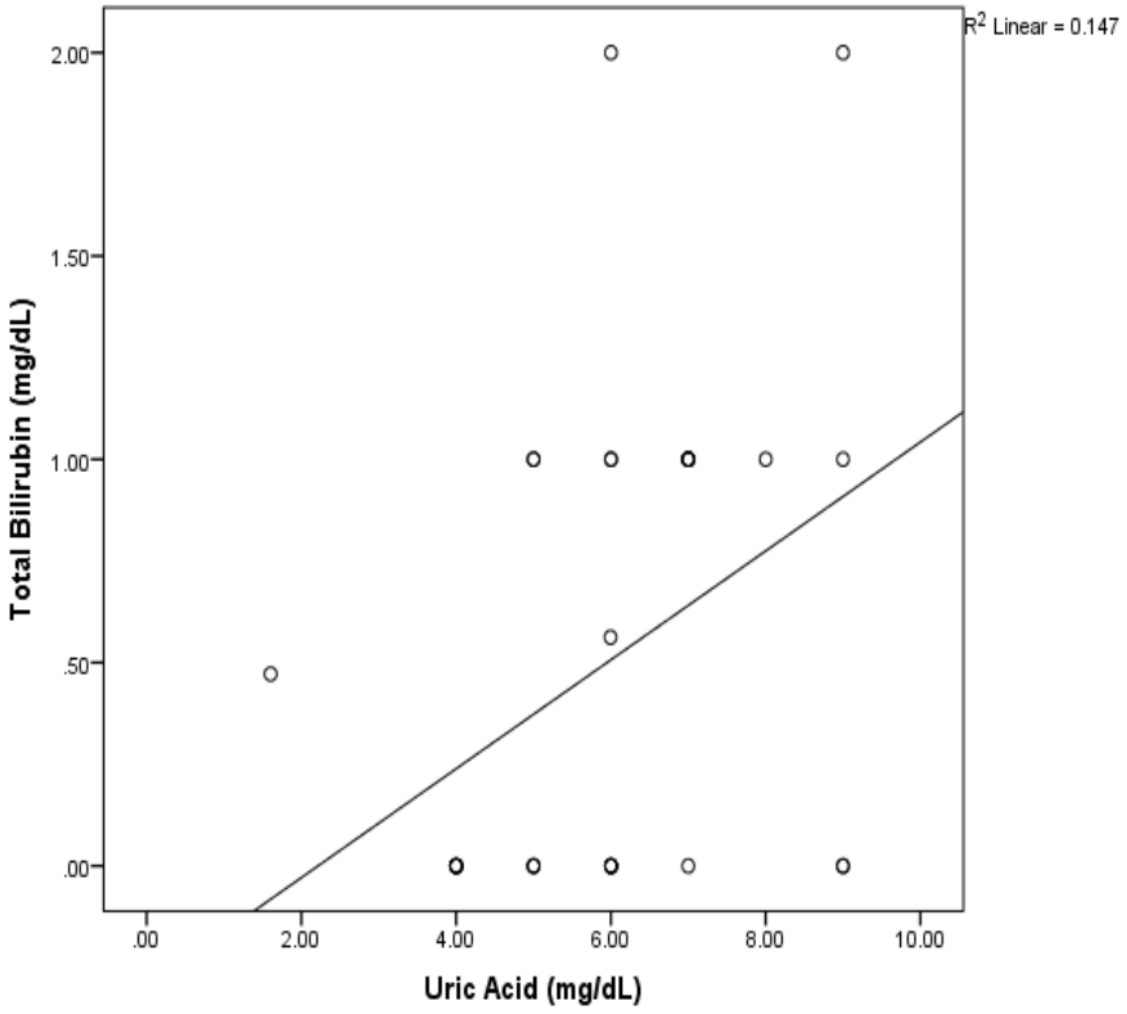
There was no correlation between Uric Acid and Total Bilirubin in Hypothyroidism cases.

Correlations

		Total Bilirubin (mg/dL)	Uric Acid (mg/dL)
Total Bilirubin (mg/dL)	Pearson Correlation	1	.383*
	Sig. (2-tailed)		.030
	N	32	32
Uric Acid (mg/dL)	Pearson Correlation	.383*	1
	Sig. (2-tailed)	.030	
	N	32	32

*. Correlation is significant when p< 0.05 level (2-tailed).

A scatter plot between levels of Total bilirubin and Uric acid



Discussion

Clinical hypothyroidism, or overt hypothyroidism, occurs when TSH levels are higher and free T4 levels are low, resulting in more noticeable symptoms (Jameson et al., 2018). Studies conducted in India as reported by Velayutham et al.,2017 and Unnikrishnan et al., 2013 have shown a higher prevalence of hypothyroidism among female patients.

Few studies have been done on serum uric acid & bilirubin in diagnosed cases of hypothryroidism, with varying outcomes. These studies showed the relationship between TSH, serum uric acid (SUA) & bilirubin levels were insignificant (Abebe et al., 2016). In a previous research, there were elevated levels of serum uric acid concentrations in patients diagnosed with clinical hypothyroidism. This is the result of low thyroid hormone secretion affecting proper functioning of the liver, thus increasing the liver metabolic rate. More purine is synthesized and uric acid, the by-product is released more (Khan et al., 2010).

Similar to our findings, Rafat, et al., (2019) found that uric acid level was elevated in hypothyroidism patients. In his study, he notes that increased Uric acid levels coincided with abnormalities in the parameters tested for chronic kidney disease. Thus, it can be deduced that the lack of efficiency of the renal system to excrete uric acid was the leading pathogenetic condition elevated in hypothyroidism. Khan et al., (2010) & Giordano et al., (2001) also found higher uric acid in hypothyroidism.

In a study done by Soliman, A. T et al, (2013), there was no significant correlation between bilirubin levels and thyroid disorders. This is contrary to our study findings. We found an insignificant increase in serum bilirubin levels in diagnosed hypothyroid patients ($p=>0.05$) with a mean concentration of 0.56 ± 0.47 in cases compared to 0.50 ± 0.26 in control groups.

A similar study on children age 2 to 14 years showed a slight increase in serum uric acid concentration in subclinical hypothyroidism compare to control though the difference was statistically negligible (Sayari et al., 2018). In another study, it was discovered that hyperuricemia in females with hypothyroid status is not related. (Satyajit, K et al., 2017).

We enrolled hypothyroidism patients along with apparently healthy controls to reduce the effect of confounding factors. In our study we did not find any statistically significant difference between cases and controls with respect to the levels of uric acid (p=0.5137) correlation and total bilirubin (p= 0.54).

There are increased levels of uric acid & bilirubin in hypothyroidism cases that show that uric acid and bilirubin could both be affected by thyroid gland disorders in one way or the other. In our investigation, we did not find any correlation between uric acid and total bilirubin in cases of hypothyroidism. However, we found that the BMI of patients with hypothyroidism was significantly raised as compared to controls (p=0.0001).

Further research needs to be done specifically on the role hypothyroidism plays in bilirubin biosynthesis to determine association, if any.

Summary-

In the study designed to evaluate serum Uric acid and Total Bilirubin in diagnosed patients of hypothyroidism and apparently healthy controls, the parameters estimated were:

- Serum Uric acid
- Total Bilirubin

From this, the following were observed:

- The concentration of serum Uric acid was not statistically significant in cases(p=0.5137) compared to controls.
- The level of Total Bilirubin was not significant though raised in cases (p=0.54) when compare to controls.
- There was no correlation between serum Uric acid and Total Bilirubin(r=0.383,p=>0.05) among cases.

Conclusion-

There was no significant difference between cases and controls with regard to the levels of Uric Acid and Total Bilirubin. We also observed no correlation between Uric Acid and Total Bilirubin in hypothyroidism. This may be attributed to the small sample size undertaken in this project. There is a necessity for further research to confirm findings but on a larger sample size

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