"ESTIMATION OF FRUCTOSAMINE AND GLYCATED HEMOGLOBIN IN NEWLY DIAGNOSED SUBCLINICAL HYPOTHYROID, CLINICAL HYPO AND HYPERTHYROID PATIENTS WITHOUT DIABETES MELLITUS:A ONE YEAR CROSS SECTIONAL STUDY"

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Seal & Signature of the HOD

Dr. Smita S Sonoli **M.D,Ph.D**
Professor and Head,
Department of Biochemistry,
KLE University's J. N. Medical College,
Belagavi-590010

Date :
Place : **Belagavi**

Seal & Signature of the Principal

Dr. N S Mahantashetti **M.D**
Principal,
KLE University's J. N. Medical College,
Belagavi-590010

Date :
Place: **Belagavi**

LIST OF ABBREVIATIONS USED

ABBREVIATION	EXPANSION
AGEs	Advanced Glycation End products
ADA	American Diabetes Association
ATA	American Thyroid Association
BMR	Basal Metabolic Rate
D_2 Gene	5-deiodinase type 2 gene
DIT	Diiodotyrosine
DUOX1	Dual Oxidase1
DUOX2	Dual Oxidase2
FPG	Fasting Plasma Glucose
HbA1c	Glycated Haemoglobin
H_2O_2	Hydrogen Peroxide
I	Iodine
IUPAC-IUB	International Union of Pure andApplied Chemistry-Intrnational Union of Biochemistry
kDa	Kilo Dalton
MIT	3-Monoidotyrosine
mg/dL	Milligram per decilitre
µmol/L	Micro mole per litre

NBT	Nitro Bue Tetrazolium
NIS	Sodium-Iodide Symporter
NHANES-III	Third National Health and Nutrition Examination Survey
OS	Oxidative Stress
PBI	Total Protein Bound Iodine
PVN	Peri Venricular Nucleus
ROS	Reactive Oxygen Species
SCH	Subclinical Hypothyroidism
sRAGE	Soluble form of Receptor for Advanced Glycation End Product
TBG	Thyroid Bound Globulin
T_g	Thyroglobulin
TH	Thyroid Hormone
T_3	Triiodothyroxine
T_4	Thyroxine
TPO	Thyroid Peroxidase
TRH	Thyrotropin Releasing Hormone
TSH	Thyroid Stimulating Hormone

ABSTRACT

Background and Objectives

Thyroid disease is the most common endocrine disorder encountered in clinical practice,it can present as hypo, hyper or subclinical hypothyroidism. Circulating sugars, primarily glucose and fructose when they come in contact with proteins and lipids cause damaging reactions and forming compounds called Advanced Glycation End Products (AGEs).Fructosamine and Glycated Hemoglobin (HbA1c) known AGEs are useful indicators to measure the peripheral metabolic function in patients with thyroid disorder.

We have undertaken this study to evaluate and correlate the role of Glycated Hemoglobin and Fructosamine in thyroid disorders.

Materials And Methods

This one year cross sectional study was done from January 2016 to December 2016 attending medicine (Endocrinology) Outpatient Department of KLE'S Dr Prabhakar Kore Hospital and Medical Research Centre, Belagavi. Newly diagnosed Subclinical hypothyroid,Clinical hypo and hyperthyroid patients were part of the study,along with age matched healthy individuals.The study consists of 110 subjects (80 cases+30 controls) who were included after obtaining informed and written consent.Suspected cases after clinical examination underwent TSH,T_3,T_4 investigations by Chemiluminescence method .After the confirmation of diagnosis,all the cases were asked to come in fasting state on the day of their choice and the FPG,Glycated Hemoglobin and Fructosamine assays were done.If FPG levels were more than reference interval (70-100 mg/dl) then those were excluded from the study.

The study was conducted in four groups.30 participants in control or euthyroid group,20 patients in Subclinical Hypothyroid group,Clinical hypothyroid and hyperthyroid group which consisted of 30 patients each,of either age and sex aged 20-60 years. Results were tabulated and subjected to appropriate statistical analyses.

Results:

In subclinical hypothyroid group the mean FPG (88.80±2.59),HbA1c (5.32±0.38) and Fructosamine values (485±40.16) were high when compared with the controls by one way ANOVA.Pair wise comparison of FPG (p=0.009),HbA1c (p=0.001) and Fructosamine levels (p=0.001) with controls by Tukeys multiple Post-hoc Bonferroni test showed statistically significant difference. There was a positive significant correlation between FPG and HbA1c (r=0.4704) ,while there was no significant correlation between FPG and Fructosamine (r=0.1317) levels by Karl Pearson's correlation coefficient method.

In clinical hypothyroid group the mean FPG (89.23±4.44), HbA1c (5.44±0.14) and Fructosamine (576.77±37.33) levels were high when compared with the controls by one way ANOVA. Pair wise comparison of FPG (p=0.001),HbA1c (p=0.001) and Fructosamine levels (p=0.001) with controls by Tukeys multiple Post-hoc Bonferroni test showed statistically significant difference. There was a positive significant correlation between FPG and HbA1c (r=0.466) and FPG and Fructosamine (r=0.421) levels by Karl Pearson's correlation coefficient method.

In clinical hyperthyroid group the mean FPG (93.67±0.92) and HbA1c (5.53±0.24) and low Fructosamine (269.43±7.90) levels when compared with the controls by one way ANOVA. Pair wise comparison of FPG (p=0.001), HbA1c (p=0.001) and Fructosamine levels (p=0.001) with controls by Tukeys multiple

Post-hoc Bonferroni test showed statistically significant difference.There was a positive significant correlation between FPG and HbA1c (r=0.406) and negative correlation between FPG and Fructosamine (r=-0.437)) levels by Karl Pearson's correlation coefficient method.

Interpretation and conclusion:

In the present study Subclinical hypothyroid ,Clinical hypothyroid and Clinical hyperthyroid groups had higher levels of FPG when compared with the Controls.HbA1c and Fructosamine levels were higher in Subclinical hypothyroid and Clinical hypothyroid groups when compared with the Controls.However in clinical hyperthyroid group FPG and HbA1c levels were higher while Fructosamine levels were low when compared with the Controls which was statistically significant.

The severity, prevalence and pathogenesis of abnormalities of carbohydrate metabolism in thyroid disorders are incompletely defined. To study the effect of thyroid hormones on glucose metabolism in non diabetic patients is an area for extensive research.Glycated Hemoglobin and Fructosamine could be useful indicators to measure the peripheral metabolic functions in patients with thyroid disorder.

Hence we suggest HbA1c and Fructosamine could be included in the thyroid work up of the patients.

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INTRODUCTION

Thyroid disease is the most common endocrine disorder encountered in clinical practice.[1] Thyroid hormone is produced by thyroid gland which regulates a wide range of genes after its activation from the prohormone, thyroxine (T_4) to the active form, triiodothyronine (T_3).[2] Thyroid disorder can present as hypothyroid, hyperthyroid or subclinical thyroid dysfunction.[1] Sub clinical Hypothyroidism (SCH) is defined as a serum thyroid-stimulating hormone (TSH) concentration above the statistically defined upper limit of the reference range and the serum free thyroxine within the reference range.[3] According to American Thyroid Association Hypothyroidism is a condition with underactive thyroid gland with decreased production of thyroid hormones. Hyperthyroidism is a clinical condition resulting from the action of excess thyroid hormone on tissues.[4]

Thyroid hormones have considerable effects in the regulation of glucose homeostasis, including modification of circulating insulin levels and other counter regulating hormones.[5] Thyroid disorders have got a major impact on glucose control. In thyroid dysfunctions, the glucose homeostatic balance is broken and it is mainly associated with increased hepatic gluconeogenesis which is a characteristic of hyperthyroidism.[6] In hypothyroidism glucose homeostasis also gets affected although it's clinical impact is less because of less disposal of glucose.[7] At the same time insulin resistance has been reported in subclinical hypothyroidism with altered glucose levels.[8] T_4 stimulation causes increase in protein synthesis.Higher concentration of T_3 causes protein catabolism and negative nitrogen balance.[9]

Subclinical hypothyroidism and clinical hypo and hyperthyroidism are inflammatory processes.[10] Circulating sugars primarily glucose and fructose are culprits of mitochondrial dysfunction in inflammatory process and when these blood sugars when they come in contact with proteins and lipids, a damaging reaction occurs forming compounds called Advanced Glycation End products (AGEs).The term glycation by the IUPAC-IUB Joint commission on biochemical Nomenclature for any reaction linking glucose to protein.[11] Glucose molecules are joined to protein molecules to form stable ketoamines through glycation,a nonenzymatic mechanism involving a liable Schiff base intermediate and amadori rearrangement. Examples of proteins subject to non-enzymatic glycation are Glycated Hemoglobin (HbA1c), Glycated Albumin is more correctly known as Fructosamine albumin.[12]

HbA1c is widely used for the assessment of glycemic status of the diabetic patients and the American Diabetes Association (ADA) recommended its use for diagnosing diabetes.[13] The glycated hemoglobin represents the fraction of hemoglobin that undergoes non-enzymatic glycation over the circulatory life span of the erythrocytes (usually 120 days).[14]

Fructosamine is also known as plasma protein ketoamine.[15] Fructosamine is glycated protein formed by a non-enzymatic reaction between a carbonyl group of a glucose molecule with an amino group of a protein.[16] In blood, Fructosamine is primarily glycated albumin, as it is the most abundant protein present. Fructosamine is also known as glycated serum proteins or glycated albumin. It is used to monitor the plasma glucose concentration over 2–3 weeks (shorter period) to assess diabetes management. [17]

Possibly Diabetes and thyroid disorders have a propensity to appear together in patients.[18] The severity,prevalence and pathogenesis of abnormalities of carbohydrate metabolism in thyroid disorders are incompletely defined.The extent of glycation of proteins provides an objective, retrospective index of glycemic control in thyroid disorders.[19]

NEED FOR THE STUDY

A positive association between thyroid and diabetes mellitus is well recognized. To study the effect of thyroid hormones on glucose metabolism in non diabetic patients is an area for extensive research.

Fructosamine and Glycated Haemoglobin are useful indicators to measure the peripheral metabolic functions in patients with thyroid disorder. There are lack of studies to correlate the effects of thyroid hormone on glycation. Fructosamine and Glycated Haemoglobin (HbA1c) can be included in the thyroid work up of the patients to assess the metabolic function and the subsequent response after the initiation of the therapy.

OBJECTIVES OF THE STUDY

- Estimation of Fructosamine and Glycated Haemoglobin levels in newly diagnosed subclinical hypothyroid, clinical hypo and hyper thyroid disorders without Diabetes Mellitus

- To study the correlation between Fructosamine levels and Glycated Haemoglobin levels in subclinical hypothyroid, clinical hypo and hyper thyroid patients.

REVIEW OF LITERATURE

THYROID GLAND

Thyroid gland is an important endocrine organ. Thyroid gland secretes T_3 and T_4 hormones which are essential for life. The structure and functions of thyroid gland were discovered during 17^{th} to 20^{th} centuries. Some secreting glands were described by Galen, adjacent to thyroid cartilage which were named laryngeal gland by Eustachius. Wharton in 1656 named them glandulae thyroidaeae (Thyroid glands) because of their anatomic proximity to thyroid cartilage.Goitre was established during 1700s.[20] Gley in 1891 was able to differentiate the functions of the thyroid from those of the parathyroid glands.[21] In 1895, Magnus-Levy established the effect of the thyroid on the metabolic rate, the low metabolic rate in patients with hypothyroidism, and the fact that the administration of thyroid extracts to these patients and to individuals with normal thyroid function increased their oxygen consumption.[22] Hashimoto's disease was described in 1912.[23]

In 1827,by experimental thyroidectomy. The association between thyroid and different body functions was studied. Thyroid gland internal secretory function was formulated by King 9 years later.[24] Thyrotoxicosis was first described in 1786 by C H Parry but was reported in 1825 .R J Graves described exophthalmic goiter and the disease named after him.[25]

ANATOMY

The thyroid is butterfly shaped gland located in the front of neck just above the trachea .The adult thyroid gland weighs between 15 to 20 gm.It is normally

bilobed, with right lobe somewhat larger than the left lobe.The lobes are connected by the isthamus. Microscopically, thyroid gland is composed of follicles or acini.

The secretory units of the thyroid gland are thyroid follicle.It is composed of an outer layer of epithelial cells that encloses an amorphous material known as colloid.colloid is composed of mainly thyroglobulin (Tg) and small quantities of iodinated thyroalbumin.

Thyroid gland also contains another type of cell known as parafollicular or c cells. These cells produce the polypeptide hormone calcitonin.[26]Thyroid gland anatomy as shown in Figure 1.

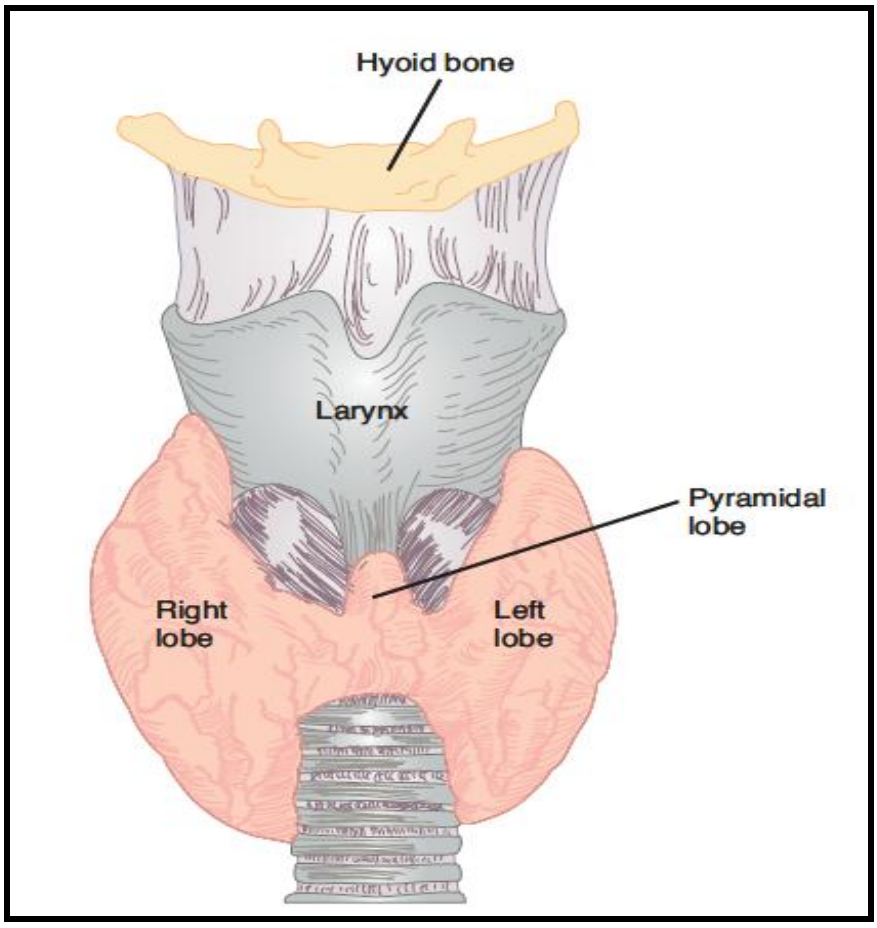


Figure 1: Human Thyroid Gland [27]

THYROID HORMONES

CHEMISTRY

The thyroid gland secretes thyroxine(3,5,3,5-L-tetraiodothyronine) and triidothyronine (3,5,3-L-triidothyronine hormones commonly known and T_4 and T_3 respectively as shown in Figure 2.

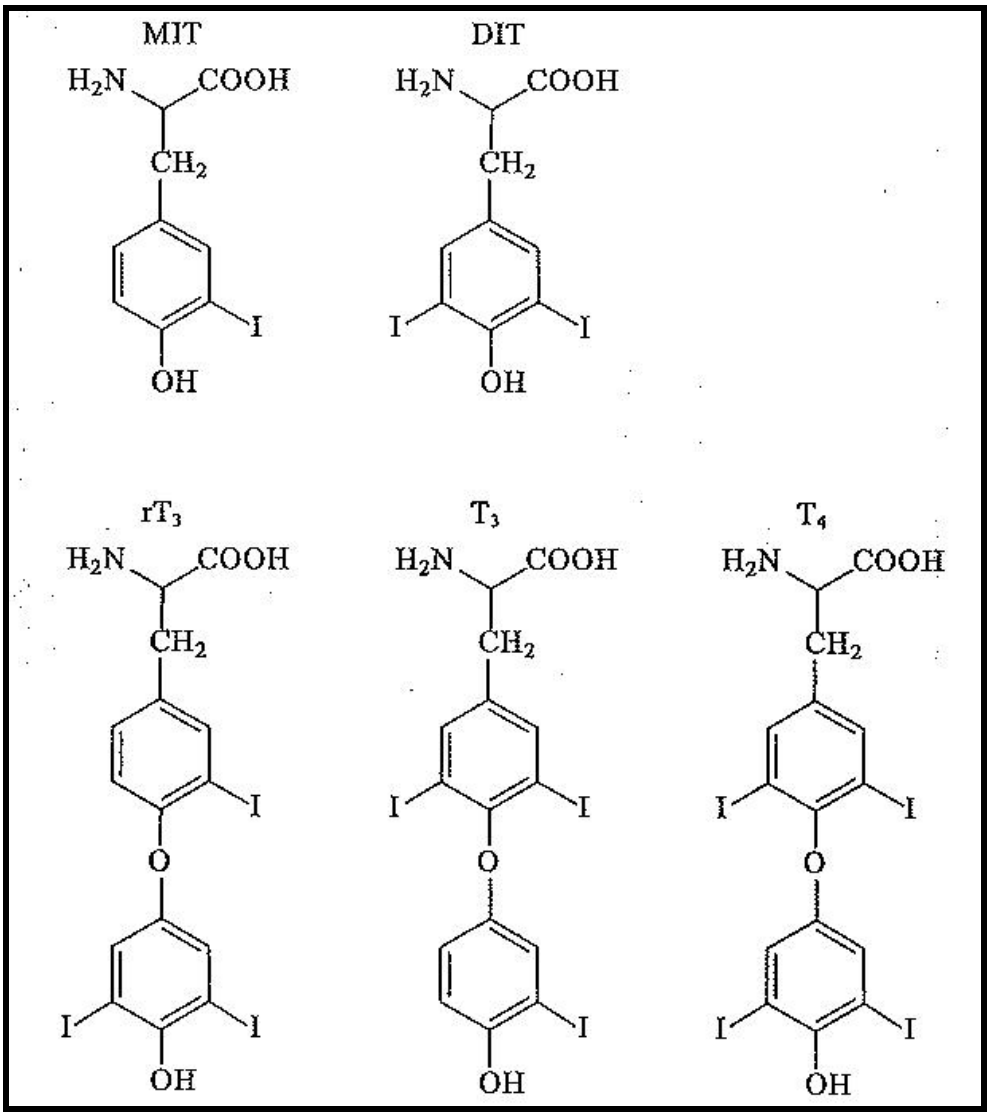


Figure 2 : Structure of Thyroid Hormones and their precursors

MIT-Monoiodotyrosine,DIT-Diiodotyrosine,rT_3-3,3'5'-L- Triiodotyrosine,T_3-3,5,3'-L- Triiodothyronine,T_4-3,5,3',5'-L-Tetraiodothyronine. [28]

THYROID HORMONE SYNTHESIS

Thyroid hormones are derived from Thyroglobulin (Tg).Tg is a large iodinated glycoprotein. It is ionitated after secretion into the thyroid follicle. Iodide uptake by the thyroidal Sodium-Iodide Symporter (NIS).Then its transfer to the colloid,and its oxidation by thyroid peroxidise(TPO).Specific tyrosine residues of Tg homodimers it is then iodinated at the apical border of the thyroid cell to form 3- monoiodotyrosine (MIT) and Diiodotyrosine(DIT).This requires formation of hydrogen peroxide by dual oxidase 1 (DUOX1) and (DUOX2) and of TPO,which catalyzes the oxidation of iodide and its transfer to tyrosine.[29]

These are subsequently coupled via an ether linkage.Reuptake of Tg into the thyroid follicular cell initiates proteolysis and release of newly synthesised T_4 and T_3.Increased demand for thyroid hormones,usually signalled by thyroid stimulating hormone (TSH) binding to its receptor on the basolateral surface of the follicular cells,leads to Tg reabsorption from the follicular lumen and proteolysis within the cell to yield thyroid hormones for secretion into the bloodstream.[30]

SYNTHESIS AND SECRETION OF THYROXINE

Step 1:Uptake of iodine

Thyroid gland concentrates Iodine .This step is stimulated by TSH and inhibited by thiocyanate and perchlorate.

Step 2:Oxidation of iodine

The iodide which is taken up by the thyroid cell is oxidised to active iodine.This step is catalysed by the enzyme thyroperoxidase.This reaction needs

hydrogen peroxide,which is produsedby an NADPH-dependent reaction.This step is stimulated by TSH and inhibited by anti thyroid drugs such as thiourea,thiouracil and methimazole

Step 3:Iodination

Thyroglobulin is synthesised by thyroid follicular cells.This is iodinated. Thyroglobulin contains 10% carbohydrates and 115 tyrosine residues out of which 35 residues can be iodinated.Thus 3-monoiodotyrosine (MIT) and 3,5-di-iodotyrosine (DIT) are produced.

Step 4:Coupling

Tyrosine residues in the thyroglobulin are aligned opposite each other and they are coupled. When two DIT molecules couple, one molecule of tetraiodothyronine (T_4) is formed.T3 is formed by de-iodination of outer of outer ring of T_4 by 5'-deiodinase.T_4 residues are attached to thyroglobulin molecule.

Step 5:Storage

Thyroglobulin contains about 8 T_4 residues per molecule.It is stored as colloid in the thyroid acini.

Step 6:Utilization

When necessary arises,thyroglobulin is released into the cell by pinocytosis.

Step 7:Hydrolysis

The T_4 is liberated by specific proteases.

Step 8:Release

The T_4 generated is released into the bloodstream.The T_3 is produsedby de-iodination at 5' position either inside the thyroid cell or in the peripheral tissues.

Step 9:Salvaging of iodine

MIT and DIT are not utilised and they are de-ionised and salvaged for the reutilisation inside the cell itself.

Step 10:Transport of Thyroid Hormones

They are transported in plasma by proteins.Bound form is biologically inactive, but they can be rapidly released.Total protein bound iodine (PBI) is about 10µg/dl and T_4 constitutes 8µg/dl.TBG Carries 80% of T_4 and 60% of T_3.

Step 11:Catabolism of Thyroid Hormones

Half life of T_3 is 1 day and T_4 has half life of 4-7 days.T_3 is biologically more active.T_4 is prohormone which is deiodinated to T_3.De- iodination takes place in peripheral tissues.This is done by selenium containing enzyme de-iodinase. Part of T_3 and T_4 are conjugated with glucuronic acid and excreted through bile and through urine.[31] shown in Figure 3.

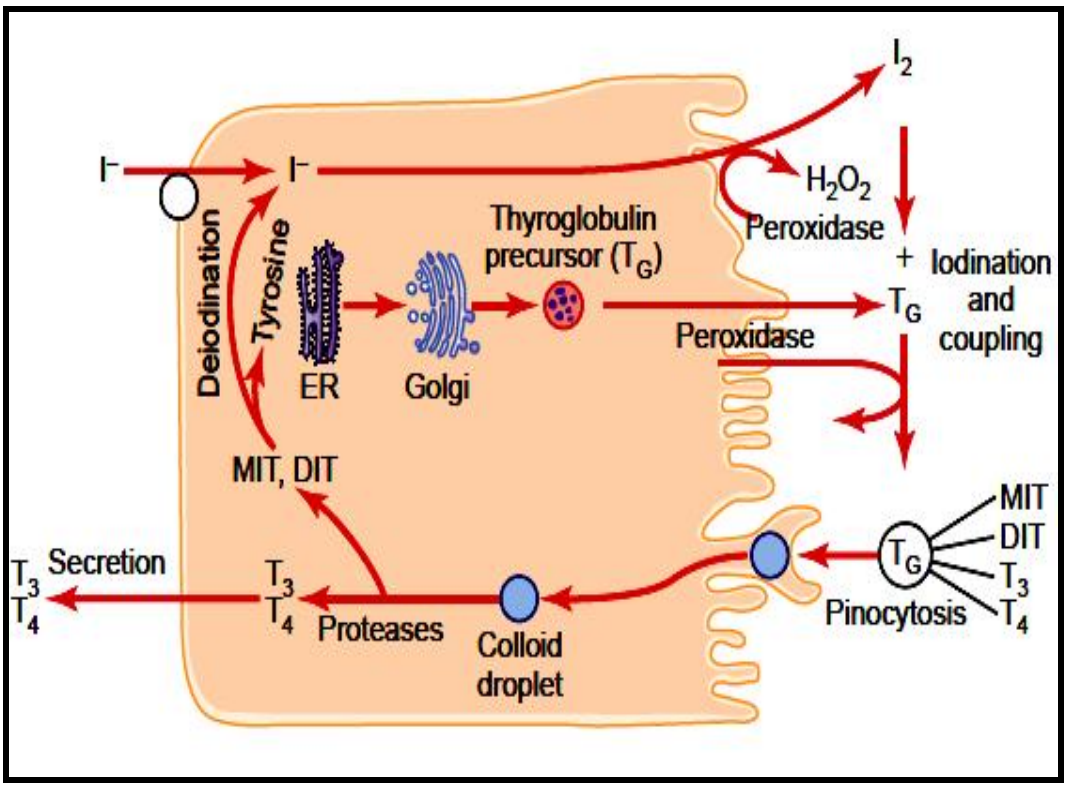


Figure 3: Thyroid cellular mechanisms for iodine transport, Thyroxine(T_4), Triiodothyronine (T_3) Formation and their release into the blood.

MIT-Mono iodo tyrosine DIT-Diiodotyrosine Tg-Thyroglobulin.[32]

The Thyroid Feedback Mechanism

The concentration of thyroid hormones in plasma and tissues is under the control of the thyroid hormone axis which consists of the following:

1. Thyrotropin-releasing hormone (TRH) secretion by cells in the hypothalamus
2. TSH secretion by cells of the anterior pituitary
3. The plasma binding proteins
4. Cellular uptake mechanisms,
5. Intracellular deiodinases
6. Nuclear thyroid hormone receptors.[33]

TSH is a 31-kDa hormone.It is composed of α and β subunits. α subunit is common to other glycoprotein hormones . β subunit is unique to TSH.TSH axis is regulated by thyrotropin releasing hormone (TRH).It influences the biologic activity of the hormone.[34]

Thyroid Hormone (TH) regulates TRH gene expression and production through a negative feedback mechanism.Thyroid releasing Hormone (TRH) expression is high when TH levels are low.TRH expression is suppressed when TH levels are increased. TRH expression is regulated by thyroid hormone in the Peri Ventricular Nucleus (PVN).[35,36] This cell specific action of TH suggests that the TRH neurons in the PVN have all the elements necessary to sense and respond to circulating peripheral TH levels. Clearly, the basal level of TRH gene expression is important in determining the set-point for regulation by TH through either a direct or indirect mechanism.Thyroid feed back mechanism as shown in Figure 4.

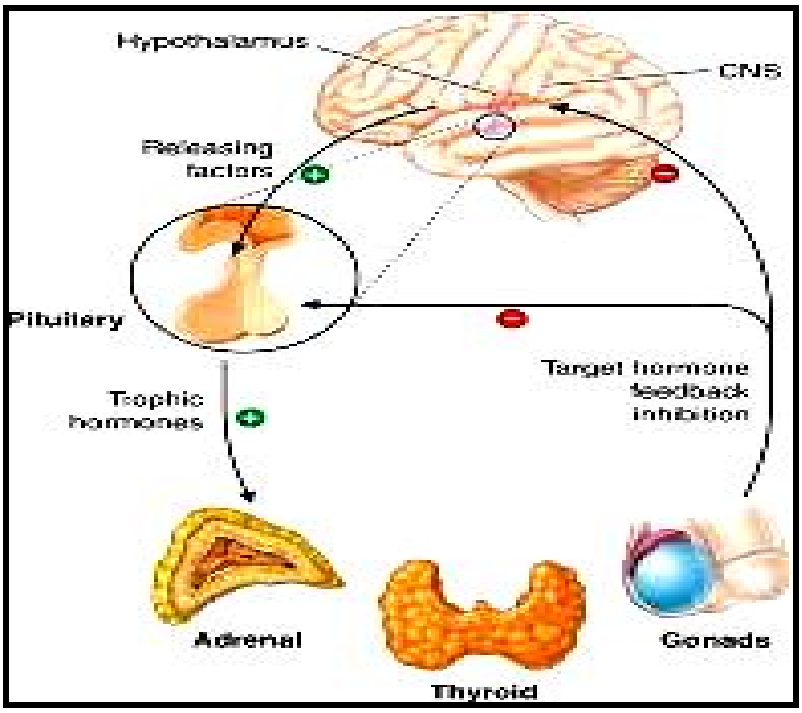


Figure 4: Feed Back Loop Of Thyroid Axis [37]

TSH has long plasma half life of 50 mins. It is released in a pulsatile manner and exhibits a diurnal rhythm. Highest levels of TSH occur at night. Single measurements are adequate to assess its circulatory levels. Immunoradiometric assays are used which are highly sensitive and specific for TSH assay. These assays readily distinguish between normal and suppressed TSH values. They are used for the diagnosis of hyperthyroidism (low TSH) as well as hypothyroidism (high TSH).[38]

Metabolic Effects of Thyroid Hormones

1. T_4 stimulation causes increase in protein synthesis.
2. Higher concentration of T_3 causes protein catabolism and negative nitrogen balance.
3. Thyroid Hormones increase Gluconeogenesis and Glycogenolysis.
4. Major effect of thyroid hormone is calorigenic or thermogenesis.
5. Thyroxine increases cellular metabolism so Basal Metabolic Rate (BMR) is increased.
6. Fatty acid metabolism is increased. Cholesterol degradation is decreased.

Actions of thyroid hormones is shown in Figure 5.

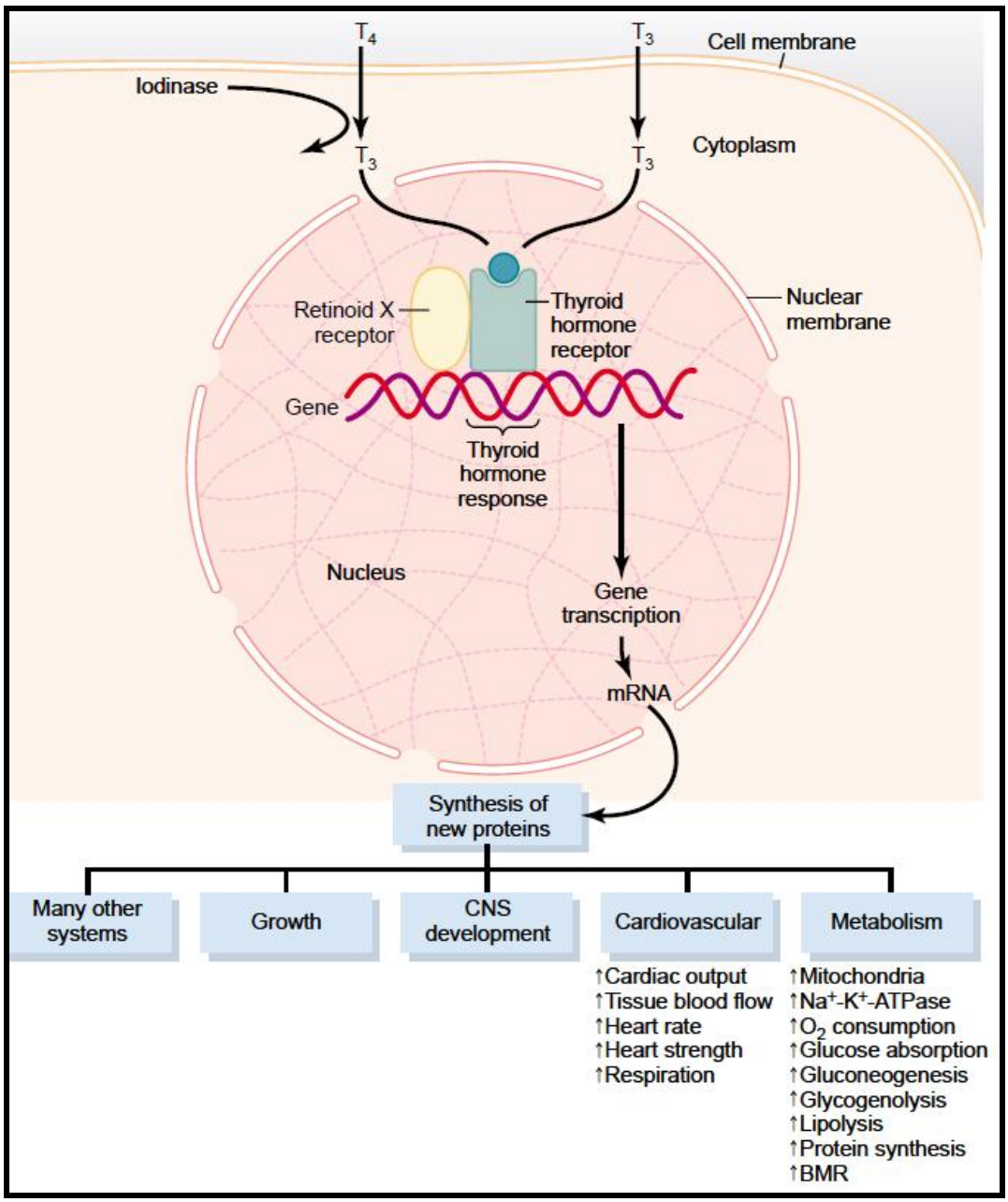


Figure 5: Actions of Thyroid Hormone on cells of different systems [39]

Thyroxine (T_4) and Triiodothyronine (T_3) readily diffuse through the cell membrane.Most of the T_4 is deiodinated to form T_3.This T_3 interacts with the thyroid hormone receptor,bound as a heterodimer with a retinoid x receptor of the thyroid hormone responce element of the gene. This ultimately causes either increases or decreases in transcription of genes that lead to formation of proteins,thus producing thyroid hormone response to cell.

TSH Assay to diagnose thyroid disorder was shown in Figure 6.

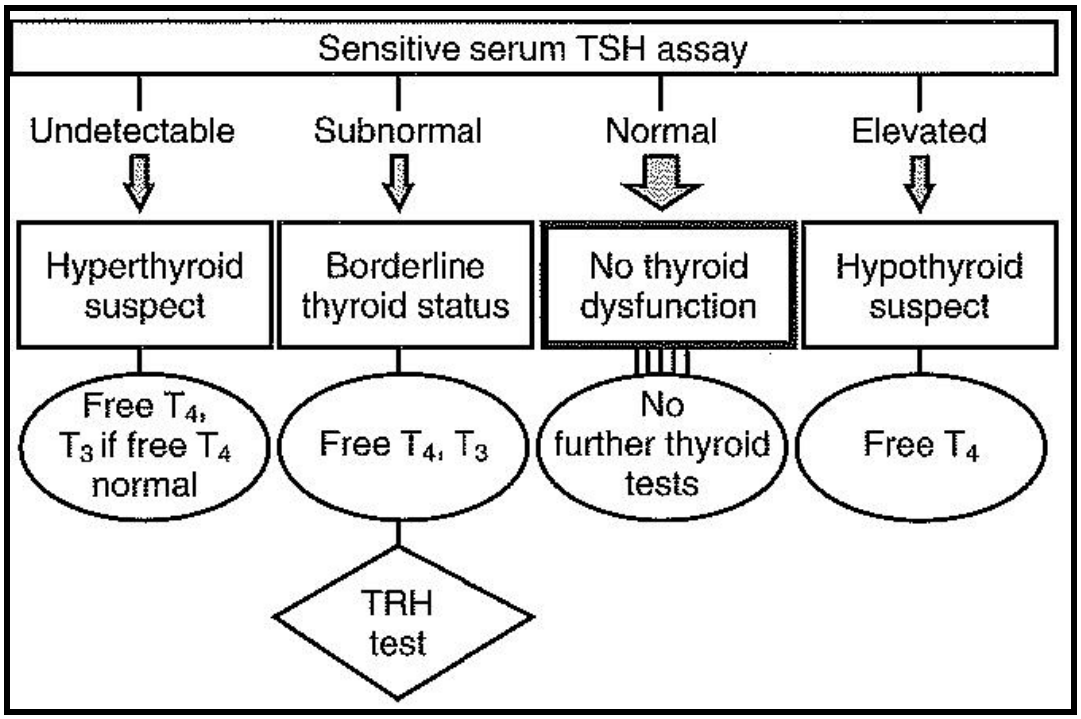


Figure 6: TSH Assay [40]

SUBCLINICAL HYPOTHYROIDISM

Subclinical hypothyroidism (SCH) is a condition where there is increased serum TSH levels above the defined upper limit of the reference range and the serumT_3,T_4 levels are within the reference range.[3] Reference range for TSH for serum TSH is 0.45 to 4.50 μU per mL.[41] The third National Health and Nutrition Examination Survey (NHANES III) screened, normal subjects who were thyroid antibody negative.[42] In this population, the median TSH concentration was 1.39 mIU/l, with the 95% TSH reference limits between 0.45 and 4.12 mIU/l. The above-mentioned consensus conference on subclinical thyroid diseases essentially agreed with this reference range. Serum TSH varies over time in healthy subjects, leading to occasional abnormal values. Then repeat serum TSH along with free thyroxine measurements within 3–4 months is required.[43,44] If elevated serum TSH

concentrations are confirmed, and free thyroxine values are within the normal range, the diagnosis of SCH is made.

Patients with SCH may have vague symptoms of hypothyroidism which are not specific.It is not easy to identify these patients on the basis of such non-specific symptoms and signs. Thus this disorder can only be diagnosed on the basis of laboratory test results.The worldwide prevalence of SCH ranges from 1-10%.[45]

Thyroid hormones (T_4 and T_3) they bind to intranuclear receptors that function as transcription factors and regulate gene expression. They have special effect on growth and regulate calorigenesis and metabolic rate throughout life. At molecular level thyroid hormones enhance mitochondrial metabolism, stimulate protein synthesis and carbohydrate metabolism. Thyroid hormones maintains the basal metabolic rate. Regulates the metabolism of endogenous and exogenous substances. Any alterations in the concentrations of thyroid hormone-binding proteins can profoundly affect the total concentrations of T_4 and T_3 without significant changes in the free hormone concentrations.[46]

HYPOTHYROIDISM

Hypothyroidism is defined as failure of the thyroid gland to produce sufficient thyroid hormone to meet the metabolic demands of the body.[47] Hypothyroidism may occur as a result of primary gland failure or insufficient thyroid gland stimulation by the hypothalamus or pituitary gland.[48] The best laboratory assessment of thyroid function, and the preferred test for diagnosing primary hypothyroidism,is a serum TSH test.[49] If the serum TSH level is elevated, testing should be repeated with a serum free thyroxine (T_4) measurement.[50] Overt primary hypothyroidism is indicated with an elevated serum TSH level and a low serum free T_4 level.[47]Evaluation of suspected hypothyroidism was shown in Figure 7.

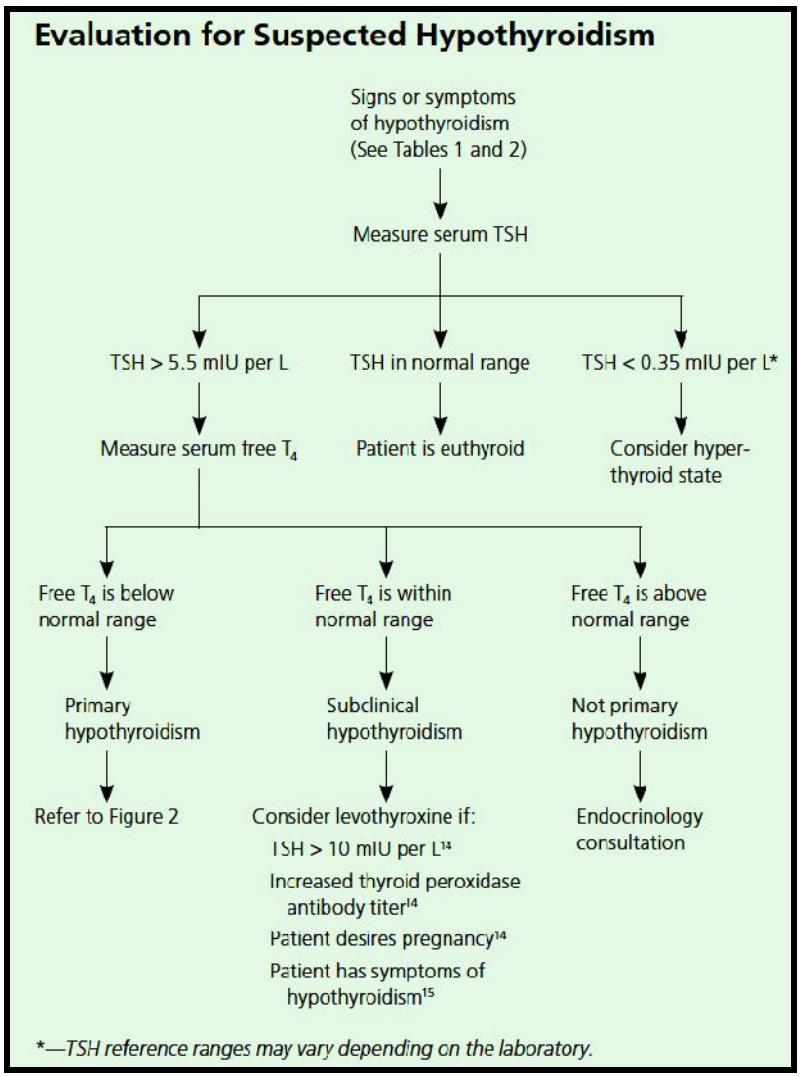


Figure 7: T_4-Thyroxine,TSH-Thyroid stimulating hormone [48]

PROTEIN,CARBOHYDRATE METABOLISM IN HYPOTHYROIDISM

In hypothyroidism, there is decrease in energy metabolism and heat production which is reflected in the low basal metabolic rate (BMR), protein synthesis and degradation are decreased. Permeability of capillaries to protein is increased. In addition, the albumin pool is increased because of the greater decrease in albumin degradation compared with albumin synthesis. In hypothyroidism, degradation of insulin slowed and the sensitivity to exogenous insulin is increased. A further influence on glucose uptake may occur at the tissue level. There is polymorphisms in

the 5-deiodinase type 2 (D2 gene), which will affect local T_3 production. It shown to be associated with impaired glucose disposal [51] which is shown in Figure 8.

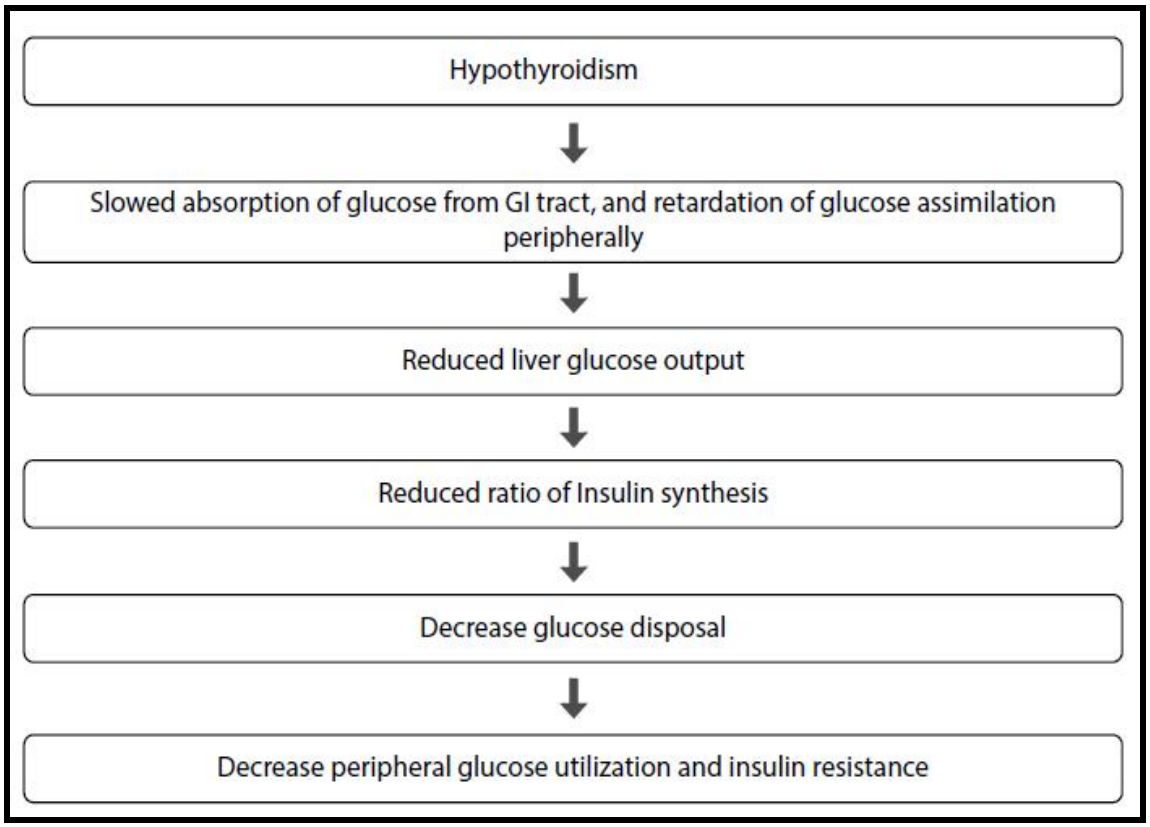


Figure 8 : Relation between hypothyroidism and insulin resistance [52]

OXIDATIVE STRESS IN HYPOTHYROIDISM

Oxidative stress (OS) results are an imbalance between the antioxidant defence systems and the rate of production of reactive oxygen species (ROS).It not only leads to lipid peroxidation and oxidative Deoxy Ribo nucleic Acid (DNA) damage but also interferes with physiologic adaptation and intracellular signal transduction. The resulting change in the intracellular redox status leads to the activation of protein kinases, for example, tyrosine kinase,protein kinase C and the mitogen-activated protein kinase cascade leading to altered cellular functions.[53]

The thyroid hormones (THs), namely, tetraiodothyronine (T_4) and a much smaller proportion of triiodothyronine, exert actions at the cellular level by binding to a set of specialized receptors that couple to both genomic and non genomic signaling pathways. They are subjected to transformations in the peripheral tissues, mainly in the form of deiodination. The general metabolic effect of THs is a relative acceleration of the basal metabolism that includes an increase of the rate of both catabolic and anabolic reactions. While ROS production depends largely on the mitochondria, THs do not directly determine the respiratory state of the mitochondria.[54]

Hypothyroidism-associated ROS is the consequence of both increased production of free radicals and reduced capacity of the antioxidative defense. Excess thyroid-stimulating hormone (TSH) might alter oxidative stress processes.[55] Hypothyroidism-induced dysfunction of the mitochondrial respiratory chain can lead to the accelerated production of free radicals.[56]

Lipid peroxidation is reported to be high in hyperlipidemia,which is a consistent biochemical feature in hypothyroidism.The presence of OS in hypothyroidism correlates with the lipid risk factors of atherosclerosis.[57] Metabolic disorder from autoimmune-based hypothyroidism can also increase oxidative stress.[58] Low-grade chronic inflammation causes endothelial dysfunction and impairs nitric oxide availability, leading to increased OS in Hashimoto's thyroiditis.[59] While some authors suggest that tissues may be protected from oxidant damage because of a hypometabolic state in hypothyroidism, others report increased oxidative stress in hypothyroidism.[60]

There is positive correlation between TSH and MDA level was found.[61]They postulated the role of excess TSH to directly produce oxidative stress. Hypothyroidism is associated with increased oxidative stress response. Treatment with L-thyroxine is effective in bringing reduction in the level of stress markers.[62]

Oxidative stress (OS) in majority of studies it is consistently associated with hypermetabolic states such as hyperthyroidism.[63] In hyperthyroidism thyroid hormones they promote mitochondrial utilization of oxygen and leads to excess generation of free radicals.Multiple pathways forming oxidative stress in hypothyroidism is shown in Figure 9.

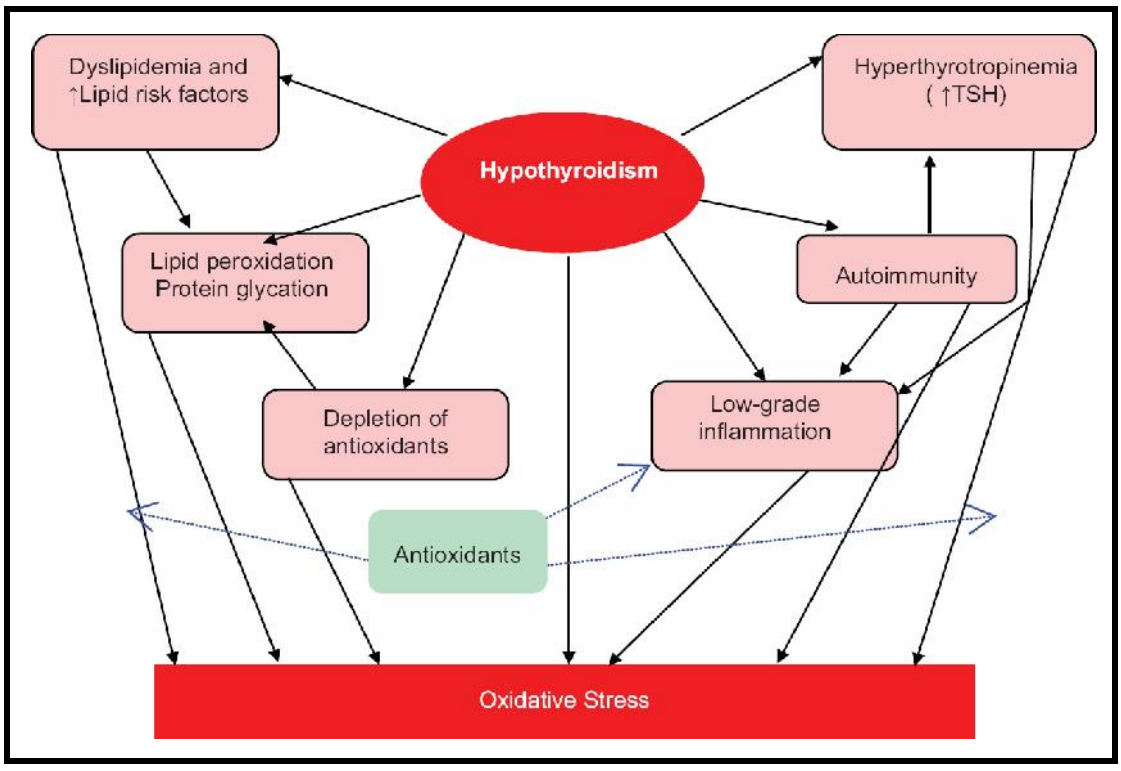


Figure 9:Multiple pathways operating alone or in concurrence leading to oxidative stress in hypothyroidism. Black arrows show the proposed mechanisms and blue arrows represent various proposed targets of anti oxidants preventing build up of oxidative stress [64]

HYPERTHYROIDISM

Clinical hyperthyroidism is caused by the effects of excess thyroid hormones, also called as thyrotoxicosis.[65] Most common cause for hyperthyroidism is Grave's disease. Which accounts for 60 to 80 percent of all cases.[66] It is an autoimmune disease caused by an antibody,active against the thyroid-stimulating hormone (TSH) receptor.[67] TSH receptor stimulates the gland to synthesize and secrete excess thyroid hormone.Thyroid hormones have profound metabolic effects and hyperthyroidism is characterised by energy expenditure with increased energy expenditure with increased oxidation of protein,glucose and lipid. [68,69] Hyperthyroidism is a catabolic state with increased energy expenditure, increased glucose turnover,increased lipolysis and increased protein turnover .The increase in protein turnover includes increased protein breakdown at the whole-body level and increased muscle protein breakdown.[70]

Protein,Carbohydrate metabolism in Hyperthyroidism

In hyperthyroidism there is stimulation of metabolism and heat production, so there will be increased Basal Metabolic Rate (BMR).So despite of increased food intake there will be caloric and nutritional inadequacy.Protein synthesis and degradation rates are increased.Studies of protein metabolism in hyperthyroid patients before and after treatment suggested that there is net protein catabolism is mainly due to depressed rates of whole body protein synthesis with low or normal rates of proteinolysis.[71]Degradation rates are more compared to synthesis.So there will be net decrease in tissue protein,as indicated by loss of weight,muscle wasting,proximal muscle weakness and mild hypoalbuminemia.In hyperthyroidism,there is accelerated turnover of insulin.[71]

Oxidative stress (OS) in majority of studies it is consistently associated with hypermetabolic states such as hyperthyroidism .[72] In hyperthyroidism thyroid hormones they promote mitochondrial utilization of oxygen and leads to excess generation of free radicals. [73] Interaction between advanced glycation end products (AGEs) and with receptor, generates reactive oxygen specie s(ROS). In their study on hyperthyrodism Caspar-Bell ,G Dhar concluded that if levels of serum sRAGE are low then the levels of AGEs and AGEs/sRAGE are high..Low levels of sRAGE and high levels of serum AGEs will generate more ROS leading to hyperthyroidism and its complications.[74]

Glycated Haemoglobin (HbA1c)

Blood oligosaccharides are attached to many proteins after translation, forming glycoproteins. HbA1c is composed in the red blood cells of the diabetic patient by combination of the glucose to the amino acid molecule of the haem, one of the component of haemoglobin so the name glycated Haemoglobin.[75] Glycation refers to a monosaccharide (usually glucose) attaching non enzymatically to the amino group of a protein. Glycated haemoglobin is formed by the condensation of glucose with select amino acid residues, commonly lysine, in haemoglobin to form an unstable Schiff base (aldimine, pre-HbA_{1c}).The Schiff base may dissociate or may undergo an Amadori rearrangement to form a stable ketoamine.[76] Formation of Glycated haemoglobin was shown in Figure 10.

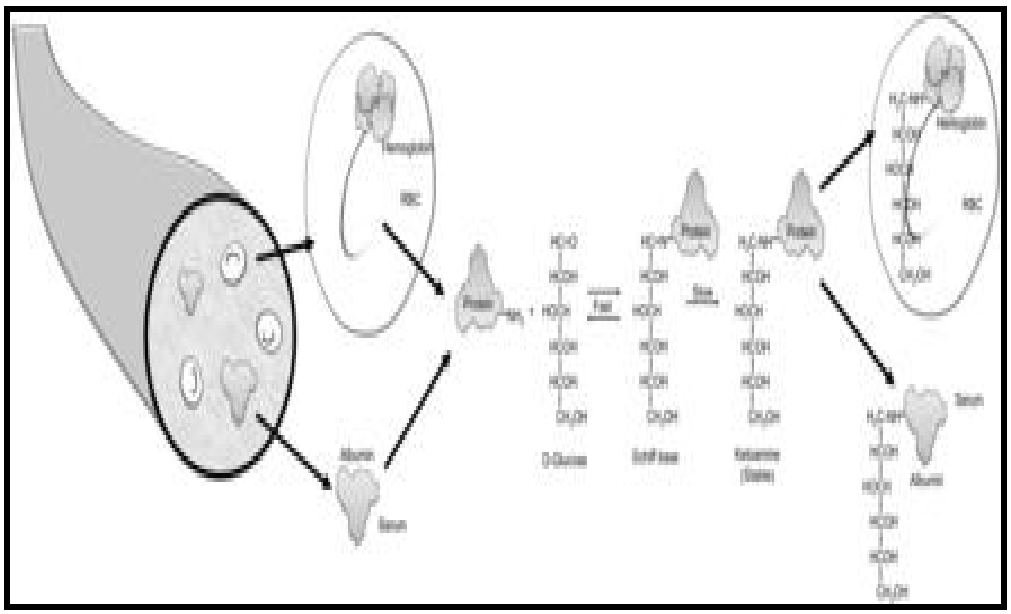


Figure 10: Mechanism in formation of Glycated Haemoglobin

An important attribute is that glycation occurs continuously over the lifetime of the protein, so the concentration of the glycated protein reflects the average blood glucose value over a period of time. While HbA1c is by far the most extensively used and studied.Other glycated proteins that have been evaluated in clinical studies include Fructosamine, glycated albumin, and advanced glycation end products (AGEs).[77-79]

FRUCTOSAMINE

Fructosamine is formed by joining of glucose molecules to protein molecules through glycation.Glycation is non-enzymatic mechanism which involves a liable Schiff base intermediate and Amadori rearrangement. Fructosamine is chemical name for 1-amino -1-deoxy fructose.It is also called isoglucosamine by Emil Fischer who first synthesised the compound in 1886.[81] Fructosamine is a ketoamine,a derivative of the non enzymatic reaction product of a sugar(usually glucose) and a protein(usually albumin).

The terms glycated albumin or protein are commonly used and they are examples of Fructosamines.The Fructosamines arise from a post- translational modification involving non enzymatic mechanism.[10]

CHEMISTRY OF GLYCATION

Millard was first to describe Glycation. It is the reaction of amino acids and reducing sugars to form stable ketoamines.[82] The straight chain Fructosamine formed from glucose thought to undergo cyclization to a hemiketal furanose or pyranose structure for added stability.[83] Chemistry of glycation was shown in Figure 11.

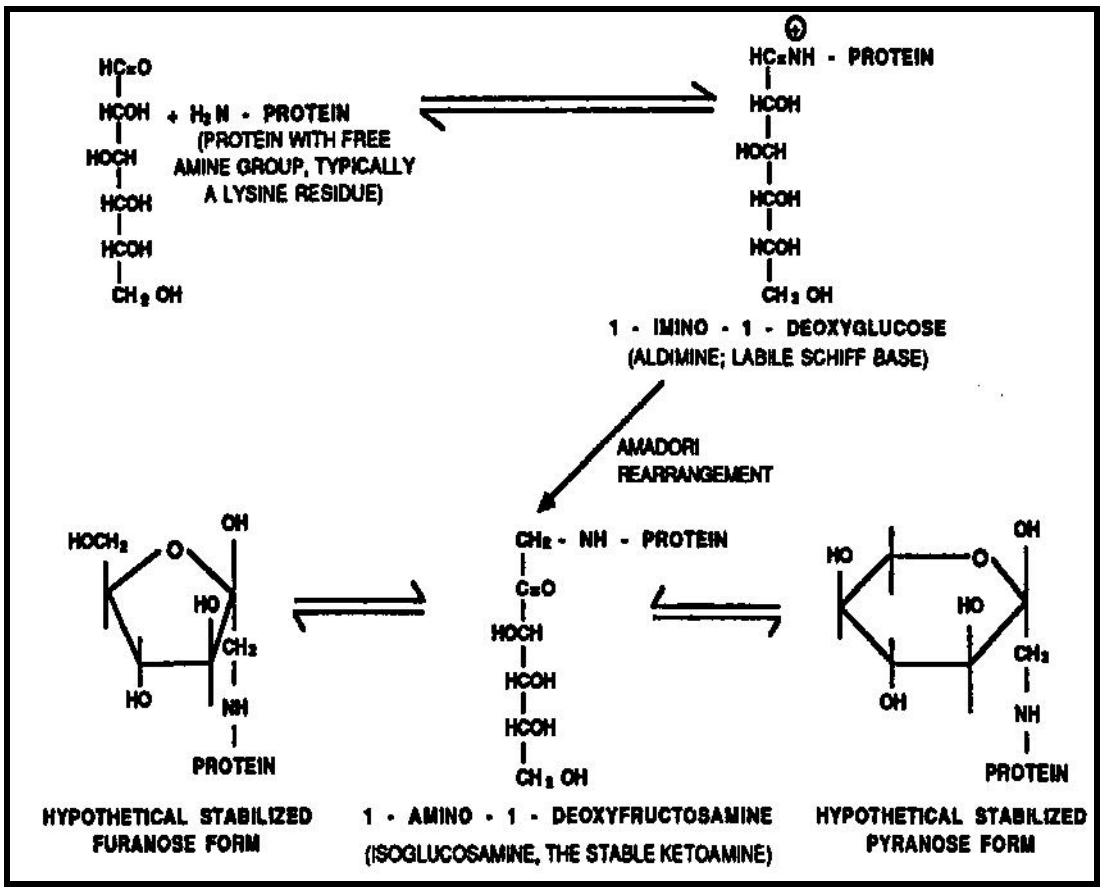


Figure 11: The Reaction Of Glucose And Protein To Form Fructosamine

Analytical Approaches For Fructosamine Estimation [11]

Different methods have been used to measure fructosamine, these are

a) Phenylhydrazine procedure

b) Furosine procedure

c) Affinity Chromatography

d) 2-Thiobarbituric Acid colorimetric Procedure

e) Nitro blue Tetrazolium (NBT) colorimetric procedure. Different analytical approaches for Fructosamine estimation was shown in Figure 12.

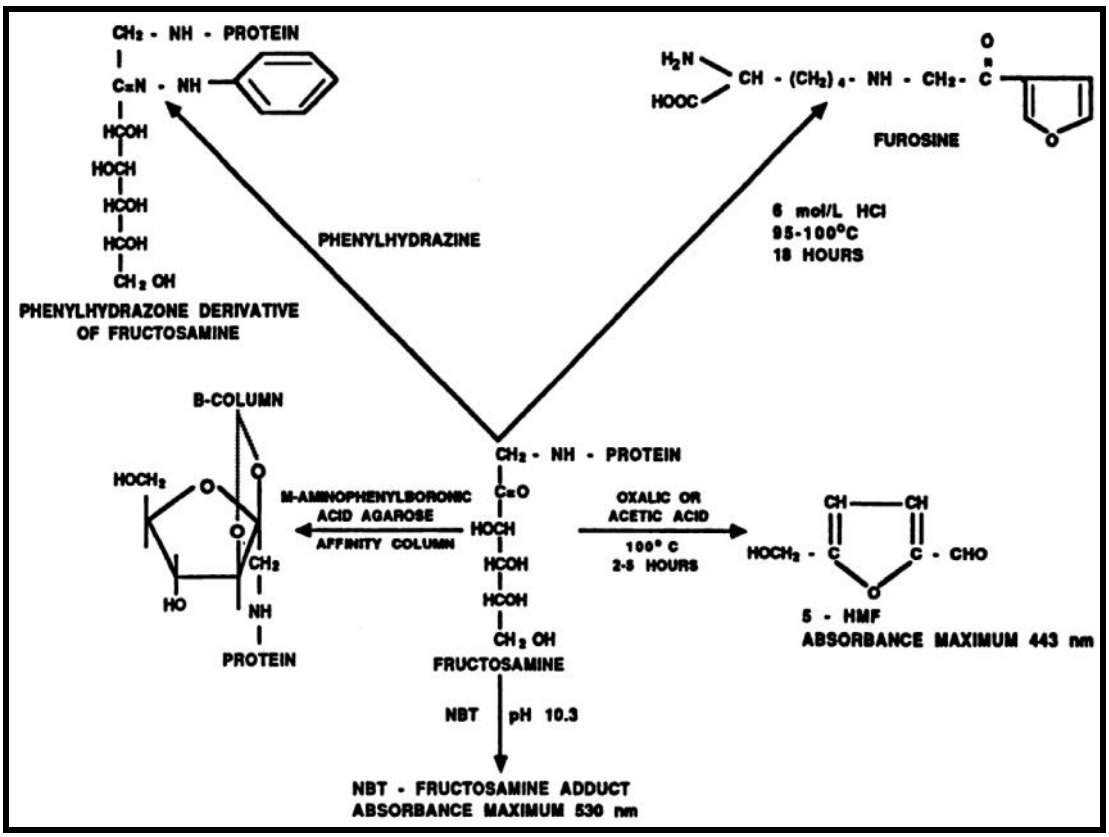


Figure 12: Analytical Approaches For Fructosamine Estimation

METHODOLOGY

SOURCE OF THE DATA

The present study comprises of newly diagnosed subclinical hypothyroid, Clinical hypothyroid and clinical hyperthyroid patients without Diabetes Mellitus, attending Endocrinology outpatient department of KLE'S Dr Prabhakar Kore Hospital and Medical Research Centre, Belagavi. Blood samples of age and sex matched normal healthy volunteers, were taken as controls.

STUDY DESIGN:

Cross sectional study

STUDY PERIOD:

Period of one year from January 2016 to December 2016

SAMPLE SIZE

Sample size (n) – $4S.D/d^2$

S.D- Standard Deviation, d - error

Considering the Mean ± S.D values from previous studies taking 95% confidence limit and 5% tolerance level along with error as 3, sample size in subclinical thyroid patients were 19 and clinical hypothyroid and hyperthyroid patients sample size were 26.

In this study 80 patients presenting with symptoms of thyroid disorder in that 20 cases of subclinical hypothyroid, 30 cases of clinical hypo and hyperthyroid patients and 30 normal subjects without the history of Diabetes Mellitus were included. Clinical data with the other relevant information was obtained as per the proforma.

CRITERIA FOR SELECTION OF THE STUDY GROUP

Inclusion Criteria

- Newly diagnosed Subclinical hypothyroid patients.
- Newly diagnosed Hypo and hyperthyroid patients.
- Age 20- 60 years.

Exclusion criteria

- Diagnosed cases of Diabetes mellitus
- Diagnosed cases of thyroid disorder who are on anti thyroid treatment.
- Hemolysed samples.
- Hyperbilirubinemia

APPROVAL FROM THE AUTHORITIES

Permission to conduct the study was obtained from all the concerned authorities viz.

1. Institutional ethics committee on human subjects research of Jawaharlal Medical College Belagavi.(Annexure-I)
2. Head of Department,Medicine KLE'S Dr Prabhakar Kore Hospital and Medical Research Center,Belagavi

OBTAINING INFORMED CONSENT

Informed consent was taken from all the participants in the study (Annexure II).

SCHEDULING

This study was carried out for a period of one year.It was undertaken during January 2016 to December 2016.

PATIENT INFORMATION

A structured proforma was used to collect socio demographic and clinical information about the study participants.(Annexure III)

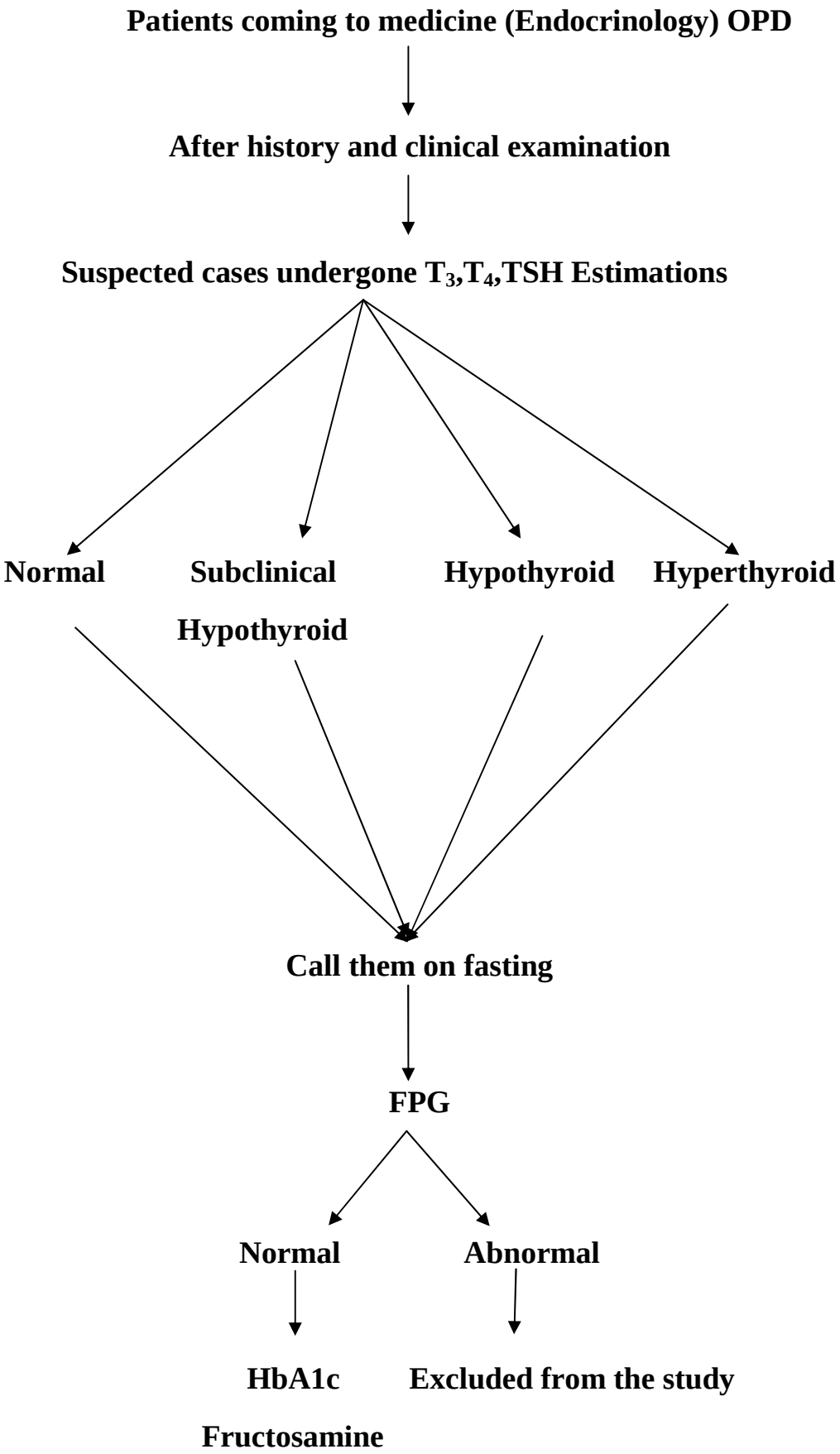
COLLECTION OF SAMPLE

Patient preparation

5ml of blood was collected from the patients and controls under aseptic precautionary measures using disposable syringe in Plain tubes. 2.5 ml separated for Fasting serum glucose and 2.5 ml for Fructosamine Estimation.

Total 110 subjects were enrolled.(Details of the enrolment, inclusion and exclusion of the participants is in the form of flow chart)

METHODOLOGY



Glucose Estimation Kit

Glucose was estimated by using a kit GenX Glucose-2R(GOD-PAP TRINDERS METHOD) in Semiautomated Erba-chem 5 analyser

Principle

$$\text{Glucose} + O_2 + H_2O \xrightarrow{\text{GOD}} \text{Gluconic Acid} + H_2O_2$$

$$H_2O_2 + \text{Phenol} + \text{4-Aminoantipyrine} \xrightarrow{\text{POD}} \text{Red quinoneimine Complex} + H_2O$$

Procedure (End point Method)

Pipette the glucose working reagent into three test tubes labelled Blank (B),Standard (S),Test(T) as follows

REAGENT	B	S	T
Glucose Working reagent	1.0 ml	1.0 ml	1.0 ml
Glucose Standard (Conc 100 mg/)	-	10µl	-
Specimen	-	-	10µl

Mix and incubate for 10 minutes at 37^{o}c or 15 minutes at room temperature Mix and read absorbance of Standard (S),and Test(T) and Blank(B) at 505 nm(490-550 nm).The final color is stable for 10 hours at room temperature.

Calculations

$$\text{Glucose Conc .in mg/dl} = \frac{\text{Abs.of T}}{\text{Abs of S}} \times 100$$

System Parameters (End point)

Reaction Type	:	End- Point
Reaction Time	:	10mins at 37ºc /15 mins at R.T
Wavelength	:	505 nm (490-550nm)
Zero setting with	:	Reagent blank
Sample volume	:	10µl
Reagent Volume	:	1.0 ml
Standard Concentration	:	100 mg/dl
Linearity	:	500 mg/dl

HbA1c Procedure

The D-10 Hemoglobin A1c Program utilizes principles of ion-exchange high performance liquid chromatography (HPLC).Automatically diluted samples on the D-10 and injected into the analytical cartridge

↓

The buffer gradient is delivered by D-10 of increasing ionic strength to the cartridge. Hemoglobins are separated depending upon the cartridge material, based on their ionic interactions

↓

The separated hemoglobins then pass through the flow cell of the filter photometer

↓

Changes in the absorbance at 415 nm are measured.

TSH Measuring Procedure

TSH was measured by electro chemiluminescence immunoassay (ECLIA) and cobas-e immunoassay analyzers.

Sandwich Principle

For this assay total incubation period is 18 minutes

- **First incubation**-50 ML of sample

- A biotinylated monoclonal TSH specific antibody and a monoclonal TSH specific antibody labelled with a ruthenium complex react to form a sandwich complex

- **Second incubation**

- The complex becomes bound to the solid phase via interaction of biotin and streptavidin after addition of streptavidin coated microparticles

- The reaction mixture is aspirated into the measuring cell where the microparticles are magnetically captured onto the surface of the electrode

- Unbound substances are then removed with Procell/Procell M

- Chemiluminescent emission is induced by application of voltage which is measured by a photomultiplier

- Results are determined via a calibration curve which is instrumentspecifically generated by 2- Point calibration and a master curve provided via the reagent barcode.

Calculation

Analyte concentration of each is calculated automatically, either in µIU/mL or mIU/L (selectable).

Triiodothyronine (T_3) Estimation

Test principle

T_3 was tested by electro chemiluminescence immunoassay (ECLIA) and cobas-e immunoassay analyzers.

Competition principle

Total duration of assay-18 minutes

- **First incubation**-30 µl of sample and a T_3 specific antibody labelled with a ruthenium complex bound T_3 is released from the binding proteins in the sample by ANS
- **Second incubation**

- Still free binding sites of the labelled antibody become occupied after addition of streptavidin-coated microparticles and biotinylated T3 and formation of an antibody –hapten complex
- The complex, bound to the solid phase via interaction of biotin and streptavidin
- The reaction mixture is aspirated by measuring cell where the microparticles are magnetically captured onto the surface of the electrode
- Unbound substances are then removed with ProCell/ProCell M
- Chemiluminescent emission is induced after application of a voltage to the electrode which is measured by a photomultiplier
- Results are determined via a calibration curve which is instrument specific.A master curve provided via the reagent barcode

Tetraiodo thyronine (T_4) Estimation

Test principle

Competion principle

- Total duration of assay 18 minutes

- **First incubation**

- 15 ml of sample and t4 specific antibody labelled with a ruthenium complex
- Bound T_4 is released from binding proteins in the sample by ANS After addition of streptavidin-coated microparticles and biotinylated T_4, the still free binding sites of the labelled antibody become occupied with formation of an antibody –hapten complex
- The complex, bound to the solid phase via interaction of biotin and streptavidin.
- The reaction mixture is aspirated into the measuring cell where the microparticles are magnetically captured onto the surface of the electrode
- Unbound substances are then removed with Procell/Procell M
- Chemiluminescent emission is induced after application of a voltage to the electrode which is measured by a photomultiplier
- Which is instrument specific and a master curve provided via the reagent barcode

Fructosamine Estimation Kit

Fructosamine was estimated in semiautomated analyser(Erba Chem-5) by Nitro Blue Tetrazolium (NBT) method

NBT METHOD

System parameters

Reaction Type	:	Fixed Time
Reaction Direction	:	Increasing
Wavelength	:	546 nm
Flow cell Temp.	:	37º c
Zero setting with	:	Distilled Water
Delay time	:	60 seconds (A1)
Measuring time	:	480 seconds (A2)
Reagent volume	:	1.0ml (R1)
Sample volume	:	50µl

Reagent	**1000µl**
Serum	**50 µl**

The final reading was expressed in µmol /L

PHOTOGRAPHS

Photo No-1: ERBA Chem 5 Semi-auto analyser

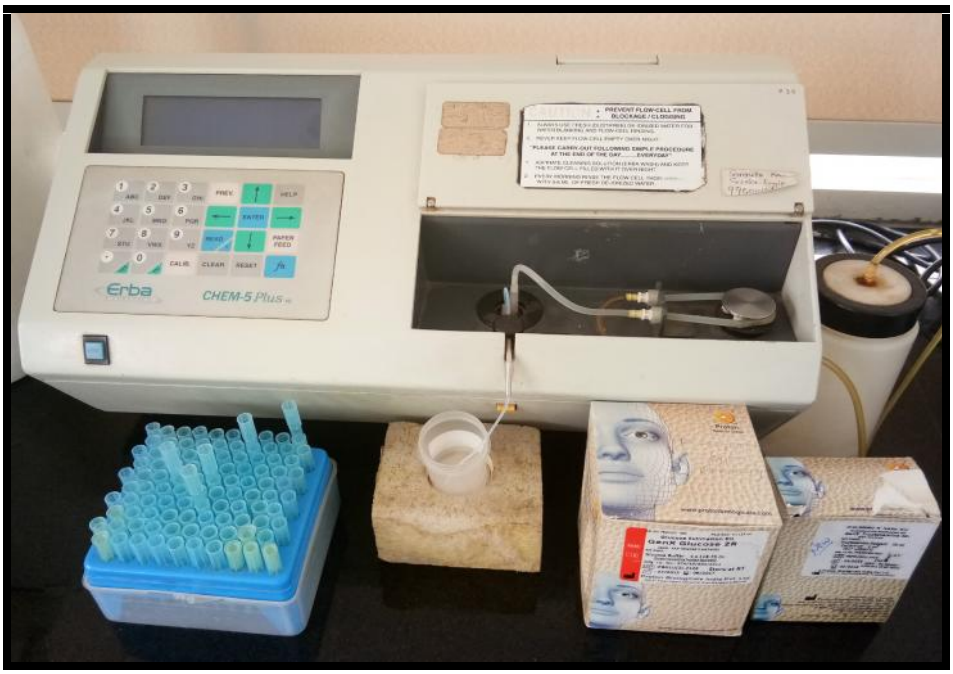
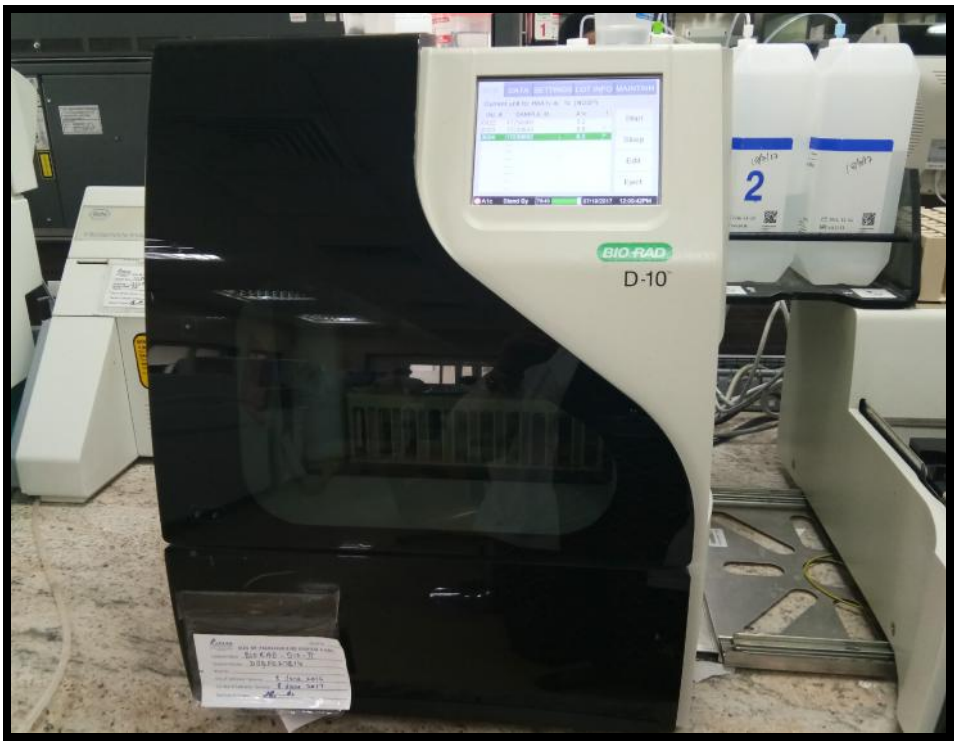


Photo No-2 Bio-Rad D-10 Dual Program Analyser



RESULTS

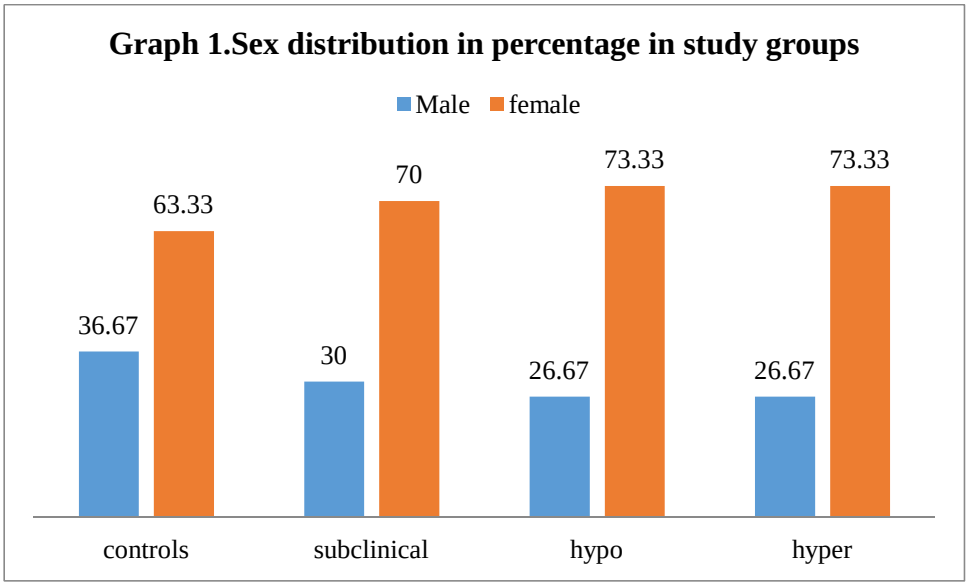
This cross sectional study consisted of four groups.

1. Controls (n=30)
2. Subclinical hypothyroidism (n=20)
3. Clinical hypothyroidism (n=30)
4. Clinical hyperthyroidism (n=30)

The data obtained was tabulated and subjected to proper statistical analyses. The results obtained were systematically described here along with the tables and graphs wherever required.

Table 1.Sex distribution of the study population

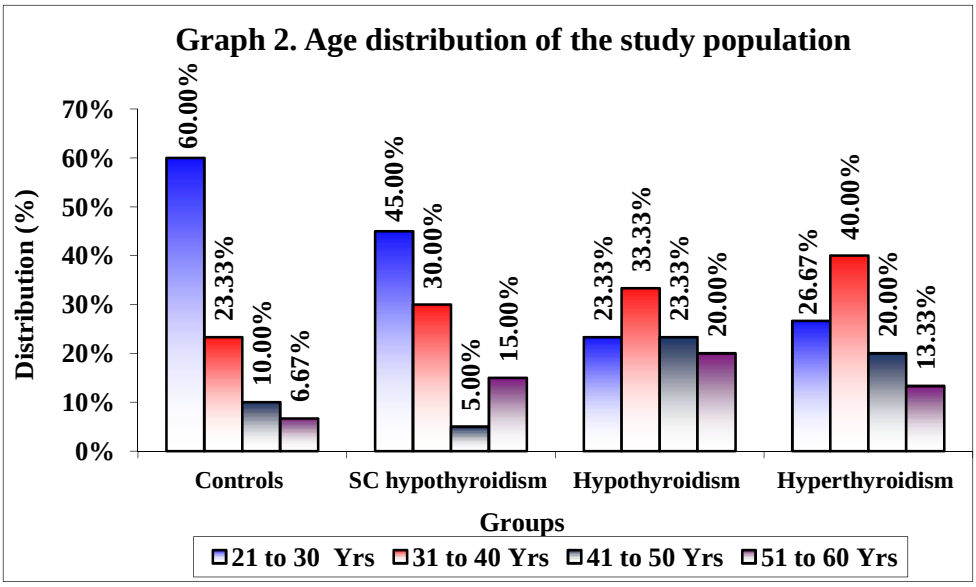
Sex	Groups									
	Controls (n=30)		Subclinical		Hypothyroidism		Hyperthyroidism		Total	
	No	%	No	%	No	%	No	%	No	%
Male	11	36.67	6	30.00	8	26.67	8	26.67	33	30.00
Female	9	63.33	14	70.00	22	73.33	22	73.33	77	70.00
Total	**30**	**100.00**	**20**	**100.00**	**30**	**100.00**	**30**	**100.00**	**110**	**100.00**



In the present study most of the participants were females. In control group 63.33% were females and 36.67% were males.In subclinical hypothyroidism group 70% were females and 30% were males.In Clinical hypothyroidism group and clinical hyperthyroidism group 73.33% were females and 26.67% were males .Sex distribution was almost equal in all the four groups as shown in Table 1 and Graph 1.

Table 2. Age Distribution of the Study Population

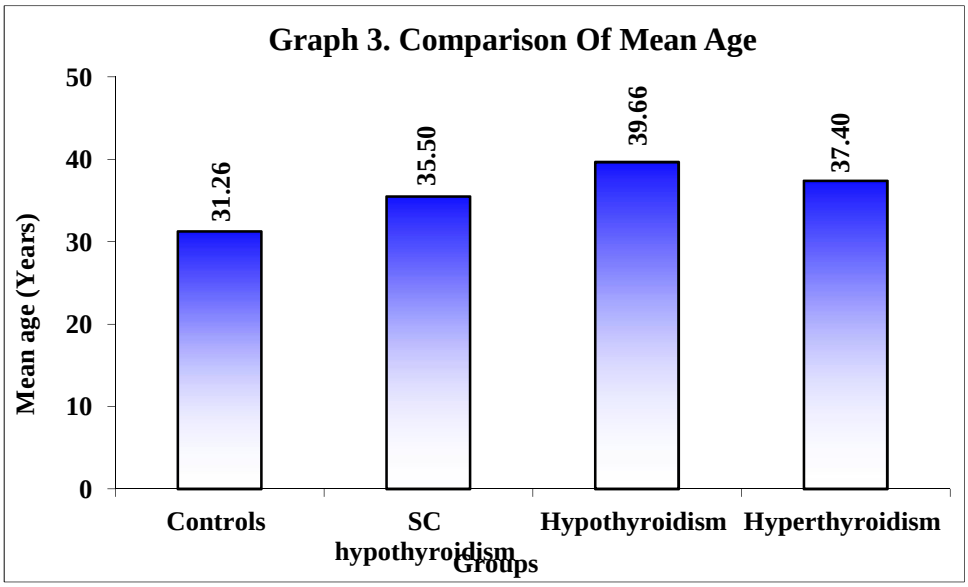
Age group (Years)	Groups									
	Controls (n=30)		Subclinical hypothyroid (n=20)		Clinical Hypothyroid (n=30)		Clinical Hyperthyroid (n=30)		Total (n=110)	
	No	%	No	%	No	%	No	%	No	%
21 - 30	18	60.00	9	45.00	7	23.33	8	26.67	42	38.18
31 - 40	7	23.33	7	35.00	10	33.33	12	40.00	36	32.73
41 - 50	3	10.00	1	5.00	7	23.33	6	20.00	17	15.45
51 - 60	2	6.67	3	15.00	6	20.00	4	13.33	15	13.64
Total	**30**	**100.00**	**20**	**100.00**	**30**	**100.00**	**30**	**100.00**	**110**	**100.00**
										p=0.140



In the present study participants belonged to the age group of 21- 30 years, which comprised of 38.18%. 60% of participants in control group who were aged between 21-30.While in subclinical hypothyroidism group it was 45%. In clinical hypothyroidism group and hyperthyroidism group 33.33% and 40% of the patients were aged between 31-40 respectively. The age distribution of the patients were comparable in four groups (p=0.140) as shown in **Table 2 and Graph 2.**

Table 3. Comparison of mean age in study groups

Groups	Total (n)	Age (Years)	
		Mean	SD
Controls	30	31.26	8.62
Subclinical hypothyroid	20	35.50	12.44
Clinical hypothyroid	30	39.66	11.08
Clinical hyperthyroid	30	37.40	10.25
"F" value		3.348	
"P" value		**0.020**	



In the present study the mean age of clinical hypothyroid group was significantly high (39.66±11.08 years) when compared with the clinical hyperthyroid (37.40±10.25 years) and subclinical hypothyroid groups (35.50±12.44 years) with the mean age of controls (31.26±8.62 years) as shown in **Table 3 and Graph 3**.

Table 4: Comparison of four study groups with respect to mean TSH scores by one way ANOVA

Groups	Mean	SD	SE
Control	2.32	0.70	0.13
Subclinical hypothyroid	13.85	8.70	1.94
Clinical Hypothyroid	56.81	47.05	8.59
Clinical Hyperthyroid	0.19	0.15	0.03
F-value	33.5643		
p-value	0.001*		

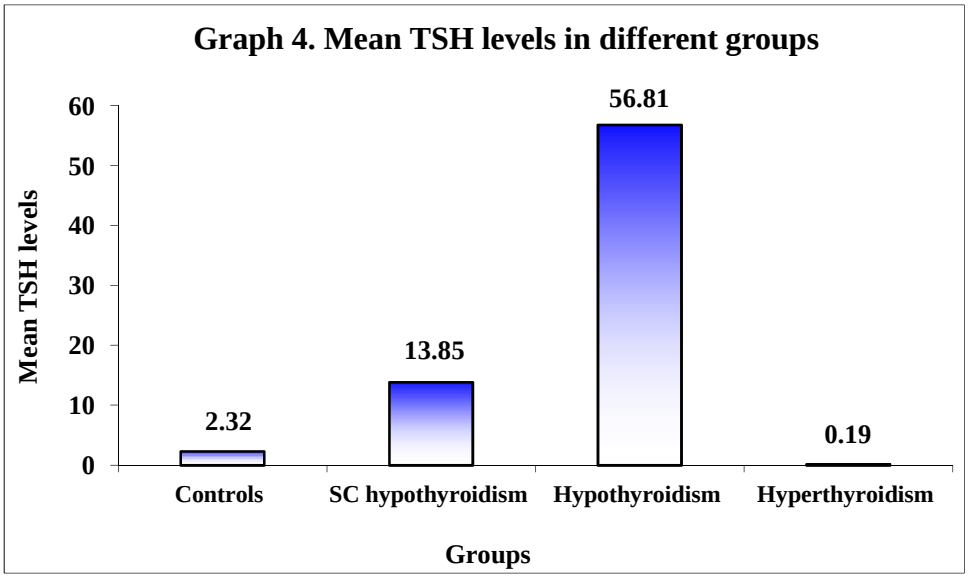


Table 4 and Graph 4 show the mean levels of TSH in controls , subclinical hypothyroid, clinical hypothyroid and hyperthyroid groups as per **one way ANOVA .**

Table 5: Comparison of four study groups with respect to mean T_3 scores by one way ANOVA

Groups	Mean	SD	SE
Control	1.05	0.36	0.07
Subclinical hypothyroid	0.91	0.29	0.07
Clinical Hypothyroid	0.71	0.82	0.15
Clinical Hyperthyroid	10.76	7.53	1.37
F-value	45.0056		
p-value	0.001*		

*p<0.05

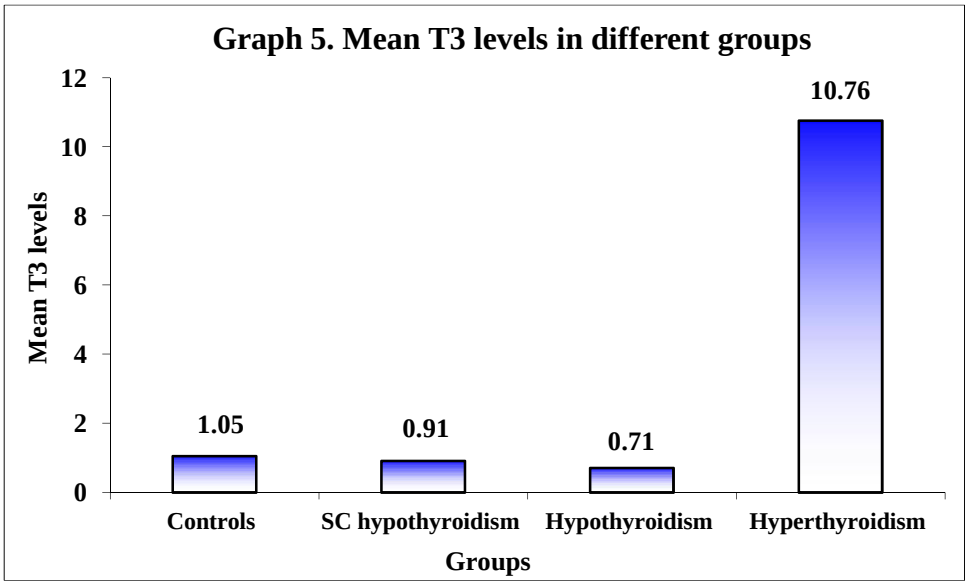


Table 5 and Graph 5 shows the Mean T_3 levels in controls ,subclinical hypothyroid, clinical hypothyroid and hyperthyroid patients as per one way ANOVA.

Table 6: Comparison of four study groups with respect to mean T_4 values by one way ANOVA

Groups	Mean	SD	SE
Controls	7.90	2.18	0.40
Subclinical hypothyroid	7.57	1.89	0.42
Clinical Hypothyroid	2.18	0.95	0.17
Clinical Hyperthyroid	16.33	8.21	1.50
F-value	49.5486		
p-value	0.001*		

*p<0.05

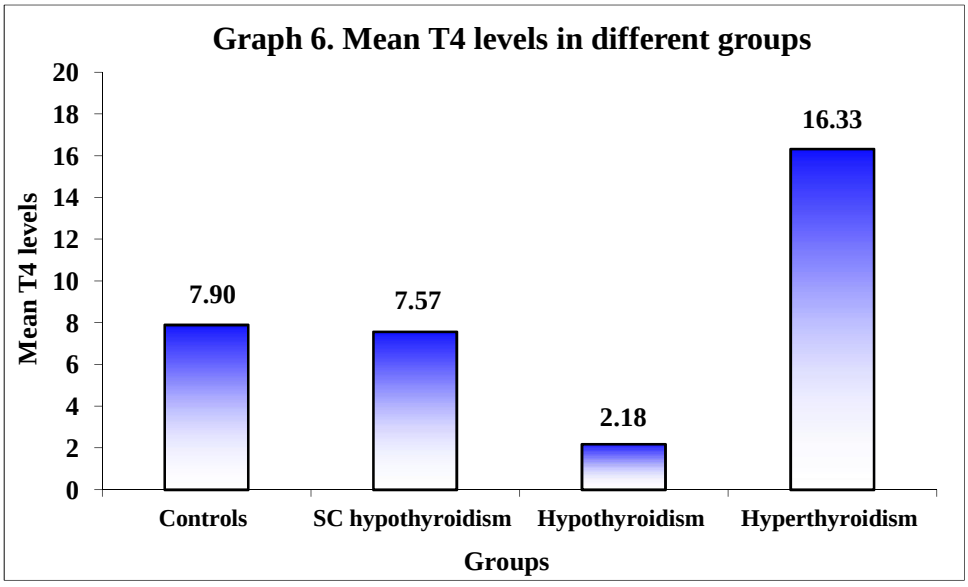


Table 6 and Graph 6 shows the levels of T_4 in controls, subclinical hypothyroid, clinical hypothyroid and hyperthyroid patients as per one way ANOVA .

Table 7: Comparison of four study groups with respect to mean Fasting Plasma Glucose (FPG) values by one way ANOVA

Groups	Mean	SD	SE
Controls	85.40	4.84	0.88
Subclinical hypothyroid	88.80	2.59	0.58
Clinical Hypothyroid	89.23	4.44	0.81
Clinical Hyperthyroid	93.67	0.92	0.17
F-value	26.037		
p-value	0.001*		

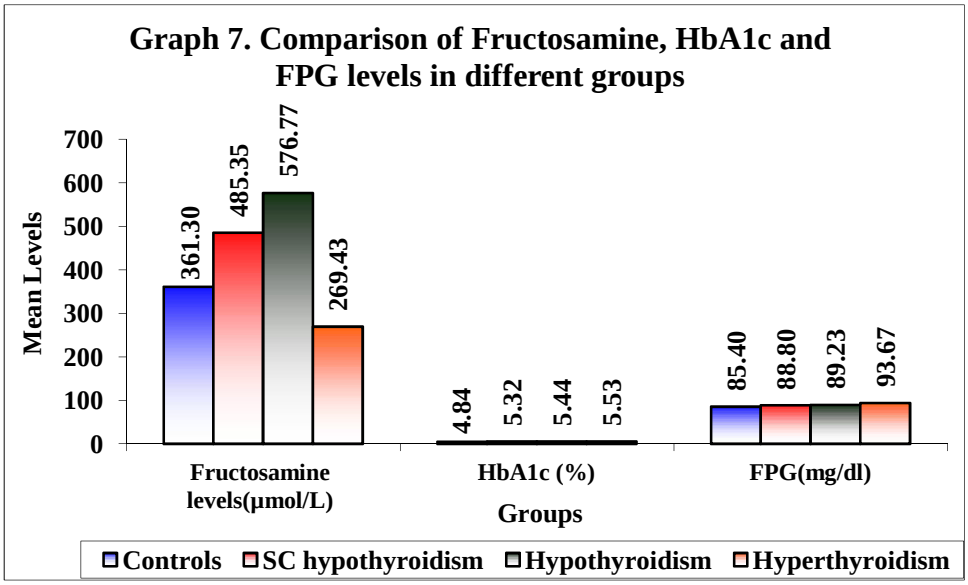


Table 7 and Graph 7 shows the levels of mean FPG values in Subclinical hypothyroid group (88.80±2.59), Clinical hypothyroid group (89.23± 4.44),and Clinical hyperthyroid group (93.67±0.92) .They were significantly high when compared with the Controls(85.40±4.84) as per one way ANOVA .

Table 8: Pair wise comparisons of FPG levels by Tukeys multiple Post-hoc Bonferroni test

Controls and Subclinical hypothyroid	p=0.009*
Controls and Clinical Hypothyroid	p=0.001*
Controls and Clinical Hyperthyroid	p=0.001*
	*p<0.05

Table 8 shows Pair wise comparison of FPG by Turkeys multiple Post-hoc Bonferroni test.There was a statistical significant difference between Controls and Subclinical hypothyroid group (p=0.009), Controls and Clinical hypothyroid group (p=0.001) and between Controls and Clinical hyperthyroid group (p=0.001).

Table 9 : Comparison of four study groups with respect to mean HbA1c levels by one way ANOVA

Groups	Mean	SD	SE
Controls	4.84	0.36	0.07
Subclinical hypothyroid	5.32	0.38	0.09
Clinical Hypothyroid	5.44	0.14	0.06
Clinical Hyperthyroid	5.53	0.24	0.04
F-value	34.555		
p-value	0.001*		

Table 9 and Graph 7 shows the levels of mean HbA1c values in Subclinical hypothyroid group(5.32±0.38), Clinical hypothyroid group (5.44± 0.14),and hyperthyroid group (5.53±0.24). They were significantly high when compared with the Controls (4.84±0.36) as per one way ANOVA .

Table 10: Pair wise comparisons of HbA1c levels by Tukeys multiple Post-hoc Bonferroni test

Controls and Subclinical hypothyroid	p=0.001*
Control and Clinical Hypothyroid	p=0.001*
Control and Clinical Hypothyroid	p=0.001*
	*p<0.05

Table 10 shows Pair wise comparison of HbA1c by Tukeys multiple Post-hoc Bonferroni test. There was a statistical significant difference between Controls and Subclinical hypothyroid group (p=0.001), Controls and Clinical hypothyroid group (p=0.001) and between Controls and Clinical hyperthyroid group (p=0.001).

Table 11: Comparison of four study groups with respect to mean Fructosamine levels by one way ANOVA

Groups	Mean	SD	SE
Controls	361.30	7.88	1.44
Subclinical hypothyroid	485.35	40.16	8.98
Clinical Hypothyroid	576.77	37.23	6.80
Clinical Hyperthyroid	269.43	7.90	1.44
F-value	761.9263		
p-value	0.001*		

Table 11 and Graph 7 shows the levels of mean Fructosamine values in Subclinical hypothyroid group (485.35±40.16), Clinical hypothyroid group (576.77± 37.23).They were significantly high when compared with the Controls (361.30±7.88). While mean Fructosamine levels were low in Clinical hyperthyroid group (269.43±7.90) when compared with the Controls (361.30±7.88) as per one way ANOVA.

Table 12: Pair wise comparisons of Fructosamine levels by Tukeys multiple Post-hoc Bonferroni test

Controls and Subclinical hypothyroid	0.001*
Control and Clinical Hypothyroid	0.001*
Controls and Clinical Hyperthyroid	0.001*

Table 12 shows Pair wise comparison of Fructosamine values by Tukeys multiple Post-hoc Bonferroni test. There was a statistical significant difference between Controls and Subclinical hypothyroid group (p=0.001), Controls and Clinical hypothyroid group (p=0.001) and between Controls and Clinical hyperthyroid group (p=0.001).

Table 13: Correlation between different variables by Karl Pearson's correlation coefficient method

Groups	Variables	HbA1c	Fructosamine	FPG	TSH	T_3	T_4
Subclinical hypothyroid	HbA1c	-					
	Fructosamine	r=0.2782	-				
	FPG	r=0.4704*	r=-0.1317	-			
	TSH	r=0.2665	r=0.2802	r=0.2297	-		
	T_3	r=-0.4292	r=-0.4130*	r=0.0058	r=0.0153	-	
	T_4	r=-0.1667	r=-0.5756	r=-0.0664	r=0.0787	r=0.2882	-
Clinical hypothyroid	HbA1c	-					
	Frucstosamine	r=0.3041	-				
	FPG	r=0.466*	r=0.421*	-			
	TSH	r=0.2562	r=0.1437	r=-0.0264	-		
	T_3	r=-0.1334	r=-0.0298	r=-0.2204	r=0.0742	-	
	T_4	r=0.0846	r=0.3717*	r=0.1893	r=0.1163	r=-0.4065*	-
Clinical hyperthyroid	HbA1c	-					
	Fructosamine	r=-0.1680	-				
	FPG	r=0.406*	r= -0.437*	-			
	TSH	r=-0.1709	r=-0.2240	r=-0.2885	-		
	T_3	r=0.0111	r=-0.1728	r=-0.0402	r=0.1334	-	
	T_4	r=-0.0520	r=0.1942	r=-0.0743	r=0.0556	r=-0.6021*	-

Once we found that there was a statistical significant difference in the level of FPG,HbA1c and Fructosamine in our groups, then we wanted to know the correlation in FPG,HbA1c and Fructosamine groups to see whether they are positively associated or negatively associated by Correlation between different variables by Karl Pearson's correlation coefficient method as shown **in Table 13.** In Subclinical hypothyroid group a significant and positive correlation was observed between FPG and HbA1c (r=0.4704) .But there was no significant correlation between FPG and Fructosamine (r=0.1317) levels at 5% level of significance.It means that FPG and HbA1c Values were depending on each other.

In Clinical hypothyroidism a significant positive correlation was observed between FPG and HbA1c (r=0.466) and FPG and Fructosamine (r=0.421) at 5% level of significance .It means that FPG and HbA1c and FPG and Fructosamine levels were depending on each other.

In Clinical hyperthyroidism a significant positive correlation was observed between FPG and HbA1c (r=0.406) and negative correlation was observed between FPG and Fructosamine (r=-0.437) at 5% level of significance. It means that FPG and HbA1c values were depending on each other whereas there was a negative association in FPG and Fructosamine levels.

DISCUSSION

Thyroid hormones T_3 and T_4 exert profound effects in the regulation of glucose homeostasis including modification of circulating insulin levels and other counter regulating hormones.In thyroid dysfunctions the glucose homeostatic balance is broken. Higher concentration of T_3 causes protein catabolism and negative nitrogen balance.Circulating sugars primarily glucose and fructose when they come in contact with proteins and lipids form Advanced glycation End Products(AGEs).Examples of proteins subject to non-enzymatic glycation are Glycated hemoglobin,and glycated albumin is also known as Fructosamine albumin.A positive association between thyroid disorders and Diabetes Mellitus is well recognized. To study the effect of thyroid hormones on glucose metabolism in non diabetic patients is an area for extensive research .There are lack of studies to correlate the effects of thyroid hormones on glycation. Fructosamine and Glycated Hemoglobin (HbA1c) can be included in the thyroid work up of the patients to assess the metabolic function and the subsequent response after the initiation of the therapy.Hence this study was undertaken to estimate and compare the Glycated haemoglobin and Fructosamine levels in subclinical hypothyroid, clinical hypo and hyperthyroid patients without DM along with apparently healthy controls.

Subclinical hypothyroidism

In the present study the mean FPG, HbA1c and Fructosamine levels were high when compared with the Controls and they were statistically significant. These findings are in accordance with the previous studies.[84]

Clinical Hypothyroidism

In the present study all clinical hypothyroid cases had normal FPG values as per the reference range 70-100 mg/dl but the mean value was higher when compared with the controls. Despite of normoglycemia, the Fructosamine levels and HbA1c levels were greatly increased in clinical hypothyroid patients when compared with the controls which was statistically significant. This is in accordance with many previous studies.[84,10]

Raised Fructosamine and HbA1c values in clinical hypothyroidism could be due to hypometabolic state leading to decreased turnover of the plasma proteins and prolonging their half- life.As hypothyroidism is an inflammatory process there is generation of free radicals which leads to increased oxidative stress causing increased glycation of proteins. As thyroid gland functions subnormally and not enough iodination of the thyroid hormones takes place, the thyroid gland becomes a major site of a dangerous H_2O_2 generation. The cascade gets activated, resulting in raised TSH levels, thus increasing the production of H_2O_2,depleting the defense mechanisms like glutathione peroxidase, reducing the synthesis of T_3 and T_4, further increasing the TSH levels and worsening the functioning of the thyroid gland and increases the glycation of proteins.[85]

As there is altered glucose homeostasis with decreased absorption and utilization leads to hyper insulinemia and insulin resistance in peripheral tissue causing transient elevations in the glucose concentrations thus contributing to increased glycation of serum proteins.The tendency of glycated proteins to accumulate in tissues resisting easy proteolysis and being further source of free radicals.[9]

Clinical Hyperthyrodism

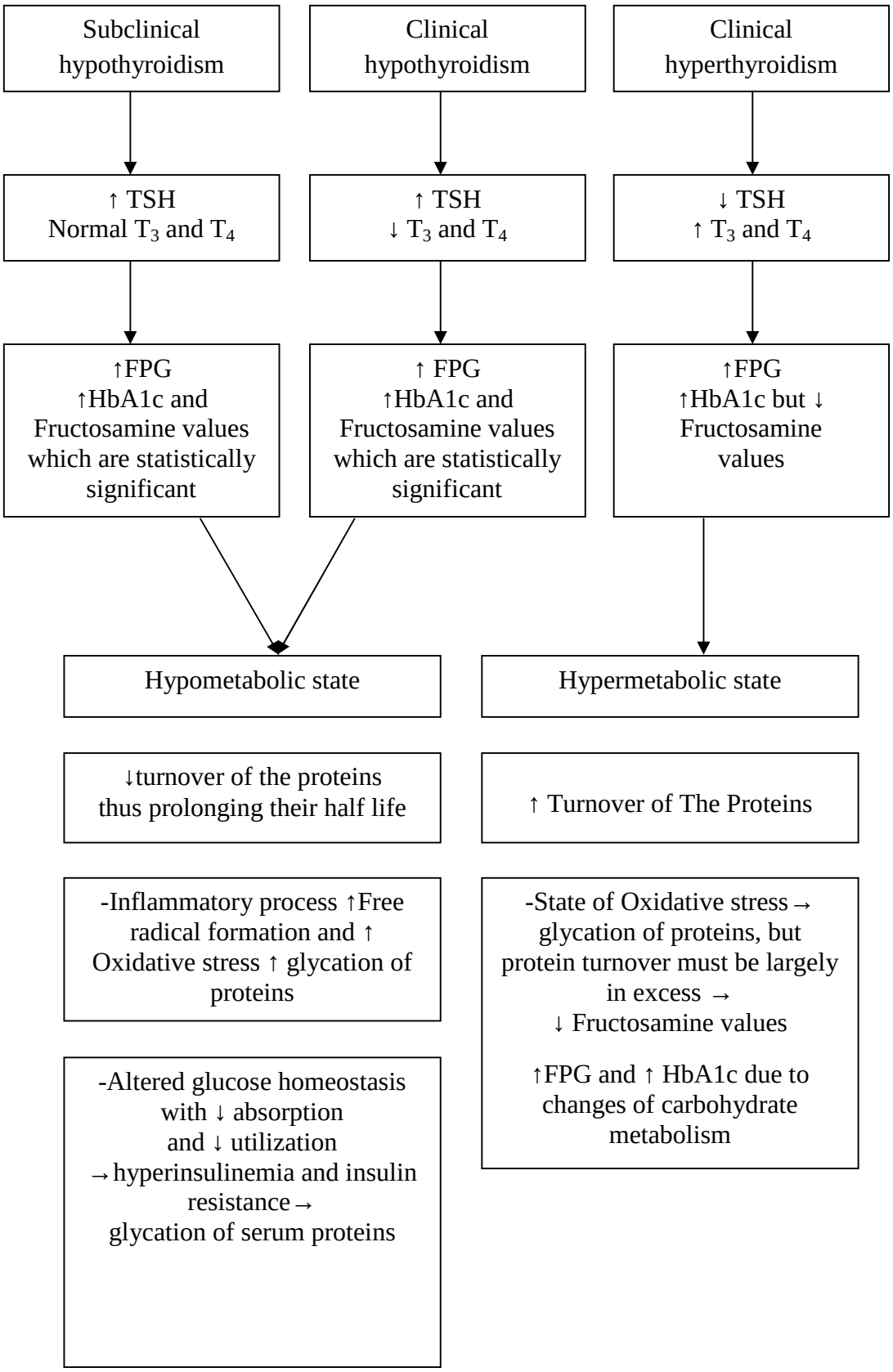
In the present study the mean FPG values and mean HbA1c levels were higher than the control group, however mean Fructosamine levels were lower than the control group and were statistically significant. These findings were in accordance with previous studies.[84,85]

Hyperthyroidism is a hypermetabolic state with increased muscle protein breakdown.There is increased metabolic activity with increased protein turnover, however the Fructosamine concentrations were significantly lower in clinical hyperthyroid patients as compared to controls. In hyperthyroidism also there is a state of oxidative stress which increases the possibility of proteins getting glycated. The higher levels of FPG and HbA1c in hyperthyroid group as compared to normal control groups was due to change in carbohydrate metabolism.[84] Thyroid hormone (T_3) influences the glycogenolytic and gluconeogenesis effects of epinephrine and glucagon and also increases hepatic expression of glucose transporter (GLUT2) thus increasing hepatic glucose output.Thyroid hormones promote albumin metabolism and the mean concentration of albumin and total serum proteins are lower in hyperthyroidism.[10]

In previous studies by Cirillo et al and Larsen et al reported marked decrease in serum Fructosamine concentrations in hyperthyroidism patients as compared to controls.[86] Ford et al in their study showed an increase in HbA1c level which corresponds to raised glucose concentrations,but also decrease Fructosamine levels and albumin concentration and concluded that these conflicting findings and results ,where one class of proteins resisted glycation and the other corresponded to the plasma glucose concentration,need to be explored. [86]

In hyperthyroidism, the protein turnover must be largely in excess of the evidence of oxidative stress and the altered glucose homeostasis, which explains the lower Fructosamine levels in this group.[84]

Probable reasons for of our study is as shown below.



CONCLUSION

In the present study HbA1c levels and Fructosamine levels were found to be significantly higher in normoglycemic subclinical hypothyroid and clinical hypothyroid patients than healthy controls.In Hyperthyroid patients HbA1c levels were higher but Fructosamine levels were low when compared with the controls which is statistically significant.

Hence we suggest HbA1c and Fructosamine could be included in the thyroid work up of the patients.The higher levels of FPG were found in all the groups of thyroid disorders.There is significant positive correlation among all the groups which suggests the altered complex carbohydrate metabolism in them.In hyperthyroidism to understand the decreased Fructosamine levels the exact mechanism needs to be explored.

LIMITATIONS OF THE PRESENT STUDY:

- In this study gender matching was not done with controls.
- Sample size was less
- We did not consider the effect of treatment in thyroid disorders after estimation of Glycated haemoglobin and Fructosamine
- We did not consider estimation of oxidative stress markers in thyroid disorders

SCOPE FOR FURTHER STUDY:

- Further studies are required to know the exact mechanism in carbohydrate and oxidative stress in thyroid disorders
- Standard procedure and mechanism of Fructosamine estimation
- The mechanism behind low Fructosamine values in hyperthyroidism needs to be explored

SUMMARY

Thyroid disease is the most common endocrine disorder encountered in clinical practice. Thyroid disorder can present as hypothyroid, hyperthyroid or subclinical thyroid dysfunction. Thyroid hormones have considerable effects in the regulation of glucose homeostasis, including modification of circulating insulin levels and other counter regulating hormones. Possibly diabetes and thyroid disorders have a propensity to appear together in patients. The severity, prevalence and pathogenesis of abnormalities of carbohydrate metabolism in thyroid disorders are incompletely defined.The extent of glycation of proteins provides an objective, retrospective index of glycemic control in thyroid disorders. There are lack of studies to correlate the effects of thyroid hormone on glycation. Fructosamine and Glycated Haemoglobin (HbA1c) can be included in the thyroid work up of the patients to assess the metabolic function and the subsequent response after the initiation of the therapy.

Objective of the present study was to Estimate and correlate the Fructosamine and Glycated Haemoglobin levels in newly diagnosed subclinical hypothyroid, clinical hypo and hyper thyroid disorders without Diabetes Mellitus.

This one year cross sectional study was conducted in KLES Dr Prabhakar Kore Hospital and Medical Research centre, Belagavi after obtaining required clearances. This study included 110 subjects of either sex,of which 20 cases of subclinical hypothyroid,30 cases of clinical hypo and 30 cases of hyperthyroid patients and 30 normal healthy controls after obtaining informed and written consent.

Initially blood samples were drawn from patients after clinical diagnosis. Blood was analysed for T_3,T_4,TSH by Chemiluminescence method.

Patients with deranged thyroid function tests were included in this study. 30 age matched healthy volunteers were taken as controls. Four groups, Controls or euthyroid group consists of 30 subjects, Subclinical Hypothyroid group consists of 20 subjects. Clinical Hypothyroid and Hyperthyroid group which consisted of 30 subjects each, of either age and sex, who were aged 20-60 years. They underwent T3,T4 and TSH, Fasting Plasma Glucose (FPG),HbA1c and Fructosamine estimations. Results were tabulated and subjected to appropriate statistical analyses.

Few important observations of the present study are as follows;

- In Subclinical hypothyroid group the mean FPG,HbA1c and Fructosamine levels were higher than the controls and were statistically significant and there was a positive significant correlation between FPG and HbA1c.

- In Clinical hypothyroid group the mean FPG,HbA1c and Fructosamine levels were higher than the controls and were statistically significant,and there was positive significant correlation between FPG and HbA1c and FPG and Fructosamine.

- In clinical hyperthyroid group mean FPG,HbA1c levels were high while Fructosamine levels were lower than the controls and were statistically significant.

- In clinical hyperthyroid there was a positive significant correlation between FPG and HbA1c levels while there was negative significant correlation between FPG and Fructosamine.

Thus we conclude that estimation of HbA1c and Fructosamine levels in thyroid disorders to know the extent of glycation and could be included in the thyroid work up of the patients to assess the metabolic function and subsequent response after the initiation of the therapy.

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ANNEXURE – I – ETHICAL CLEARANCE LETTER

K.L.E.UNIVERSITY'S
JAWAHARLAL NEHRU MEDICAL COLLEGE,
NEHRU NAGAR, BELAGAVI-590010 (KARNATAKA-INDIA)
(Accredited 'A' Grade by NAAC)

Website: http//www.jnmc.edu
E-Mail : dome@jnmc.edu

Phone: (+ 91-(0)831 Office : 2471350
Principal: 2471701
Fax No. +91 (0)831 – 2470759

Ref: MDC/DOME/ 437 **Date: 18/11/2015**

To,

Sub: Institutional Ethical Clearance for the study.

With reference to the above, we wish to inform you that your proposed research project titled **"ESTIMATION OF FRUCTOSAMINE AND GLYCATED HEMOGLOBIN (HBA1C) IN NEWLY DIAGNOSED SUBCLINICAL HYPOTHYROID AND CLINICAL HYPO AND HYPERTHYROID PATIENTS WITHOUT DIABETES MELLITUS – A ONE YEAR CROSS SECTIONAL STUDY",** is ethical and justifiable. The proposed research project has been cleared by the JNMC Institutional Ethics Committee on Human Subjects Research.

(Dr. Arathi Darshan)
Member Secretary
JNMC Institutional Ethics Committee
on Human Subjects Research,
J.N.Medical College, Belagavi.

(Dr. Ganga Pilli)
Chairman,
JNMC Institutional Ethics Committee
on Human Subjects Research,
J.N.Medical College, Belagavi.

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ANNEXURE-II

INFORMED CONSENT OF THE PATIENT

Title: Estimation of Fructosamine and Glycated Hemoglobin (Hba1c) In Newly Diagnosed Subclinical Hypothyroid and Clinical Hypo and Hyperthyroid Patients without Diabetes Mellitus -A One Year Cross Sectional Study.

Study investigator :

Guide :

OBJECTIVES AND PURPOSE: Patient is being invited to participate in this study on estimation of levels of Fructosamine and HbA1c in thyroid disorders.

In this study we are going to estimate the levels of Fructosamine and HbA1c in patients with thyroid disorders without diabetes mellitus. Thyroid disease is the most common endocrine disorder encountered in clinical practice. Sub clinical hypothyroidism and clinical hypo and hyperthyroidism are inflammatory processes. Circulating sugars primarily glucose and fructose are culprits of mitochondrial dysfunction in inflammatory process and when these blood sugars come in contact with proteins and lipids, a damaging reaction occurs forming compounds called advanced glycation end products AGEs. Fructosamine and glycated hemoglobin (HbA1c) are useful indicator to measure the peripheral metabolic function in patients with thyroid disorder.Fructosamine and glycated hemoglobin can be included in the thyroid work up

of the patients to assess the metabolic function and the subsequent response after the initation of the therapy.

EXPLANATION OF PROCEDURE: Under aseptic precautions, 5 ml of venous blood will be collected by using a thin 22 Gauge needle prick. The collected blood will be transferred to a plain vacutainer tube and sent to the biochemical laboratory.

POSSIBLE BENEFITS: The investigator does not promise or guarantee that your patient will receive any benefit by being in the study; however, it will be aimed at better understanding of the glycemic control in thyroid disorder patients.

CONFIDENTIALITY: All information collected during the course of study will be kept confidential.

WITHDRAWAL: Participation in this study is voluntary. If you don't wish to participate in this study; you will not lose benefits to which you are entitled. After starting the study, anytime during the study, if you feel to withdraw from the study, you are free to do so.

COST OF PARTICIPATION: The cost of the study will be borne by the researcher. There will be no additional cost to you for participation in the study.

PAYMENT OF PARTICIPATION: No incentive will be paid to the patient for participating in this study

QUESTIONS:

If you have any question about rights as a study participant, you may also contact, Dr.(Mrs) N.S. Mahantashetti. Principal, J. N. Medical College, Belagavi Or Dr. Ganga S.Pilli, Chairman, Institutional Ethics Committee on human subjects' research, JNMC, KLE University, Belagavi Phone no.:0831-2471702(O).

LEGAL RIGHTS: By signing this consent form, you are not waiving any of your patient's legal rights.

PUBLICATION RIGHTS: The result of this study will be used for teaching and medical publication; however the patient's identify will be kept confidential

Name of the patient: IP/OP No.:

CONSENT STATEMENT:

"I am giving consent on behalf of my patient that, my patient will volunteer and participate in this study. I have read the content or it has been read to me and my patient in the language I can understand. This study has been fully explained to me and my patient and I may ask any questions at any time."

Name of patient's Relative:

Signature/ Thumb impression of patient's Relative:

Relation to the patient:

Name of witness:

Signature of witness:

Date:

Place: Signature of the investigator

ANNEXURE-III

PROFORMA FOR PATIENT DATA

Name:

Age/sex:

Address:

Socioeconomic status:

OP no.

Weight: Height:

History

Presenting symptoms:

Brief History of present illness:

Past history:

Family history:

Personal history:

Treatment history:

General physical examination

Pulse:

RR:

BP:

Temperature:

Neck examination

Systemic Examination

Central nervous system

Respiratory system

Cardiovascular system

Per abdomen

Provisional Diagnosis:

BIOCHEMICAL INVESTIGATION RESULTS:

T_3

T_4

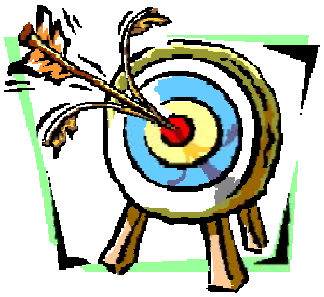
TSH

FPG

FRUCTOSAMINE

HbA1c

Introduction



Objectives

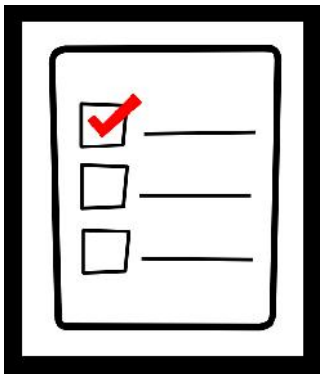
Review of Literature

Methodology

Results

Discussion

Conclusion

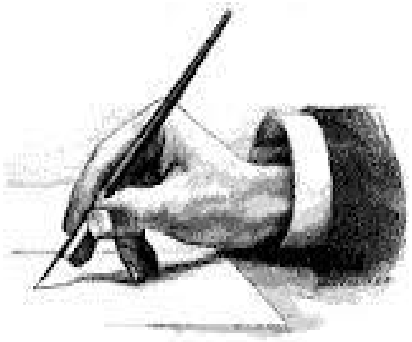


Limitations

Scope for further study:

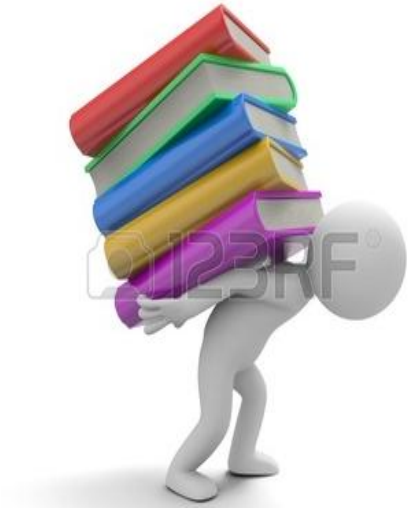
Summary

Bibliography



Annexure-I

Annexure-II



Annexure-III

Annexure-IV

Annexure-V