

Addis Ababa University
School of Graduate studies
M. Sc. Thesis

Title: ASSESSMENT OF THYROID FUNTION TESTS FOR THE DIAGNOSIS OF SUBCLINICAL HYPOTHYROIDISM AND DETECTION OF CLNICAL HYPOTHYROIDISM IN TIKUR ANBESSA SPECIALIZED HOSPITAL

By
Habtamu Azene

Department of Biochemistry
School of Medicine

July 2011
Addis Ababa, Ethiopia

ASSESSMENT OF THYROID FUNTION TESTS FOR THE DIAGNOSIS OF SUBCLINICAL HYPOTHYROIDISM AND DETECTION OF CLNICAL HYPOTHYROIDISM IN TIKUR ANBESSA SPECIALIZED HOSPITAL

A thesis submitted to Department of Biochemistry for partial fulfillment of the requirement for the degree of masters of Science in Biochemistry

By
Habtamu Azene

Advisors
1. Melaku Umeta (PhD)
Department of Biochemistry, School of Medicine, Addis Ababa University
2. Hassen Fayed (PhD)
Department of Biochemistry, School of Medicine, Addis Ababa University
3. Solomon damene (PhD)
Department of Internal Medicine, School of Medicine, Addis Ababa University
4. Fikre Enquselassie (PhD)
School of public health, School of medicine, Addis Ababa University

July 2011

Acknowledgement

I am indebted to my advisors: Dr. Melaku Umeta for his being real mentor and giving comments throughout all stages of this thesis.; and Dr. Hassen Fayed for being patient in correcting this thesis and giving clear guidance. I am thankful to Dr. Solomon Damene for his constructive comments and help. Dr. Fikre Enquselassie, for his support in statistical aspect of the study is gratefully acknowledged.

Nuclear Medicine staff members, nurses working in endocrine clinic, and TASH record office staff members were committed to cooperation during data collection. I am really grateful to them for without their collaboration this work would not be real.

Last but not least I would like to thank the Department of Biochemistry, Addis Ababa University for establishing a ground for me to carry out this study.

Thank you all, the staff members of Biochemistry department, my family, my colleagues and friends, whom I could not completely list their names have been supportive in an endless activities. May God bless you all. I will not come to end without mentioning the priceless encouragement of my wife Kidist Meneberetsehay. Thank God above all who made this to happen this far!

Table of content

Acknowledgment.....i

Table of contents.....ii

List of tables.....v

List of figures.....v

Acronyms.....vii

Abstract.....viii

1. Introduction 7

1.1. Synthesis and secretion of thyroid hormones 8

1.2. Subclinical hypothyroidism 11

1.3. Etiology of subclinical hypothyroidism 12

1.4. Prevalence of subclinical hypothyroidism 18

1.5. Proposed adverse consequences of subclinical hypothyroidism 19

1.5.1. Progression to Overt Hypothyroidism 19

1.5.2. Systemic Symptoms of Hypothyroidism 19

1.5.3. Lipid Abnormalities and Other Cardiac Risk Factors 20

1.5.4. Adverse effect ischaemic heart disease 20

1.5.5. Cardiac Dysfunction 21

1.5.6. Adverse Fetal Effects 21

1.5.7. Neuromuscular Dysfunction 21

1.5.8. Psychiatric and Cognitive Dysfunction 22

2. Significance of the study 22

3. Hypothesis 23

There is a high prevalence of SCH and its progression to overt hypothyroidism among patients attending in TASH. 23

4. Objectives 23

4.1. General objective 23

4.2. Specific objectives 23

5. Material and methods 24

5.1. Study design 24

5.2. Study area 24
5.3. Study subjects 24
5.4. Sample size determination 24
5.5. Selection criteria 25
5.6. Sample collection 26
5.7. Ethical issues 26
5.8. Data collection 26
5.9. Biochemical analysis of laboratory variables 26
5.9.1. Serum T4 coated bead RIA 28
5.9.1.1. Assay reagents and materials 28
5.9.2. Serum TSH coated bead IRMA 31
5.9.2.1. Assay reagents and materials 32
5.9.3. Free T4 coated bead IRMA 35
5.9.3.1. Assay reagents and materials 36
5.9.4. Serum T3 coated bead RIA 39
5.9.4.1. Assay reagents and materials 40
6. Statistical Analysis 43
7. Results 44
7.1. Study Subjects 44
7.2. Prevalence of subclinical hypothyroidism 45
7.3. Assessment of thyroid function tests 45
7.4. BMI 48
7.5. Hypertension 49
7.6. Thyroid function test of subclinical hypothyroid patients follow up for one year 51
7.6.1. Normalization 51
7.6.2. Progression to overt hypothyroidism 52
7.7. Symptoms 52
8. Discussion 54
8.1. Prevalence 54
8.2. Assessment of thyroid function test 55
8.3. Hypertension 55
8.4. BMI 56

8.5. Thyroid function test of subclinical hypothyroid patients follow up for one year 57
8.5.1. Progression 57
8.5.2. Normalization 58
8.6. Symptoms 59
9. Limitation of the study 61
10. Conclusion 62
11. Recommendation 63
12. References 64

Introduction

The thyroid gland is located in the neck, anterior to the trachea, between the cricoid cartilage and the suprasternal notch. The thyroid consists of two lobes that are connected by an isthmus. It is normally 12 to 20 g in size, highly vascular, and soft in consistency. The gland is composed of closely packed spherical follicles, which are invested with a rich capillary network. The interior of the follicle is filled with the clear, proteinaceous colloid that normally is the major constituent of the total thyroid mass. On cross-section, thyroid tissue appears as closely packed, ring-shaped structures consisting of a single layer of thyroid cells surrounding a lumen shown in figure 1. The diameter of the follicles varies considerably, even within a single gland, but averages about 200 μm. The mature thyroid gland contains numerous spherical follicles composed of thyroid follicular cells that surround secreted colloid, a proteinaceous fluid that contains large amounts of thyroglobulin, the protein precursor of thyroid hormones. The thyroid also contains parafollicular cells (C cells) that are the source of the calcium-lowering hormone, calcitonin. Parafollicular cells differ from the cells of the follicular epithelium in never bordering on the follicular lumen and in being rich in mitochondria.

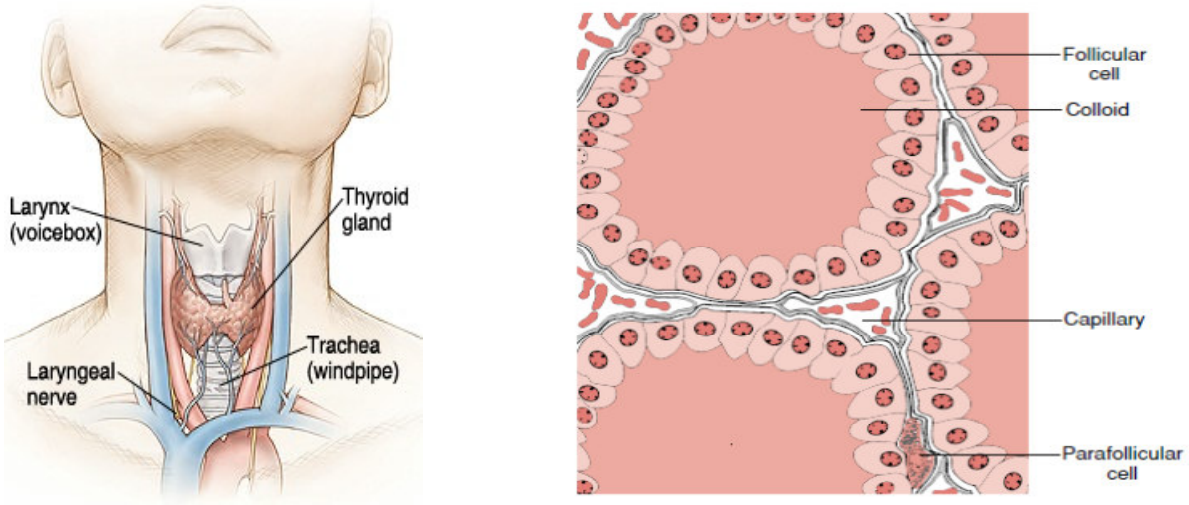


Figure 1. Structure of thyroid and cross-sectional view of follicles

1.1. Synthesis and secretion of thyroid hormones

The steps involved in the synthesis of iodinated thyroglobulin are shown in Figure 2. This process involves the synthesis of a thyroglobulin precursor, the uptake of iodide, and the formation of iodothyronine residues. The synthesis of the protein precursor for thyroglobulin is the first step in the formation of T4 and T3. Thyroglobulin substance is a 660-kDa glycoprotein composed of two similar 330- kDa subunits held together by disulfide bridges. The subunits are synthesized by ribosomes on the rough ER and then undergo dimerization and glycosylation in the smooth ER. The completed glycoprotein is packaged into vesicles by the Golgi apparatus. These vesicles migrate to the apical membrane of the follicular cell and fuse with it. The thyroglobulin precursor protein is then extruded onto the apical surface of the cell, where iodination takes place.

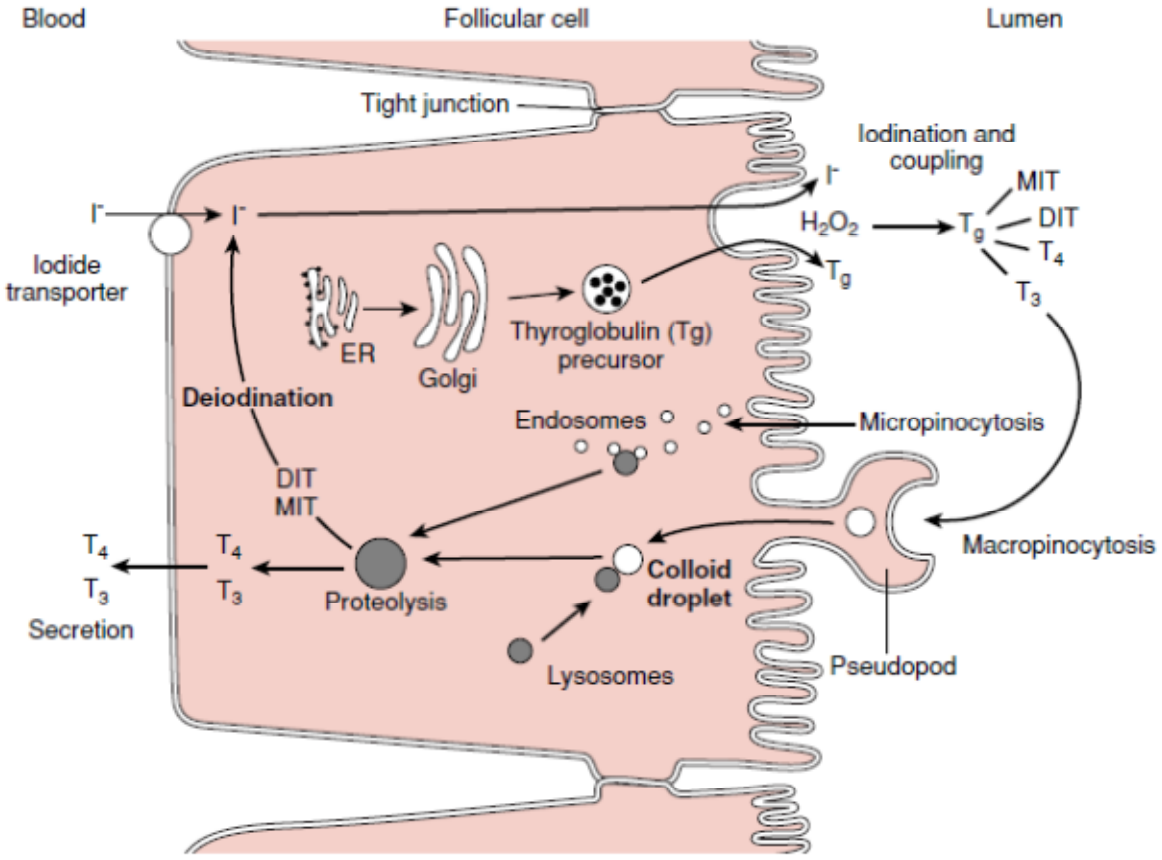


Figure 2. Thyroid cellular mechanisms for iodine transport, thyroxine and triiodothyronine formation, and thyroxine and triiodothyronine release into the blood.

The iodide used for iodination of the thyroglobulin precursor protein comes from the blood perfusing the thyroid gland. The basal plasma membranes of follicular cells, which are near the capillaries that supply the follicle, contain iodide transporters [1].These transporters move iodide across the basal membrane and into the cytosol of the follicular cell. The iodide transporter is an active transport mechanism that requires ATP, is saturable, and can also transport certain other anions, such as bromide, thiocyanate, and perchlorate. It enables the follicular cell to concentrate iodide many times over the concentration of iodide present in the blood; therefore, follicular cells are efficient extractors of the small amount of iodide circulating in the blood. Once inside follicular cells, the iodide ions diffuse rapidly to the apical membrane, where they are used for iodination of the thyroglobulin precursor.

The next step in the formation of thyroglobulin is the addition of one or two iodine atoms to tyrosine residues in the precursor protein. The precursor of thyroglobulin contains 134 tyrosine residues, but only a small fraction of these become iodinated. A typical thyroglobulin molecule contains only 20 to 30 atoms of iodine. The iodination of thyroglobulin is catalyzed by the enzyme thyroid peroxidase, which is bound to the apical membranes of follicular cells [2]. Thyroid peroxidase binds an iodide ion and a tyrosine residue in the thyroglobulin precursor, bringing them in close proximity. The enzyme oxidizes the iodide ion and the tyrosine residue to short lived free radicals, using hydrogen peroxide that has been generated within the mitochondria of follicular cells. The free radicals then undergo addition. The product formed is a monoiodotyrosine (MIT) residue, which remains in peptide linkage in the thyroglobulin structure. A second iodine atom may be added to a MIT residue by this same enzymatic process, forming a diiodotyrosine (DIT) residue.

Iodinated tyrosine residues that are close together in the thyroglobulin precursor molecule undergo a coupling reaction, which forms the iodothyronine structure. Thyroid peroxidase, the same enzyme that initially oxidizes iodine, is believed to catalyze the coupling reaction through the oxidation of neighboring iodinated tyrosine residues to short-lived free radicals. These free radicals undergo addition. The addition reaction produces an iodothyronine residue and a dehydroalanine residue, both of which remain in peptide linkage in the thyroglobulin structure [3]. For example, when two neighboring DIT residues couple by this mechanism, T4 is formed.

After being iodinated, the thyroglobulin molecule is stored as part of the colloid in the lumen of the follicle. Only about 20 to 25% of the DIT and MIT residues in the thyroglobulin molecule become coupled to form iodothyronines. For example, a typical thyroglobulin molecule contains five to six uncoupled residues of DIT and two to three residues of T4. However, T3 is formed in only about one of three thyroglobulin molecules [4]. As a result, the thyroid secretes substantially more T4 than T3.

When the thyroid gland is stimulated to secrete thyroid hormones, vigorous pinocytosis occurs at the apical membranes of follicular cells. Pseudopods from the apical membrane reach into the lumen of the follicle, engulfing bits of the colloid. Endocytotic vesicles or colloid droplets formed by this pinocytotic activity migrate toward the basal region of the follicular cell [5]. Lysosomes, which are mainly located in the basal region of resting follicular cells, migrate toward the apical region of the stimulated cells. The lysosomes fuse with the colloid droplets and hydrolyze the thyroglobulin to its constituent amino acids shown in figure 3. As a result, T4 and T3 and the other iodinated amino acids are released into the cytosol.

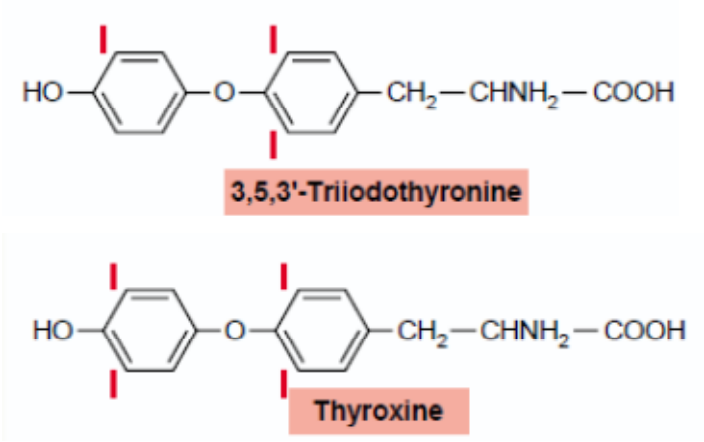


Figure 3. Structure of thyroid hormones

T4 and T3 formed from the hydrolysis of thyroglobulin are released from the follicular cell and enter the nearby capillary circulation, however, the mechanism of transport of T4 and T3 across the basal plasma membrane has not been defined. The DIT and MIT generated by the hydrolysis of thyroglobulin are deiodinated in the follicular cell [6]. The released iodide is then reutilized by the follicular cell for the iodination of thyroglobulin.

Most of the T4 and T3 molecules that enter the bloodstream become bound to plasma proteins. About 70% of the T4 and 80% of the T3 are noncovalently bound to thyroxine-binding globulin (TBG), a 54-kDa glycoprotein that is synthesized and secreted by the liver. Each molecule of TBG has a single binding site for a thyroid hormone molecule. The remaining T4 and T3 in the blood are bound to transthyretin or to albumin. Less than 1% of the T4 and T3 in blood is in the free form, and it is in equilibrium with the large protein bound fraction. It is this small amount of free thyroid hormone that interacts with target cells.

The protein-bound form of T4 and T3 represents a large reservoir of preformed hormone that can replenish the small amount of circulating free hormone as it is cleared from the blood. This reservoir provides the body with a buffer against drastic changes in circulating thyroid hormone levels as a result of sudden changes in the rate of T4 and T3 secretion. The protein-bound T4 and T3 molecules are also protected from metabolic inactivation and excretion in the urine. As a result of these factors, the thyroid hormones have long half-lives in the bloodstream. The half-life of T4 is about 7 days; the half-life of T3 is about 1 day.

Thyroid hormones are important regulators of central nervous system development. It stimulates growth by regulating growth hormone release from the pituitary and by direct actions on target tissues, such as bone. Thyroid hormones also regulate the basal metabolic rate and intermediary metabolism through effects on mitochondrial ATP synthesis and the expression of genes encoding metabolic enzymes.

1.2. Subclinical hypothyroidism

Biochemical tests of thyroid function can reveal evidence of hypothyroidism before it is clinically apparent. The term subclinical hypothyroidism is defined biochemically as the finding of a raised serum TSH (≥4.5mIU/L) above the reference range with normal circulating levels of total thyroxine (T_4) and triiodothyronine(T_3) (TT4=62-141 nmol/L and TT3=1.1-3.2 nmol/L).

The term subclinical hypothyroidism (SCH) was coined by Bastenie et al in 1967, and the entity has remained a subject of a heated debate among clinicians ever since [7]. There appears to be agreement on the basic definition of SCH as the finding of a TSH level above the upper limit of the reference range in the presence of normal reference range levels of serum total thyroxine

(TT_4) and total triiodothyronine (TT_3)[8]. The controversy relates in part to just what is the normal reference range for TSH level, specifically the upper limit of the range beyond which a diagnosis of hypothyroidism would obtained. That means upper limit has been falling in recent years from values of 6.5 or 7.0 mIU/L down to 4.1 to 4.5 mIU/L in most laboratories.

The symptoms of abnormal thyroid function in cases of mild thyroid failure are not clearly defined. Most studies use symptom scores and results are inconsistent. The Colorado survey found that certain symptoms such as dry skin, cold intolerance and easy fatigability appear more often in subclinical hypothyroid than in euthyroid subjects [10]. These findings, although statistically significant, were not clinically useful in separating individual subjects.

1.3. Etiology of subclinical hypothyroidism

The etiology of SCH is the same as the etiology of overt hypothyroidism presented in (Table 1) [8,9].

Table 1. Causes of subclinical hypothyroidism

Primary Hypothyroidism with Goiter
Acquired
Hashimoto's thyroiditis
Iodine deficiency (endemic goiter)
Drugs blocking synthesis or release of T4 (e.g., lithium, ethionamide, sulfonamides, iodide)
Goitrogens in foodstuffs or as endemic substances or pollutants
Cytokines (interferon-α , interleukin-2)
Thyroid infiltration
Congenital
Iodide transport or utilization defect (NIS or pendrin mutations)
Iodotyrosine dehalogenase deficiency
Organification disorders (TPO deficiency or dysfunction)
Defects in thyroglobulin synthesis or processing
Atrophic Hypothyroidism

Acquired Hashimoto's disease Postablative due to ^{131} I, surgery, or therapeutic irradiation for nonthyroidal malignancy Congenital Thyroid agenesis or dysplasia TSH receptor defects Thyroidal Gs protein abnormalities (pseudohypoparathyroidism) Idiopathic TSH unresponsiveness
Transient Hypothyroidism Following subacute, painless, or postpartum thyroiditis
Consumptive Hypothyroidism Rapid destruction of thyroid hormone due to D3 expression in large hemangiomas or hemangioendotheliomas
Central Hypothyroidism Acquired Pituitary origin (secondary) Hypothalamic disorders (tertiary) Congenital TSH deficiency or structural abnormality TSH receptor defect

Subclinical hypothyroidism is most often caused by chronic lymphocytic thyroiditis (goitrous Hashimoto's thyroiditis and atrophic thyroiditis), an autoimmune disorder of the thyroid gland that is the most common cause of decreased thyroid hormone production in patients with acquired, subclinical, or overt hypothyroidism[9,11-13].

The term endemic goiter denotes any goiter occurring in a region where goiter is prevalent [14, 15]. As mentioned, endemic goiter almost always occurs in areas of environmental iodine deficiency. Goiter is a public health problem with a prevalence rate as high as 38 % in the high

lands of Northern Ethiopia [16]. Recently, an overall prevalence of gross goiter of 22.8 % has been reported from sekota District, Northern Ethiopia [17]. In Ethiopia Iodine deficiency disorders has been recognized as a public health for many decades [18-22].A recent study that did both clinical and biochemical assessment has confirmed that the situation of iodine deficiency disorder has deteriorated [23]. In high goiter endemic regions the prevalence of goiter in children was greater than 30 % while in less endemic regions it was less than 15 % [24].Although endemic goiter condition is estimated to affect more than 200 million people throughout the world and is of major public health significance, it is most common in mountainous areas, such as the Alps, Himalayas, and Andes, or in the Great Lakes and Mississippi Valley regions of the United States, owing to the depletion of iodine consequent to the persistent glacial run-off in these regions[15]. The causative role of iodine deficiency in the genesis of endemic goiter is supported by the inverse correlation between the iodine content of soil and water and the incidence of goiter, the kinetics of iodine metabolism in patients with the disorder, and a decrease in incidence after iodine prophylaxis.

A research made by Zewdie Woldegebriel and Teshome Demeke in 1993 showed a positive correlation between goiter prevalence and altitude amongst school children as well as house hold members. The correlation was relatively stronger among the household which may be related to the longer period of exposure to a fixed iodine intake. In addition to the relationship between goiter prevalence and altitude, there was variation in the prevalence within the same altitude among the different study sites, particularly in lowlands areas. Thus, the prevalence in the household members in Felegeneway which lies at an altitude of 1430 m was 33 % while it was less than 8 % at all the other sites below 1500 m [20].

Ingestion of compounds that block thyroid hormone synthesis or release may cause goiter with or without hypothyroidism. Apart from the agents used in the treatment of hyperthyroidism, antithyroid agents may be encountered either as drugs for the treatment of disorders unrelated to the thyroid gland or as natural agents in foodstuffs [25]. Lithium carbonate, which is prescribed for the treatment of manic-depressive disorders, may impair thyroid hormone synthesis and release, and may be associated with the development of goiter (40–60% of cases) and mild or moderate hypothyroidism [26,27]. Patients who develop persistent hypothyroidism during

lithium treatment are more likely to have underlying chronic Hashimoto thyroiditis. Hypothyroidism may also develop during treatment with aminoglutethimide, ethionamide, sulfonamides, and sulfonylureas, which interfere with thyroid hormone synthesis [28].

Antithyroid agents also occur naturally in foods such as cabbages, turnips, kale, kohlrabi, rutabaga, mustard, and various plants that are not eaten by humans but that serve as animal fodder. It is likely that some thiocyanate is present in such plants (particularly cabbage). Cassava meal, a dietary staple in many regions of the world, contains linamarin, a cyanogenic glycoside, the metabolism of which leads to the formation of thiocyanate which influences the thyroid iodine metabolism thereby causing mild and overt hypothyroidism. Health problems associated with cassava consumption was studied in three villages (Kodowono, Lotte and Woidewashe) of Gamo-Gofa, south Ethiopia. Total goiter rate is increased with increasing rate of cassava consumption while, urinary iodine excretion was found to be in the normal range for the three villages [29].

A number of synthetic chemical pollutants have been implicated in causing goitrous hypothyroidism, including polychlorinated biphenyls and resorcinol derivatives. Perchlorate has also been noted in high concentrations in geographic regions in which explosives were made. It is not clear whether the concentrations are significant enough to produce mild and overt hypothyroidism [30-32].

Interferon-α, used for the treatment of hepatitis or certain tumors, alone or in combination with IL-2, may induce thyroid dysfunction due to the activation or enhancement of the autoimmune process [28,33]. Risk factors associated with the possible development of hypothyroidism include female sex, longer duration of interferon-α treatment, presence of chronic hepatitis C virus, older age, and preexisting presence of anti-TPO antibodies. The prevalence of interferon-α-associated hypothyroidism in patients with chronic hepatitis C virus is reported to be between 7 and 39%, and the presence TPO antibodies may be an important risk factor that predicts persistent hypothyroidism at the end of interferon-α treatment [28]. Thyroid autoimmunity(TA) and dysfunction frequently occur in multiple sclerosis patients during interferon-β therapy,

particularly within the first year of treatment; thyroid dysfunction is generally subclinical and transient in over half of the cases [34].

Infiltrative disease or infectious disorders of the thyroid gland (Pneumocystis carini infection and Kaposi's sarcoma in patients with AIDS) may induce thyroid hormone deficiency; however these diseases rarely cause hypothyroidism [28].

Other causes of primary hypothyroidism may result from therapies that destroy thyroid tissue such as radioactive iodine treatment or external radiation therapy. Mild and overt hypothyroidisms are common after external radiotherapy of the head and neck area and develops gradually within the first year with a risk that appears to be dose-dependent [28]. It is frequent after external radiation therapy in patients with Hodgkin's lymphoma, leukemia, aplastic anemia, brain tumors, or bone marrow transplantation. Chemotherapy also may induce hypothyroidism in patients with lymphoma [35]. Women with breast cancer may have an increased risk of autoimmune thyroid disease after adjuvant chemotherapy and tamoxifen [36,37].Surgical reports on thyroid disease are scanty in Ethiopia. Hospital based studies on Addis Ababa as well indicate goiter and its complication are frequent causes of outpatient visit and admission [38-40]. A report from Gondar [41] showed that thyroid disorder constitute 3.7 % of the surgical admissions. A previous analysis of surgical admission in the department of surgery, TASH, showed goiter as one of the top ten surgical conditions [42].

Inherited defects in hormone biosynthesis are rare causes of goitrous hypothyroidism and account for only about 10% to 15% of the 1 in 3500 newborns with congenital hypothyroidism [43]. Specific defects in the pathways of hormone synthesis caused by iodide transport defect, defects in expression or function of thyroid peroxidase, iodotyrosine dehalogenase defect, pendred's syndrome, and defects in thyroglobulin synthesis may result in hypothyroidism. Atrophic hypothyroidism of congenital origin is caused by thyroid agenesis or dysplasia,TSH receptor defects, thyroidal Gs protein abnormalities (pseudohypoparathyroidism), idiopathic TSH unresponsiveness result in hypothyroidism. A research made in 2002 in the pattern of hypothyroid in Ethio-Swedish children's hospital showed that one hundred twenty six new patients from all over the country were referred to endocrinology clinic of Ethio-Swedish

children's hospital for thyroid disorder and thirty one patients (24%) were found to have hypothyroidism, seventeen (13%) had congenital hypothyroidism while the rest fourteen (11.5%) were categorized to have juvenile hypothyroidism [44].

Transient increases in serum TSH may occur after subacute, postpartum, or painless thyroiditis and after partial thyroidectomy and eventually followed by euthyroid state is the cause for transient SCH. The postpartum period is associated with an increased risk of developing subclinical or overt hypothyroidism.

Consumptive hypothyroidism is the term given to an unusual cause of hypothyroidism that has been identified in infants with visceral hemangiomas or related tumors [45,46,47]. The first patient reported with this syndrome presented with abdominal distention caused by a large hepatic hemangioma with respiratory compromise secondary to upward displacement of the diaphragm. However, clinical signs suggested hypothyroidism, which was confirmed by finding a markedly elevated TSH level and undetectable T4 and T3 levels. Rapid destruction of thyroid hormone due to D3 expression in large hemangiomas or hemangiomasendotheliomas.

SCH is frequently observed in patients with overt hypothyroidism receiving inadequate replacement therapy due to poor compliance, drug interactions, or inadequate monitoring of therapy. In fact, between 17.6 and 30% of patients with overt thyroid failure were reported to have SCH due to inadequate thyroid hormone supplementation [10,48].

Central hypothyroidism is due to TSH deficiency caused by either acquired or congenital hypothalamic or pituitary gland disorders. In addition to pituitary tumors, hypothalamic disorders, and the like, an unusual cause of secondary hypothyroidism occurs in individuals given bexarotene (a retinoid X [RXR] receptor agonist) for T-cell lymphoma [49]. This drug suppresses the activity of the human TSH -subunit promoter in vitro. Serum T4 concentrations are reduced about 50%, and patients experience clinical benefit from thyroid hormone replacement. Dopamine, dobutamine, high-dose glucocorticoids, or severe illness may suppress TSH release transiently, leading to a pattern of thyroid hormone abnormalities suggesting central hypothyroidism. Germline loss-of-function mutations in one or both alleles of the TSH-receptor

gene can cause SCH [50,51]. TSH was slightly (6.6 –14.9 mIU/liter) to moderately (24–46 mIU/ liter) increased in these patients and was associated with normal free thyroid hormone concentrations, normal thyroid size, and normal biochemical parameters of TA [51]. Recently, a low prevalence of TSH receptor mutations was reported in a large series of subjects with sporadic and familial non autoimmune SCH [52].

1.4. Prevalence of subclinical hypothyroidism

The prevalence of SCH has been reported to be between 4 and 10% of adult population worldwide [10,53-58].Two large population-based screening studies have provided important epidemiological data about SCH: the Whickham Survey , and NHANES III .In the Whickham Survey (2779 subjects), SCH, defined by serum TSH levels above 6 IU/liter, was identified in 7.5% of females and 2.8% of males [54] .The sample examined in NHANES III (16,353 people aged ≥ 12 yr) represented the geographic and ethnic distribution of the U.S. population . SCH was found in 4.3% of this population [53].

In the Colorado study (over 25,000 state residents attending a series of state wide health fairs), 9.5% of all subjects had an elevated serum TSH concentration, and 89% of these were not on thyroid hormone therapy. Seventy-five percent of these individuals had serum TSH levels in the 5–10 mIU/liter range. In each age decade, a higher percentage of women than men had an elevated serum TSH concentration; the difference was significant after age 34 yr ($P < 0.01$). In the ninth decade of life, the prevalence of elevated TSH was as high as 15–20% [10].

The prevalence of mild thyroid dysfunction was higher in older populations in all epidemiological surveys conducted so far [53-59]. The highest age- and sex-specific rates are in women older than 60 years of age, approaching 20 percent in some reports [10,54]. In a recent survey, the prevalence of subclinical hypothyroidism in men over the age of 74 years (16 percent) was almost as high as it was in women of the same age (21 percent) [10]. Up to 75 percent of patients have only mildly elevated serum thyrotropin values (5 to 10 mU per liter), and 50 to 80 percent of patients have positive tests for antibodies against thyroperoxidase, depending on the age, sex, and serum thyrotropin levels [10,54]. Goiter is twice as prevalent among patients with this condition as in the general population [54].

SCH is more frequent in areas of iodine sufficiency, *i.e.*, 4.2% in iodine-deficient areas compared with 23.9% in areas of abundant iodine intake. This finding was confirmed by a high prevalence of SCH in Iceland 18% and Hungary 24%, which have a high iodine intake [60].

1.5. Proposed adverse consequences of subclinical hypothyroidism

1.5.1. Progression to Overt Hypothyroidism

Patients with SCH have a high rate of progression to clinically overt hypothyroidism, 2.6% each year if thyroperoxidase (TPO) antibodies are absent and 4.3% if they are present [61]. However, some persons do not show progression and some experience normalization. A TSH level greater than 10 mIU/L predicts a higher rate of progression, and a level of less than 6 mIU/L predicts a lower likelihood of progression. In a study in men and women older than 55 years with a mean follow-up of 32 months, the TSH level normalized in 52% of those with a serum TSH of less than 10 mIU/L [62].

1.5.2. Systemic Symptoms of Hypothyroidism

The symptoms of abnormal thyroid function in cases of mild thyroid failure are not clearly defined. Most studies use symptom scores and results are inconsistent. The Colorado survey [10] found that certain symptoms such as dry skin, cold intolerance and easy fatigability appear more often in subclinical hypothyroid than in euthyroid subjects. These findings, although statistically significant, were not clinically useful in separating individual subjects. Studies involving the elderly have found no greater prevalence of symptoms among those with subclinical hypothyroidism compared with euthyroid subjects [56].

Several randomized studies of the effect of levothyroxine therapy in patients with SCH are available. One study limited to patients with serum TSH levels from 5 to 10 mIU/L did not show any benefit [59]. Some studies (range in TSH level, 3-32 mIU/L) showed improved symptom scores or improved memory in a quarter of patients. Many recent studies have not shown improvement in mood, anxiety, and cognition in older persons [63-66]. In a previous scientific review in 2004, available data were considered insufficient to support a benefit for levothyroxine

therapy in patients with SCH, in particular for the group with TSH less than 10 mIU/L, and a similar conclusion have been drawn in 2008.

1.5.3. Lipid Abnormalities and Other Cardiac Risk Factors

The adverse effects of subclinical hypothyroidism on cholesterol concentrations have long been associated with a theoretical increased risk of cardiovascular disease, but the evidence is inconsistent [67]. Most but not all studies have found an association between subclinical hypothyroidism and raised low-density lipoprotein cholesterol (LDL-C), low high-density lipoprotein cholesterol (HDL-C) and raised lipoprotein(a) [67]. A recent cross-sectional population study calculated that subclinical hypothyroidism, present in 10% of middle-aged women, increased total cholesterol by 0.5 mmol L^{-1} [69]. A re-analysis of intervention studies between 1976 and 1995 showed that subclinical hypothyroidism is up to three times more common in subjects with increased plasma cholesterol concentrations, but that total cholesterol is only slightly raised (up to 30%) in subclinical hypothyroidism [70]. T4 treatment of patients with mild thyroid failure has been estimated to lower serum total cholesterol by 0.2–0.4 mmol L^{-1} and LDL cholesterol by ≈0.2 mmol L^{-1} [68,70].

A recent double-blind study of physiological T4 replacement in 63 women with subclinical hypothyroidism (serum TSH 11.7 ± 0.8 mU L^{-1}) confirmed a reduction in total cholesterol and LDL cholesterol of 0.24 mmol L^{-1} and 0.33 mmol L^{-1}, respectively, with an estimated risk reduction of cardiovascular mortality of 9–31% [71]. Among the studies that have stratified patients by TSH level, the magnitude of the change in lipids following T4 therapy appeared to depend upon the severity of thyroid dysfunction. In the only one of five studies that was a randomized controlled trial, reductions in serum lipids were not significant in those individuals with a serum TSH 10 mU l^{-1} [71].

1.5.4. Adverse effect ischaemic heart disease

It is not known whether T4 therapy in subclinical hypothyroidism reduces the risk of ischaemic heart disease. A coronary angiographic study found greater progression of lesions in hypothyroid patients with serum TSH levels in the subclinical hypothyroid range compared with patients whose serum TSH levels were maintained within the normal range [72]. More recently, a report from a cross-sectional study of elderly women from Rotterdam concluded that mild thyroid

failure was an independent risk factor for myocardial infarction [73]. However, the only longitudinal data from a multiple logistic regression analysis from the 20-year follow up of the Whickham survey cohort showed no association between evidence of autoimmune thyroid disease (defined as either treated hypothyroidism, presence of antithyroid antibodies or raised serum TSH) documented at the first survey with mortality or the development of ischaemic heart disease [74]. Therefore, evidence to support improved cardiovascular outcomes in subjects with subclinical hypothyroidism treated with T4 to normalize serum TSH is lacking.

1.5.5. Cardiac Dysfunction

Studies have shown slowed left ventricular relaxation time, increased vascular tone at rest, and left ventricular systolic dysfunction with exercise and impaired endothelial function [75]. Some studies have shown improvement of cardiac contractibility and systolic time interval with levothyroxine therapy [75]. No evidence exists to support an association between heart failure and a serum TSH level of less than 10.0 mIU/L. Again, most studies were not categorized for degrees of TSH elevation, and data remain insufficient for a TSH level less than 10 mIU/L but strongly suggestive for a TSH level greater than 10 mIU/L.

1.5.6. Adverse Fetal Effects

There are observational data suggesting that subclinical hypothyroidism during pregnancy might be associated with suboptimal intellectual performance in children, but these observations are based on relatively small numbers of cases [76]. Some studies have even suggested that the maternal serum free T4 level is more sensitive than serum TSH in predicting the likelihood of adverse intellectual outcomes in the offspring [77]. No intervention data on the effect of T4 therapy in pregnancy exist.

1.5.7. Neuromuscular Dysfunction

It has been suggested that neuromuscular symptoms and dysfunction are common in patients with SCH and can be reversed by levothyroxine treatment [78]. A definitive answer will require more studies with TSH levels stratified as less than or greater than 10 mIU/L.

1.5.8. Psychiatric and Cognitive Dysfunction

Data related to aggravation of depression, bipolar disorder, and effects on cognitive function have been presented [79]. A recent study showed no association with anxiety, depression, or cognitive dysfunction [80]. Nonetheless, it is still reasonable to have a low threshold for therapy for SCH in patients with depression, bipolar disorder, and cognitive dysfunction.

Significance of the study

Many of the research are done on iodine deficiency, endemic goiter in school children, endemic goiter and dietary association, congenital hypothyroidism and thyrotoxicosis. A large-scale survey of the prevalence of SCH has not been performed in Ethiopia. Since no data is available on SCH, this study provides a preliminary understanding of the prevalence and the progression of the disease to different clinical severity.

In turn, this may lead to the management of subclinical hypothyroidism and its progression to an overt hypothyroidism.

Hypothesis

There is a high prevalence of SCH and its progression to overt hypothyroidism among patients attending in TASH.

Objectives

1.6. General objective

- To assess the serum levels of T3, T4 and TSH role for the diagnosis of subclinical hypothyroidism.

1.7. Specific objectives

- To assess the serum levels of T3, T4 and TSH role for the diagnosis of subclinical hypothyroidism.
- To determine the prevalence of subclinical hypothyroidism.
- To estimate the migration of subclinical hypothyroidism to clinical hypothyroidism as well as normalization of TSH.
- To correlate thyroid function test to BMI and blood pressure.
- To compare the symptoms of abnormal thyroid function between the subclinical hypothyroid patients and euthyroid.

Material and methods

1.8. Study design

Retrospective study was conducted on medical records of subclinical hypothyroidism patients.

1.9. Study area

The study was conducted in Nuclear Medicine Center at TASH in Addis Ababa. This hospital serves as a referral hospital and heterogeneous group of patients from different regions of the country for thyroid function test.

1.10. Study subjects

The subjects of the study were comprised of patients referred to Tikur Anbessa Specialized Hospital for thyroid function test. Thus, a heterogeneous group of patients from the different regions of the country were included in the study. Study groups- Those individuals who are subclinical hypothyroid patients. Control groups- Those individuals who are euthyroid patients whose age, gender, socio-cultural life style match within the study group.

1.11. Sample size determination

Sample size of the study was determined by the single population proportion formula according to Daniel, 1995 [81]

$$N = P\,(1 - P)\left(\frac{Z_{\alpha/2}}{E}\right)^2$$

Where: $Z_{\alpha/2}$ =1.96 (95 % confidence interval)

P= prevalence

E=precision

The prevalence of subclinical hypothyroidism in the general population is about 7 % [82]. Using the values of $Z_{\alpha/2}$ =1.96 (95 % confidence interval) and P=0.07 and E=0.02 the sample size was calculated as with population correction factor:

$$N = 0.07\ (1 - 0.07)\left(\frac{1.96}{0.02}\right)^2$$

N=582.

The total number of subclinical hypothyroid patients will be included in this study is about 582 and 349 euthyroid controls.

1.12. Selection criteria

Inclusion criteria

All SCH patients of both females and males whose serum TSH level ≥ 4.5mIU/L and normal TT4 and TT3 (TT4=62-141 nmol/L and TT3=1.1-3.2 nmol/L) and euthyroid individuals (with normal thyroid function tests) determined at nuclear medicine center of TASH were included for the study as a control group. The study was conducted on all subjects fulfilling the inclusion criteria on their medical records.

Exclusion criteria

Individuals that show no persistent increase in serum TSH levels-(Transient subclinical hypothyroidism) mentioned below.

- Transient SCH following subacute, painless, or postpartum thyroiditis.
- After withdrawal of thyroid hormone therapy in euthyroid patients.
- Laboratory analytical problem (assay variability, heterophilic antibodies).
- Impaired renal function.
- Recovery phase of euthyroid sick syndrome.
- Untreated adrenal insufficiency.

✓ Pregnant women.
✓ Patients with endocrine disorder (e.g. Diabetes Mellitus).
✓ Cancer patients.
✓ Alcoholics.
✓ Smokers.
✓ Patients with acute and systemic sever illness.

- ✓ Cancer patients subjected to radiotherapy.
- ✓ Patients taking thyroid medications will be excluded from the study.

1.13. Sample collection

Blood sample: Between 3-5ml of blood was drawn from antecubital vein of the arm by trained laboratory technicians working at Nuclear Medicine Center. The serum was separated after 45 minutes of collection by centrifugation at 3500 rpm for 15 minutes. Then, the serum was collected in a nanc tube and stored in refrigerator at -20^{o}C until analysis.

1.14. Ethical issues

This study was approved by the Institutional Review Board (IRB) of the School of Medicine, Addis Ababa University.

1.15. Data collection

All necessary information was collected from patient's medical record using structured formats (Annex III) consisting parts for collection of data on demographic characteristics, clinical findings, and laboratory investigations about sampled individuals. All the data collection formats were filled in nuclear medicine center of TASH by the principal investigator.

1.16. Biochemical analysis of laboratory variables

Working principle

RIA is a sensitive method for measuring small amounts of substances in the blood. Its principle is based on the Ag-Ab reaction in which tracer amount of radio labeled Ag competes with the endogenous Ag for limited binding sites of specific Ab against the antigen. The measurement of the analyte using the labels is broadly categorized into competitive and noncompetitive methods.

In **competitive formats**, unlabelled analyte in the test sample is measured by its ability to compete with labeled antigen for a limited number of antibody binding sites. The unlabeled antigen blocks the ability of the labeled antigen to bind because that binding site on the antibody is already occupied. Thus, in a competitive immunoassay, less label measured in the assay means

more of the unlabeled (test sample) antigen is present. The amount of antigen in the test sample is inversely related to the amount of label measured in the competitive format.

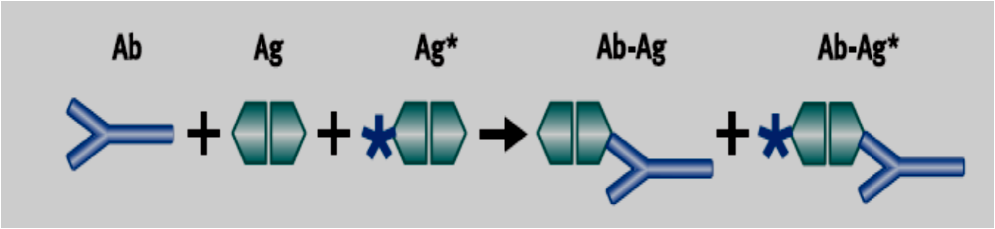


Figure 4. Competitive reaction of labeled and unlabeled antigen for an antibody

Noncompetitive (sandwich) **immunoassays** generally provide the highest level of assay sensitivity and specificity. This format is referred to as a "sandwich" assay because the analyte is bound (sandwiched) between two highly specific antibody reagents.

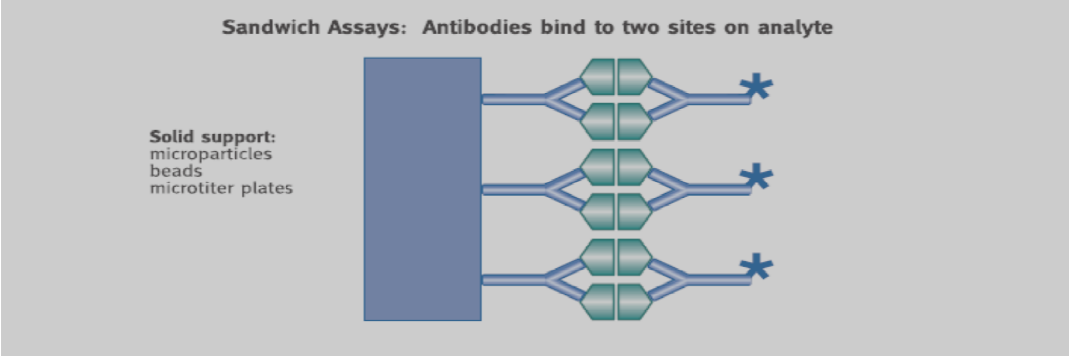


Figure 5. Noncompetitive reaction of labeled and unlabeled antibodies to two sites on an antigen

The reaction mixture typically includes an excess of labeled antibody, so that all metabolite is bound. The amount of antibody-antigen complex is then measured to determine the amount of metabolite present in the sample. The measurement of labeled analyte, usually antibody, is directly proportional to the amount of antigen present in the sample.

Various instruments are used to detect and measure radioactivity:

- scintillation counter
- Geir-muller counter
- Gas counter etc.

Both the free and bound fractions are counted. Various relationships are established between the variables and mathematical equations are defined to calculate the required data.

1.16.1. Serum T4 coated bead RIA

Principle

Unlabelled analyte antigen in the test sample is measured by its ability to compete with radio labeled antigen for a limited number of antibody binding sites. Both the free and the bound fractions are counted by gamma counter. The amount of antigen in the test sample is inversely related to the amount of radio labeled antigen measured in the competitive RIA. Less label measured in the assay means more of the unlabelled antigen is present (test sample).

Reaction:



Figure 6. T4 coated bead RIA reaction. A.Unlabeled hormone, B. Unlabeled hormone mixed with radiolabeled hormone, C. Radiolabeled and unlabeled compete for anti-T4 coated bead, D. Washing.

1.16.1.1. Assay reagents and materials

i. Materials

Serum T4 Coated Bead Reagent Pack. (SKY 1102A)

500Anti – T4 coated beads

1 set of 6x1ml T4 standards

1x6µCi ^{125}I-T4 tracer

Serum T4 Coated Bead Complete Kit. (SKY 1102B)

500Anti – T4 coated beads

1 set of 6x1ml T4 standards

1x6µCi ^{125}I-T4 tracer

6x25ml Serum T4 RIA Coated Bead Assay Buffer

2x25ml Wash solution (100xconcentrate)

1set of 3x 1ml Quality control

ii. Assay reagent Preparation

Wash Buffer

Dilute Wash solution concentrate1:99 with distilled water to give a working strength solution.

Prepare fresh, only sufficient for the assay. Do not store diluted wash solution for subsequent assays.

Tracer solution ^{125}I-T4

Reconstitute the tracer aliquot with 1ml distilled or deionised water. Prepare 100µl aliquoits. Either use immediately or store at -20^{0}C. When ready to perform an essay, dilute aliquots in assay buffer 1:99.

E.g. 100µ1+9.9ml assay buffer.

A200µl sample of this diluted material should give a count rate of between 15,000 and 20,000 counts per minute.

Prepare fresh, only sufficient for the assay. Do not store diluted tracer solution for subsequent assays.

Standards

Reconstitute each standard with 1ml distilled or deionised water. Allow to stand for 30 minutes, mixing at 10 minute intervals.

Storage: Use immediately or separate into aliquots and store at -20^{o}C.

Coated bead

Allow the coated beads to warm to ambient temperature prior to opening the bag. Re-seal unused beads in bag with dessicant. Store at 4^0C.

Procedure

A. Label and number the tubes with a marker pen. Ensure there are enough tubes for the required number of tests, Standards and QC's. Tests should be performed in duplicate for best results.

B. Add 10µ1 of standard, sample or QC to each tube.

C. Add 200µ1 of tracer solution ^{125}I-T4 to each tube. Total counts tubes should be prepared by adding 200µ1 only to two tubes.

D. Add one Anti T4 coated bead to each tube except Total counts

E. Vortex

F. Mix for 2 hours preferably using a rotating tube holder.

G. Add 2ml wash solution to all the tubes except total counts tubes.

H. Aspirate or decant the solution from all tubes except total counts tubes

I. Repeat steps G and H twice more to ensure thorough washing of the tubes.

J. Count the bound radioactivity for 60 seconds using a gamma counter.

K. Plot the graph of % (Bound/Total) Counts vs log T4 concentration for the standards.

L. Determine the concentrations of the QC's and unknown test results by interpolation from the standard curve.

Note: Steps K and L may be performed automatically using a data reduction software package.

Table 7.1. Mixing (T4 immunoassay)

Steps	Label and no. standards	Label and no. specimens	Label and no. quality controls	Label and no. total counts
Add 10 µL	✓	–	–	–
	–	✓	–	–
	–	–	✓	–
	–	–	–	–
Add 200 µL tracer solution ^{125}I-T4	✓	✓	✓	✓
Add one Anti T4 coated bead	✓	✓	✓	–
Vortex and mix for 2 hours using a rotating tube holder				
Add 2 ml wash solution	✓	✓	✓	–
Aspirate or decant the solution from all tubes except the total count tubes				
Count the bound radioactivity for 60 seconds using a gamma counter				

Plot the graph of % (Bound/Total) counts vs log T4 concentration for the standards

Using semi-logarithmic graph paper plot B/T (%) for each standard versus the corresponding logarithm of concentration of T4. Figure 7 shows a typical standard curve. Determine the T4 concentration of the unknown samples by interpolation from the standard curve. Do not extrapolate values beyond the standard curve range.

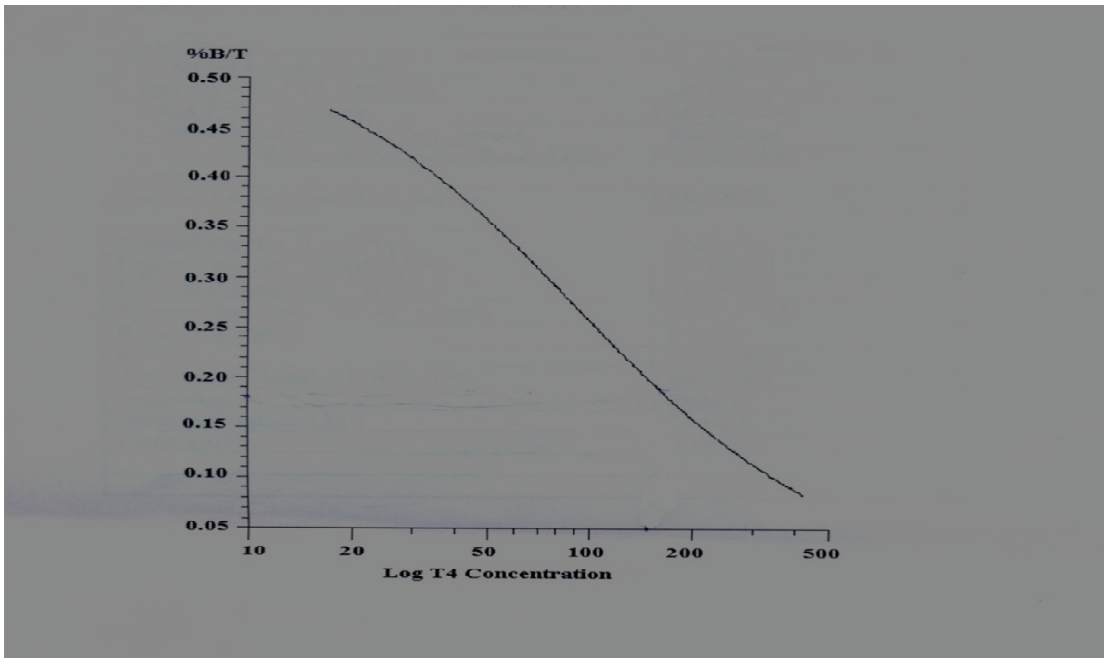


Figure 7. Percentage of Bound per Total T4 versus Logarithm of T4 concentration

Expected values

Normal 62-141 nmol/L

1.16.2. Serum TSH coated bead IRMA

Principle

Noncompetitive RIA is referred to as a sandwich assay because the analyte is bound to two highly specific antibody reagents. The reaction mixture typically includes an excess of the labeled antibody, so that the antigen in the test sample is bound. Both the free and the bound

fractions are counted by gamma counter .The amount of antibody antigen complex is then measured to determine the amount of antigen present in the sample. The measurement of labeled analyte usually antibody, is directly proportional to the amounts of antigen present in the sample.

Reaction:



Figure 8. TSH coated bead IRMA reaction. A. Unlabeled hormone, B. Unlabeled hormone mixed with radiolabeled hormone, C. Analyte sandwiched between two highly specific antibodies, D. Washing.

1.16.2.1. Assay reagents and materials

i.Materials

Serum TSH coated Bead Reagent pack. (SKY1101A)

500Anti –TSH coated beads

1 set of 8x1ml TSH stands

1x20µCi ^{125}I-Anti TSH tracer

Serum TSH coated Bead complete Kit. (SKY1101B)

500Anti –TSH coated beads

1 set of 8x1ml TSH stands

1x20µCi ^{125}I-Anti TSH tracer

6x24ml TSH serum IRMA coated Bead Assay Buffer

2x25ml Wash solution (100xconcetrate)

1set of 2x1ml Quality controls

ii.Assay reagent Preparation

Wash Buffer

Dilute Wash solution concentrate1:99 with distilled water to give a working strength solution.

Prepare fresh, only sufficient for the assay. Do not store diluted wash solution for subsequent assays.

Tracer solution ^{125}I-Anti TSH

Reconstitute the tracer alquot with 1ml distilled or deionised water. Prepare100µ1aliquoits. Either use immediately or store at -20^{0}C. When ready to perform an essay, dilute aliquots in assay buffer 1:99.

E.g. 100µ1+9.9ml assay buffer.

A200µ1 sample of this diluted material should give a count rate of between 40,000 and 60,000 counts per minute.

Prepare fresh, only sufficient for the assay. Do not store diluted tracer solution for subsequent assays.

Standards

Reconstitute each standard with 1ml distilled or deionised water. Allow to stand for 30 minutes, mixing at 10 minute intervals.

Storage: Use immediately or separate into aliquots and store at -20^{o}C.

Coated bead

Allow the coated beads to warm to ambient temperature prior to opening the bag. Re-seal unused beads in bag with dessicant. Store at 4^{0}C.

Procedure

A. Label and number the tubes with a marker pen. Ensure there are enough tubes for the required number of tests, Standards and QC's. Tests should be performed in duplicate for best results.

B. Add 100µ1 of standard, sample or QC to each tube.

C. Add 200µ1 of tracer solution ^{125}I Anti-TSH to each tube. Total counts tubes should be prepared by adding 200µ1 only to two tubes.

D. Add one Anti TSH coated bead to each tube except Total counts

E. Vortex

F. Mix for a minimum of 16 hours preferably using a rotating tube holder.

Next day:

G. Add 2ml wash solution to all the tubes except total counts tubes.

H. Aspirate or decant the solution from all tubes except total counts tubes

I. Repeat steps G and H twice more to ensure thorough washing of the tubes.

J. Count the bound radioactivity for 60 seconds using a gamma counter.

K. Plot the graph of % (Bound/Total) Counts vs log TSH concentration for the standards.

L. Determine the concentrations of the QC's and unknown test results by interpolation from the standard curve.

Note: Steps K and L may be performed automatically using a data reduction software package.

Table 7.2. Mixing (TSH immunoassay)

Steps	Label and no. standards	Label and no. specimens	Label and no. quality controls	Label and no. total counts
Add 100 µL	✓	–	–	–
	–	✓	–	–
	–	–	✓	–
	–	–	–	–
Add 200 µL tracer solution ^{125}I-anti TSH	✓	✓	✓	✓
Add one Anti TSH coated bead	✓	✓	✓	–
Vortex and mix for 16 hours using a rotating tube holder				
Add 2 ml wash solution	✓	✓	✓	–
Aspirate or decant the solution from all tubes except the total count tubes				
Count the bound radioactivity for 60 seconds using a gamma counter				
Plot the graph of % (Bound/Total) counts vs log TSH concentration for the standards				

Using semi-logarithmic graph paper plot B/T (%) for each standard versus the corresponding logarithm of concentration of TSH. Figure 9 shows a typical standard curve. Determine the TSH concentration of the unknown samples by interpolation from the standard curve. Do not extrapolate values beyond the standard curve range.

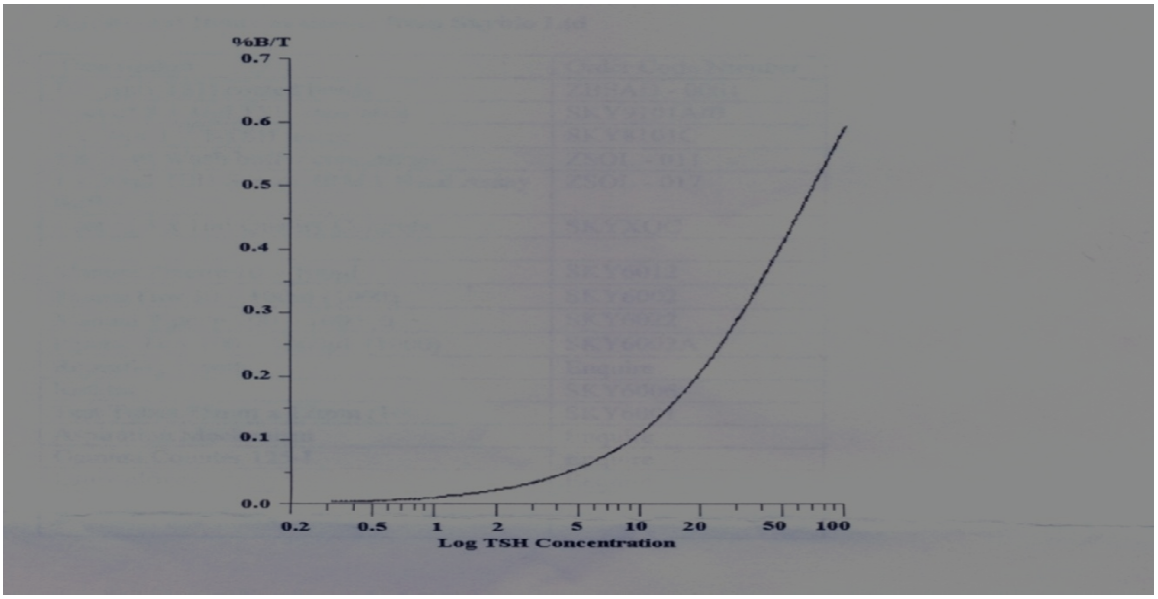


Figure 9. Percentage of Bound per Total TSH versus Logarithm of TSH concentration

Expected values

Normal 0.38-4.8 mIU/L

1.16.3. Free T4 coated bead IRMA

Principle

Noncompetitive RIA is referred to as a sandwich assay because the analyte is bound to two highly specific antibody reagents. The reaction mixture typically includes an excess of the labeled antibody, so that the antigen in the test sample is bound. Both the free and the bound fractions are counted by gamma counter .The amount of antibody antigen complex is then measured to determine the amount of antigen present in the sample. The measurement of labeled analyte usually antibody, is directly proportional to the amounts of antigen present in the sample.

Reaction:



Figure 10. FT4 coated bead IRMA reaction. A. Unlabeled hormone, B. Unlabeled hormone mixed with radiolabeled hormone, C. Analyte sandwiched between two highly specific antibodies, D. Washing.

1.16.3.1. Assay reagents and materials

i.Assay reagent ready to use

Free T4 coated Bead Reagent pack. (SKY1102H)

500Anti –Free T4 coated beads

1 set of 6x1ml Free T4 standards

4x6µCi ^{125}I-Anti Free T4 tracer

Free T4 coated Tube complete Kit. (SKY2202B)

500Anti –Free T4 coated beads

1 set of 6x1ml Free T4 stands

4x6µCi ^{125}Ianti TSH tracer

40x25ml Free T4 serum IRMA coated Bead Assay Buffer

2x25ml Wash solution (100 x concentrate)

1set of 3x1ml Quality controls

ii.Assay reagent Preparation

a. Wash Buffer

Dilute Wash solution concentrate1:99 with distilled water to give a working strength solution.

Prepare fresh, only sufficient for the assay. Do not store diluted wash solution for subsequent assays.

Tracer solution ^{125}I-T4

Reconstitute the tracer aliquot with 1ml distilled or deionised water. Prepare100µ1aliquoits. Either use immediately or store at -20^{0}C. When ready to perform an essay, dilute aliquots in assay buffer 1:24.

E.g. 100µ1+2.4ml assay buffer.

A500µ1 sample of this diluted material should give a count rate of between 40,000 and 60,000 counts per minute.

Prepare fresh, only sufficient for the assay. Do not store diluted tracer solution for subsequent assays.

Standards

Reconstitute each standard with 1ml distilled or deionised water. Allow to stand for 30 mins, mixing at 10 minute intervals.

Storage: Use immediately or separate into aliquots and store at -20^{o}C.

Coated bead

Allow the coated beads to warm to ambient temperature prior to opening the bag. Re-seal unused beads in bag with dessicant. Store at 4^{0}C.

Procedure

A. Label and number the tubes with a marker pen. Ensure there are enough tubes for the required number of tests, Standards and QC's. Tests should be performed in duplicate for best results.

B. Add 50µ1 of standard, sample or QC to each tube.

C. Add 500µ1 of assay buffer to each tube

D. Add one Anti Free T4 coated bead to each tube except Total counts.

E. Vortex.

F. Mix for a 30 minimum preferably using a rotating tube holder.

Next:

G. Add 2ml wash solution to all the tubes except total counts tubes.

H. Aspirate or decant the solution from all tubes except total counts tubes.

I. Repeat steps G and H twice more to ensure thorough washing of the tubes.

J. Add 500µl of tracer solution ^{125}I-T4 to each tube. Total counts tubes should be prepared by adding 500µl only to two tubes.

K. Mix 30 minutes preferably using a rotating tube holder.

L. Add 2ml wash solution to all the tubes except total counts tubes.

M. Aspirate or decant the solution from all tubes except total counts tubes.

N. Repeat steps L and M twice more to ensure through washing of the tubes.

O. Count the bound radioactivity for 60 seconds using a gamma counter.

P. Plot the graph of % (Bound/Total) Counts vs log Free T4 concentration for the standards.

Q. Determine the concentrations of the QC's and unknown test results by interpolation from the standard curve.

Note:Steps K and L may be performed automatically using a data reduction software package.

Table 7.3. Mixing (Free T4 immunoassay)

Steps	Label and no. standards	Label and no. specimens	Label and no. quality controls	Label and no. total counts
Add 50 µL	✓	–	–	–
	–	✓	–	–
	–	–	✓	–
	–	–	–	–
Add 500 µL assay buffer	✓	✓	✓	–
Add one Anti free T4 coated bead	✓	✓	✓	–
Vortex and mix for 30 minutes preferably using a rotating tube holder				
Add 2 ml wash solution	✓	✓	✓	–
Aspirate or decant the solution from all tubes except the total count tubes				
Add 200 µL tracer solution ^{125}I-T4	✓	✓	✓	✓
Mix for 30minute preferably using a rotating tube holder				
Add 2 ml wash solution	✓	✓	✓	–
Aspirate or decant the solution from all tubes except the total count tubes				
Count the bound radioactivity for 60 seconds using a gamma counter				

Plot the graph of % (Bound/Total) counts vs log free T4 concentration for the standards

Using semi-logarithmic graph paper plot B/T (%) for each standard versus the corresponding logarithm of concentration of freeT4. Figure 11 shows a typical standard curve. Determine the free T4 concentration of the unknown samples by interpolation from the standard curve. Do not extrapolate values beyond the standard curve range.

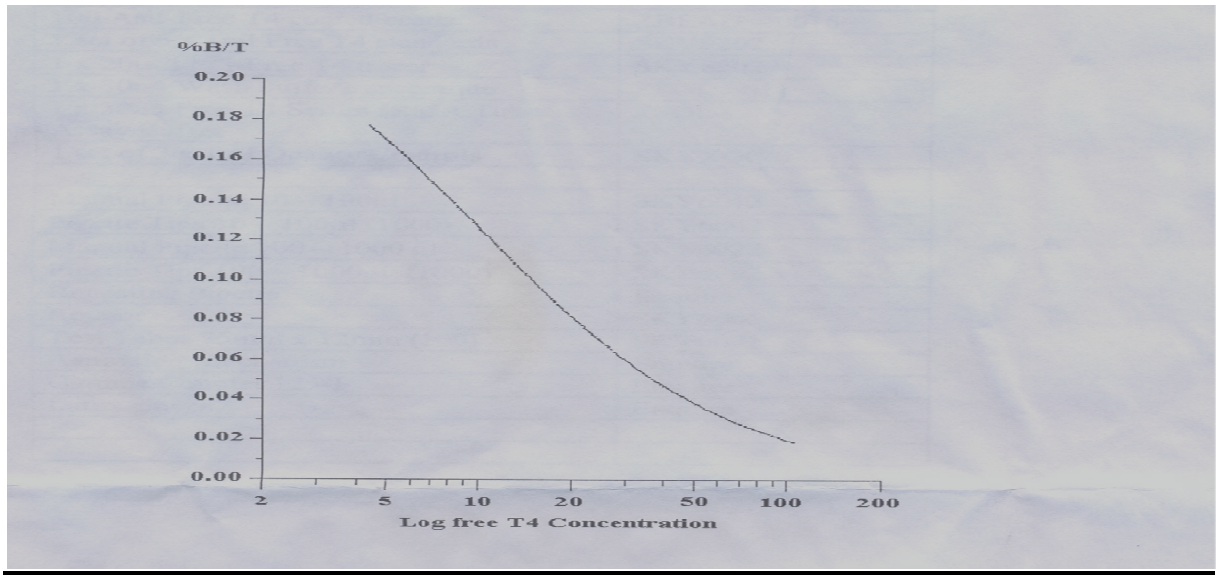


Figure 11. Percentage of Bound per total T4 versus Logarithm of Free T4

Expected values

Normal 10-20 pmol/L

1.16.4. Serum T3 coated bead RIA

Principle

Unlabelled analyte antigen in the test sample is measured by its ability to compete with radio labeled antigen for a limited number of antibody binding sites. Both the free and the bound fractions are counted by gamma counter. The amount of antigen in the test sample is inversely

related to the amount of radio labeled antigen measured in the competitive RIA. Less label measured in the assay means more of the unlabelled antigen is present (test sample).

Reaction:



Figure 12. T3 coated bead RIA reaction. A. Unlabeled hormone, B. Unlabeled hormone mixed with radiolabeled hormone, C. Radiolabeled and unlabeled compete for anti-T3 coated bead, D. Washing.

1.16.4.1. Assay reagents and materials

i.Materials

Serum T3 Coated Bead Reagent Pack. (SKY 1104A)

500Anti – T3 coated beads

1 set of 6x1ml T3 standards

1x6µCi ^{125}I-T3 tracer

Serum T3 Coated Bead Complete Pack. (SKY 1104B)

500Anti – T3 coated beads

1 set of 6x1ml T3 standards

1x6µCi ^{125}I-T3 tracer

6x25ml Serum T3 RIA Coated Bead Assay Buffer

2x25ml Wash solution (100 x concentrate)

1set of 3x 1ml Quality control

ii.Assay reagent Preparation

a. Wash Buffer

Dilute Wash solution concentrate1:99 with distilled water to give a working strength solution

Prepare fresh, only sufficient for the assay. Do not store diluted wash solution for subsequent assays.

Tracer solution ^{125}I-T3

Reconstitute the tracer alquot with 1ml distilled or deionised water. Prepare100µ1aliquoits. Either use immediately or store at -20^{0}C. When ready to perform an essay, dilute aliquots in assay buffer 1:99.

E.g. 100µ1+9.9ml assay buffer.

A200µ1 sample of this diluted material should give a count rate of between 15,000 and 20,000 counts per minute.

Prepare fresh, only sufficient for the assay. Do not store diluted tracer solution for subsequent assays.

Standards

Reconstitute each standard with 1ml distilled or deionised water. Allow to stand for 30 minutes, mixing at 10 minute intervals.

Storage: Use immediately or separate into aliquots and store at -20^{o}C.

Coated bead

Allow the coated beads to warm to ambient temperature prior to opening the bag. Re-seal unused beads in bag with dessicant. Store at 4^{0}C.

Procedure

A. Label and number the tubes with a marker pen. Ensure there are enough tubes for the required number of tests, Standards and QC's. Tests should be performed in duplicate for best results.

B. Add 100µ1 of standard, sample or QC to each tube.

C. Add 200µ1 of tracer solution ^{125}I-T3 to each tube. Total counts tubes should be prepared by adding 200µ1 only to two tubes.

D. Add one Anti T3 coated bead to each tube except Total counts

E. Vortex

F. Mix for 1 hour preferably using a rotating tube holder.

G. Add 2ml wash solution to all the tubes except total counts tubes.

H. Aspirate or decant the solution from all tubes except total counts tubes

I. Repeat steps G and H twice more to ensure thorough washing of the tubes.

J. Count the bound radioactivity for 60 seconds using a gamma counter.

K. Plot the graph of % (Bound/Total) Counts vs log T3 concentration for the standards.

L. Determine the concentrations of the QC's and unknown test results by interpolation from the standard curve.

Note: Steps K and L may be performed automatically using a data reduction software package.

Table 7.4. Mixing (T3 immunoassay)

Steps	Label and no. standards	Label and no. specimens	Label and no. quality controls	Label and no. total counts
Add 100 µL	✓	–	–	–
	–	✓	–	–
	–	–	✓	–
	–	–	–	–
Add 200 µL tracer solution ^{125}I-T3	✓	✓	✓	✓
Add one Anti T3 coated bead	✓	✓	✓	–
Vortex and mix for 1 hours using a rotating tube holder				
Add 2 ml wash solution	✓	✓	✓	–
Aspirate or decant the solution from all tubes except the total count tubes				
Count the bound radioactivity for 60 seconds using a gamma counter				
Plot the graph of % (Bound/Total) counts vs log T3 concentration for the standards				

Using semi-logarithmic graph paper plot B/T (%) for each standard versus the corresponding logarithm of concentration of total T3. Figure 13 shows a typical standard curve. Determine the total T3 concentration of the unknown samples by interpolation from the standard curve. Do not extrapolate values beyond the standard curve range.

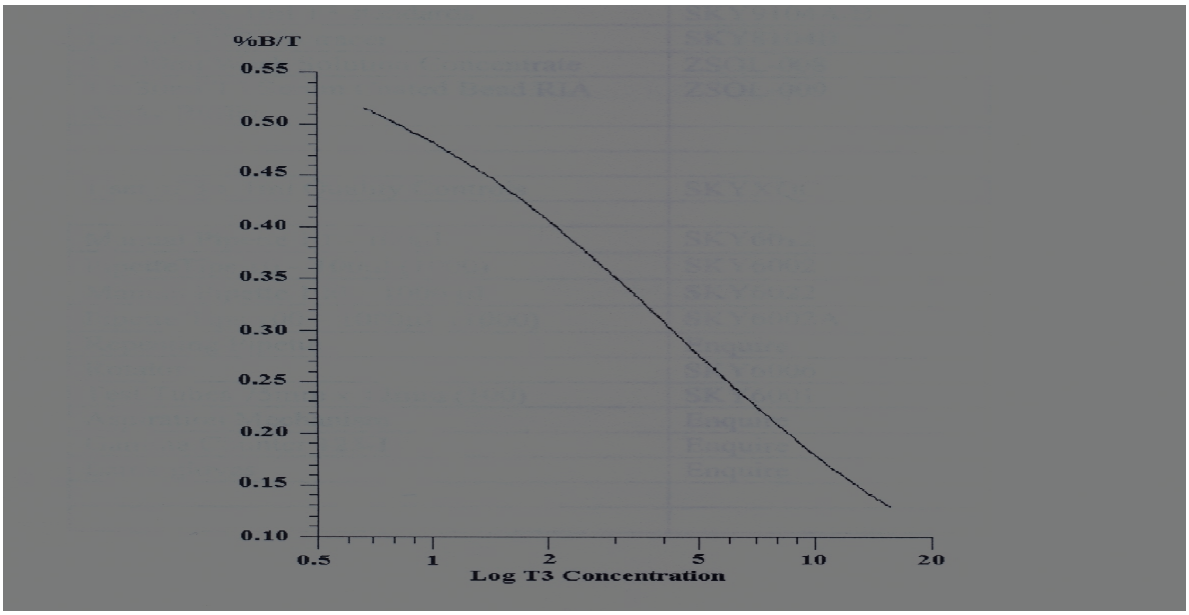


Figure 13. Percentage of Bound per Total T3 versus Logarithm of T3 concentration

Expected values

Normal 1.1-3.2 nmol/L

Statistical Analysis

Excel data sheet and SPSS version 16.0 (Chicago, IL, USA) were used to analyze data obtained from patient's medical record. The data were expressed as range, mean and standard deviation of the mean, median and interquartile range, and p-values if necessary. Correlation among parameters was made by using linear regression model. The level of significance was set at $p < 0.05$.

Results

1.17. Study Subjects

A total of 582 study subjects were included in this study. Their age ranged 18 to 90 years, with mean age of 38.83 ±14.65 years. As a control, 348 euthyroid controls with a mean age of 41.44 ±14.41were also included. Table 8.1 shows anthropometric and some health information of the study subjects and controls. All the subjects were diagnosed and found to be subclinical hypothyroid patients.

Table 8.1. Physical and some health data of study subjects (n = 582) and controls (n=348)

	Variables	Range	Mean ±SD
Subclinical hypothyroid patients	Age	72	38.83 ±14.65
	BMI	20.03	24.65± 4.39
	SBP	120	119.37±15.08
	DBP	50	76.41±8.40
Euthyroid controls	Age	55	41.44 ±14.41
	BMI	20.45	24.99± 4.23
	SBP	80	119.73±13.81
	DBP	40	75.75±7.9

BMI = body mass index, SBP =systolic blood pressure, DBP = diastolic blood pressure

Of the total studied sample 28.82 % were male and 71.18 % were female who met the inclusion criteria. About 55.6 % of the study subjects resided in Addis Ababa city and the rest 44.4 % were from other regions. Regarding euthyroid controls 65.8 % were female and 34.2 % were male and 65.8% were residents in Addis Ababa as shown in table 8.2.

Table 8.2. Demographic information of subjects

			Percentage (%)
Subclinical hypothyroid patients	Residence	Addis Ababa	55.6
		Other regions	44.4
	Sex	Female	71.18
		Male	28.82
Euthyroid controls	Residence	Addis Ababa	66.67
		Other regions	33.33
	Sex	Female	65.8
		Male	34.2

1.18. Prevalence of subclinical hypothyroidism

The medical records were reviewed for a total of 9,000 who were enrolled at Department of Nuclear Medicine for thyroid function test in, Tikur Anbessa Specialized Hospital, between January 2004 and November 2009. Five hundred and eighty two subclinical hypothyroid patients who fulfill the study criteria were recruited during the study period. It is estimated that the prevalence of subclinical hypothyroidism were 6.5% and 4.6% in female and 1.9 % in male.

1.19. Assessment of thyroid function tests

Of the total subclinical hypothyroid patients, most patients were diagnosed after investigation of typical symptoms of hypothyroidism or following screening in asymptomatic subjects. Most (74%) had serum TSH levels between 5 and 10 mIU/L and some (26%) had serum TSH level >10 mIU/L.

This study showed there was no significant difference in TT3 between those subclinical hypothyroid patients whose age $\geq$ 40 and those whose age < 40 but the mean TT4 value decreased further with age between the two groups and the difference was statistically significant. The average TSH of subclinical hypothyroid patients whose age $\geq$ 40 differ

significantly from that of subclinical hypothyroid patients whose age < 40 and it showed increment as shown in table 8.3.

Table 8.3. Comparison of thyroid function tests subclinical hypothyroid subjects

Thyroid function test	Age	Mean (±SD)	P* value
TSH	≥40	11.54(±10.29)	0.015
	<40	8.71(±6.82)	
TT4	≥40	100.79(±21.96)	0.04
	<40	104.42(±22.34)	
TT3	≥40	2.11(±0.57)	0.93
	<40	2.19(±0.53)	

*Mann-Whitney two sample rank sum test

The mean serum TT4 found in female slightly higher than that of male but the difference between the two groups was statistically insignificant. The average TSH and TT3 of subclinical hypothyroid male patients did not differ significantly from that of subclinical hypothyroid female patients as shown in table 8.4.

Table 8.4. Comparison of thyroid function tests of male and female subclinical hypothyroid subjects

Thyroid function test	Sex	Mean (±SD)	P* value
TSH	Female	10.88(±9.53)	0.96
	Male	9.66(±8.33)	
TT4	Female	103.09(±22.16)	0.49
	Male	101.48(±22.40)	
TT3	Female	2.13(±0.55)	0.12
	Male	2.21(±0.55)	

*Mann-Whitney two sample rank sum test

The mean serum TT3 and TT4 of female whose age ≥40 and <40 did not show significant difference where as the average TSH of subclinical hypothyroid female patients whose age≥40 differ significantly from that of subclinical hypothyroid female patients whose age<40.However, the mean TSH showed increment and that of TT4 were decreased as shown in table 8.5.

Table 8.5. Comparison of thyroid function tests of female subclinical hypothyroid subjects

Thyroid function test	Female whose age	Mean (±SD)	P* value
TSH	≥40	11.46(±10.38)	0.048
	<40	7.93(±5.19)	
TT4	≥40	101.24(±22.17)	0.07
	<40	105.21(±21.98)	
TT3	≥40	2.11(±0.55)	0.51
	<40	2.15(±0.55)	

*Mann-Whitney two sample rank sum test

There was no significant difference, between male whose age≥40 and age<40, in TSH, TT4, TT3.However, the mean TT4 decrease with age as shown in table 8.6.

Table 8.6. Comparison of thyroid function tests of male subclinical hypothyroid subjects

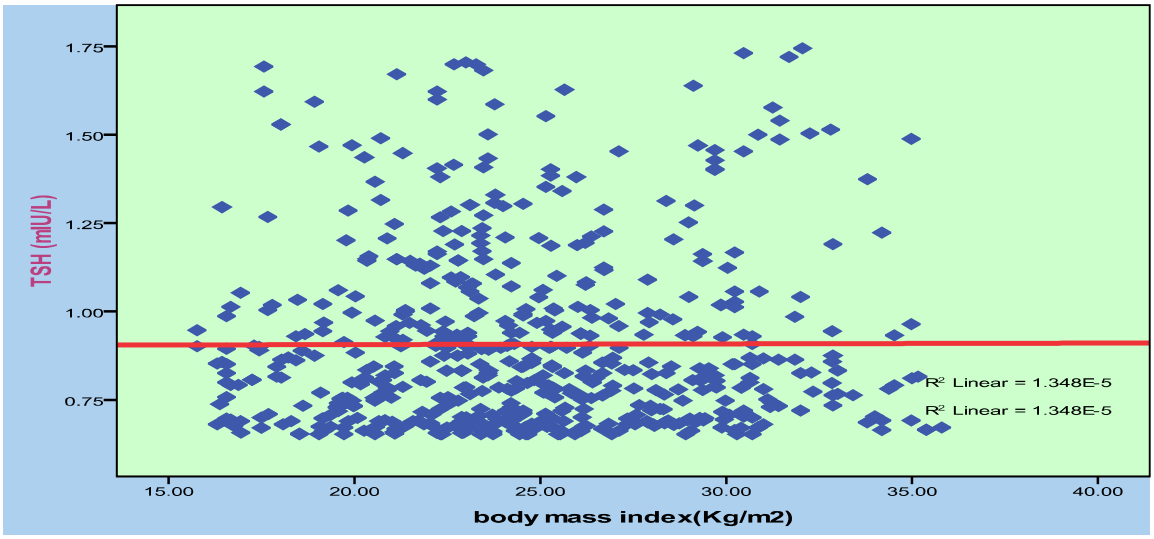
Thyroid function test	Male whose age	Mean (±SD)	P* value
TSH	≥40	11.79(±10.12)	0.14
	<40	10.31(±9.14)	
TT4	≥40	98.39(±21.39)	0.26
	<40	102.86(±23.05)	
TT3	≥40	2.12(±0.61)	0.08
	<40	2.27(±0.49)	

*Mann-Whitney two sample rank sum test

1.20. BMI

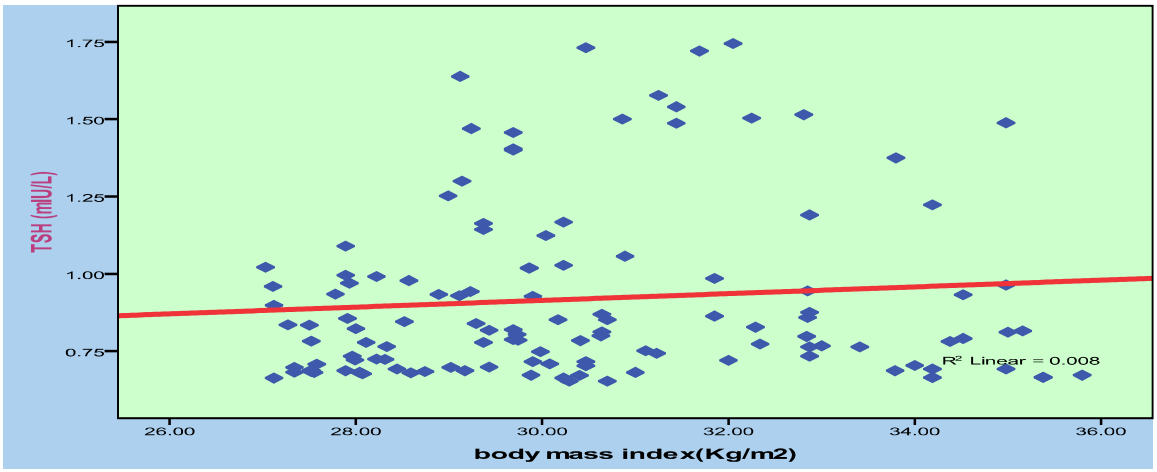
It was observed that, there is a non significant, very weak positive correlation between the thyroid stimulating hormone and body mass index. (p= 0.93, r = 0.004) as indicated in figure 14.

Figure 14. shows the correlation of thyroid stimulating hormone and body mass index.



It was observed that, there is a non significant, very weak positive correlation between the thyroid stimulating hormone and body mass index (p= 0.32, r = 0.09) of subclinical hypothyroid patients who were moderately obese (BMI ≥ 27).

Figure 15. shows the correlation of thyroid stimulating hormone and body mass index of subclinical hypothyroid patients who were moderately obese (BMI ≥ 27).



Raised serum TSH (Subclinical hypothyroidism) was not associated with significant differences in BMI compared with euthyroid subjects. When obesity was examined as a (BMI ≥ 30Kg/m^2), there was a non significant association between obesity and subclinical hypothyroidism (odds ratio [OR] 1.03, 95% CI 0.70-1.51).On further analysis, there was also non significant association of obesity and subjects with TSH 4.5-10mIU/L (odds ratio [OR] 1.04, 95% CI 0.69-1.56) and TSH >10mIU/L (odds ratio [OR] 1.01, 95% CI 0.58-1.75) as shown in table 8.7.

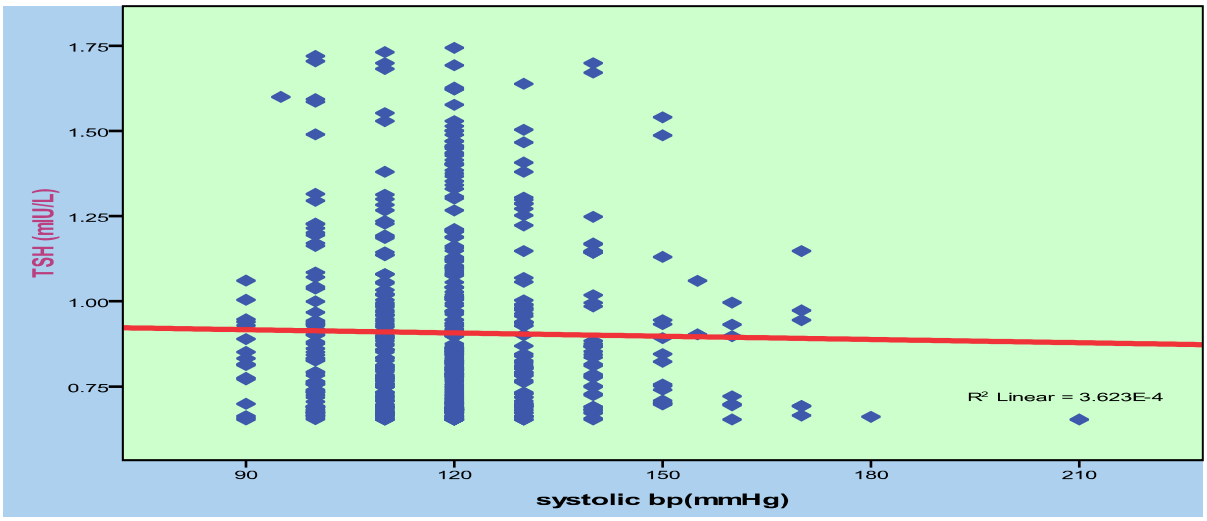
Table 8.7**.** Odds ratios for BMI (BMI ≥ 30Kg/m^2) in subjects with subclinical thyroid dysfunction compared with euthyroid subjects

	Euthyroid	Subclinical hypothyroid	Subclinical hypothyroidism	
			4.5-10mIU/L	>10mIU/L
BMI,mean±SD	24.99±4.23	24.65±4.39	24.66±4.47	24.60±4.19
Odds ratio for BMI (95% CI)	1.00 -	1.03 (0.70-1.51)	1.04 (0.69-1.56)	1.01 (0.58-1.75)
P value	-	0.88	0.86	0.98

1.21. Hypertension

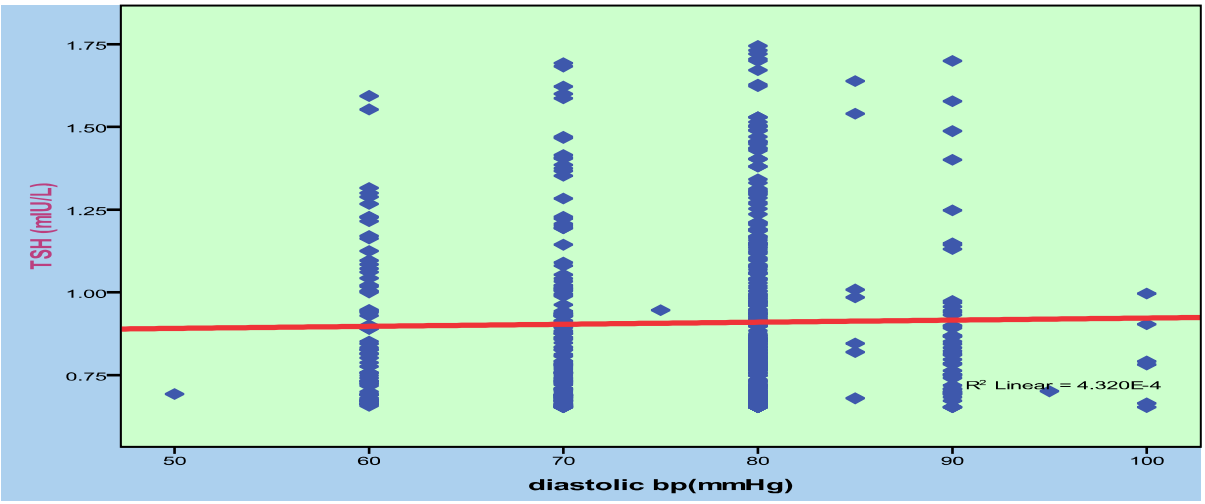
It was observed that, there is a non significant, very weak correlation between the thyroid stimulating hormone and systolic blood pressure (p= 0.65, r = -0.019) shown in figure 16.

Figure 16 shows the correlation of thyroid stimulating hormone and systolic blood pressure.



It was observed that, there is a non significant, very weak correlation between the thyroid stimulating hormone and diastolic blood pressure (p= 0.62, r = 0.021) as shown in figure 17.

Figure 17 shows the correlation of thyroid stimulating hormone and diastolic blood pressure.



Subclinical hypothyroidism was not associated with significant differences in SBP or DBP compared with euthyroid subjects. When hypertension was examined as a binary variable (SBP ≥140 mmHg, DBP ≥90 mmHg), there was a non significant association between hypertension and subclinical hypothyroidism (odds ratio [OR] 0.85, 95% CI 0.59-1.25).On further analysis, there was also non significant association of hypertension and subjects with TSH 4.5-10mIU/L (odds ratio [OR] 0.78, 95% CI 0.52-1.15) and TSH >10mIU/L (odds ratio [OR] 1.17, 95% CI 0.66-2.09) as shown in table 8.8.

Table 8.8. Odds ratios for hypertension (SBP = 140 mmHg, DBP = 90 mmHg) in subjects with subclinical thyroid dysfunction compared with euthyroid subjects.

	Euthyroid	Subclinical hypothyroid	Subclinical hypothyroidism	
			4.5-10mIU/L	>10mIU/L
SBP,mean±SD(mmHg)	119.73±13.81	119.37±15.08	119.53±15.79	118.93±12.84
DBP,mean±SD(mmHg)	75.75±7.9	76.41±8.4	76.65±8.54	75.70±7.99
Odds ratio for hypertension (95% CI)	1.00 -	0.85 (0.59-1.25)	0.78 (0.52-1.15)	1.17 (0.66-2.09)
P value	-	0.41	0.21	0.59

1.22. Thyroid function test of subclinical hypothyroid patients follow up for one year

There were 99 patients who came every 6 month for thyroid function test and follow up. The thyroid function tests of these patients were assessed before and after a year. As shown in table (8.9) the mean TSH after one year increased slightly and their FT4, TT3, and TT4 showed a slight decrease but statistically insignificant.

Table 8.9. Thyroid function test before and after a year for those who came to follow up

Thyroid function test		Before one year	After one year	P value
TSH	Mean (±SD)	8.44 (±4.69)	10.54 (±8.9)	0.14
FT4	Mean (±SD)	14.11 (±3.2)	13.59 (±3.29)	0.32
TT4	Mean (±SD)	102.04 (±25.55)	98.08 (±25.92)	0.32
TT3	Mean (±SD)	2.19 (±0.58)	2.08 (±0.53)	0.18

1.22.1. Normalization

At the end of the first year out of 99 subclinical hypothyroid patients 5 (5%) patients normalized their TSH level. In this survey, normalization was more frequent in patients with moderate TSH elevation (5.65 –8.19 mU/liter) and without thyroid autoimmunity shown in table 8.10.

Table 8.10. Thyroid function test before and after a year for those who normalize their TSH

Thyroid function test		Before one year	After one year
TSH	Mean (±SD)	6.92 (±1.27)	3.08(±0.67)
	Range	3.54	1.61
FT4	Mean (±SD)	13.59(±2.07)	14.78(±2.04)
	Range	4.91	4.33
TT4	Mean (±SD)	98.02(±28.98)	116.75(±18.62)
	Range	76.27	49.03
TT3	Mean (±SD)	2.1(±0.51)	2.48(±0.27)
	Range	1.33	0.75

1.22.2. Progression to overt hypothyroidism

Three female patients (3%) with subclinical hypothyroidism were reverted to hypothyroidism and their TSH levels remarkably increased during the first year of follow-up. These patients had a TSH value greater than 10 and with family history of thyroid disease shown in table 8.11.

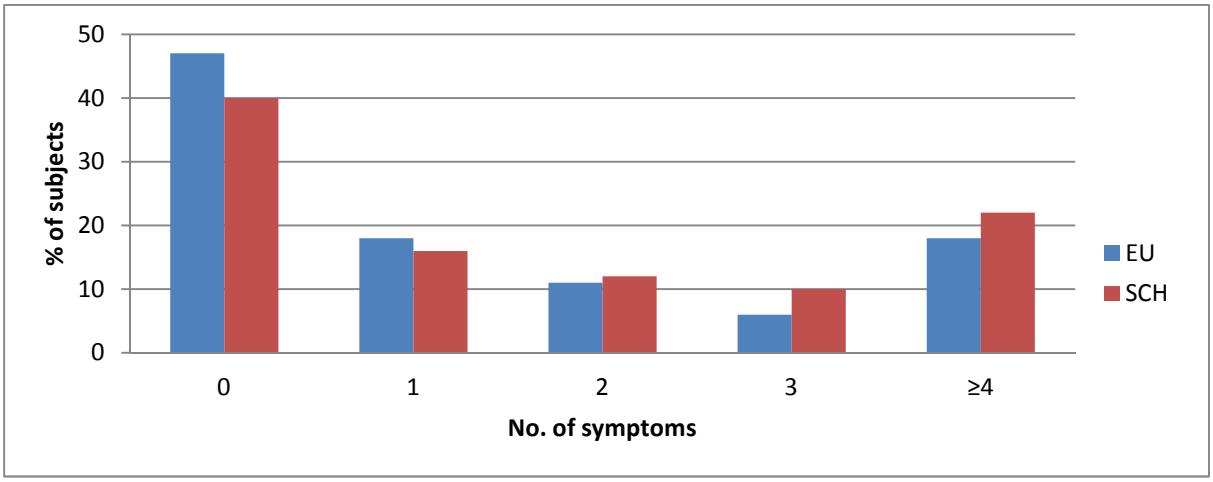
Table 8.11. Thyroid function test before and after a year for those who progress to overt

Thyroid function test		Before one year	After one year
TSH	Mean (±SD)	15.34 (±0.87)	35.53(±5.13)
	Range	1.56	10.26
FT4	Mean (±SD)	10.52(±0.45)	7.02(±0.49)
	Range	0.9	0.86
TT4	Mean (±SD)	112.36(±28.98)	41.23(±13.74)
	Range	78.22	26.70
TT3	Mean (±SD)	1.87(±0.85)	0.6(±0.2)
	Range	1.69	0.4

1.23. Symptoms

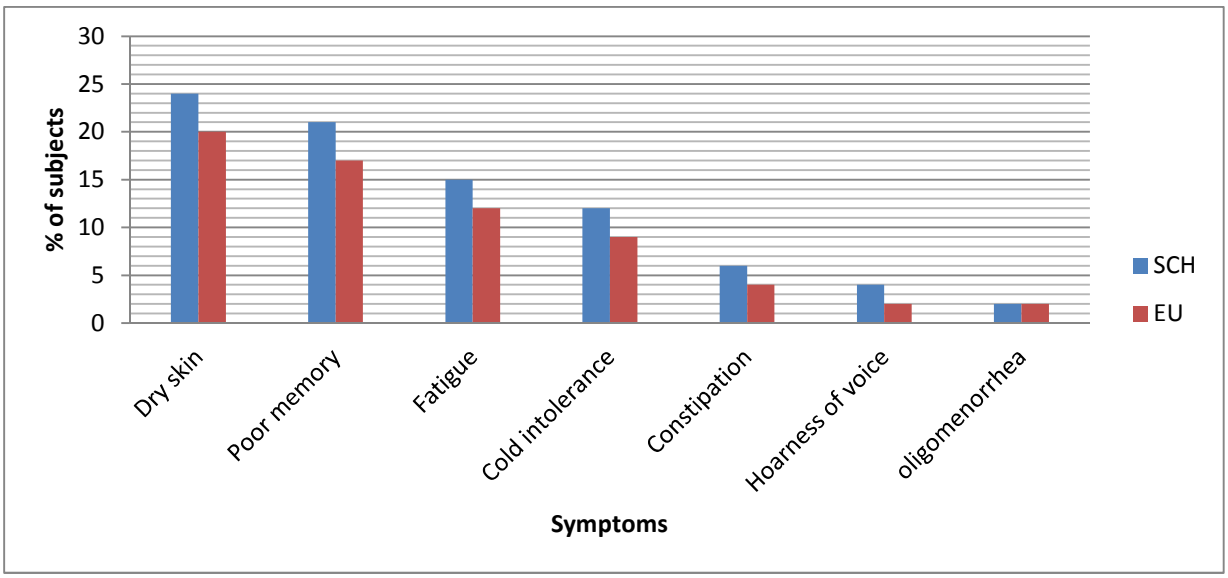
The percentage of subjects with no symptom were 40% in subclinical hypothyroid patient vs. 44% in euthyroid patients, with 1 symptom were 16% in subclinical hypothyroid patient vs. 18% in euthyroid patients, with 2 symptom were 12% in subclinical hypothyroid patient vs. 12% in euthyroid patients, with 3 symptom were 10% in subclinical hypothyroid patient vs. 6% in euthyroid patients, with 4 symptom were 22% in subclinical hypothyroid patient vs. 18% in euthyroid patients. The proportion of subclinical hypothyroid subjects having a certain number of symptoms rose as the number of symptoms increased (**Figure 18**). That is, as more symptoms were reported, the subject was more likely to be subclinical hypothyroid.

Figure 18**.** The percentage of euthyroid and subclinical hypothyroid subjects who reported no, 1, 2, 3, or 4 or more symptoms.



Subjects who were identified as subclinical hypothyroid more often reported having dry skin (24%), poor memory (21%), fatigue (15%), cold intolerance (12%), constipation (6%), hoarseness of voice (4%) than did euthyroid subjects (figure 19).

Figure 19**.** The percentage of euthyroid subjects compared with that subclinical thyroid subjects level who reported each symptom



Discussion

1.24. Prevalence

In this study the prevalence of subclinical hypothyroidism were estimated to be 6.5% and 4.6% in female and 1.9 % in male. A similar result regarding prevalence of SCH has been reported to be between 4 and 10% of adult population worldwide [10,83-88].Two large population-based screening studies have provided important epidemiological data about SCH: the Whickham Survey , and NHANES III .In the Whickham Survey (2779 subjects), SCH, defined by serum TSH levels above 6 IU/liter, was identified in 7.5% of females and 2.8% of males [84] .The sample examined in NHANES III (16,353 people aged ≥ 12 yr) represented the geographic and ethnic distribution of the U.S. population . SCH was found in 4.3% of this population [83].

In the Colorado study (over 25,000 state residents attending a series of state wide health fairs), 9.5% of all subjects had an elevated serum TSH concentration, and 89% of these were not on thyroid hormone therapy. Seventy-five percent of these individuals had serum TSH levels in the 5–10 mIU/liter range. In each age decade, a higher percentage of women than men had an elevated serum TSH concentration; the difference was significant after age 34 yr ($P < 0.01$). In the ninth decade of life, the prevalence of elevated TSH was as high as 15–20% [10].

The prevalence of mild thyroid dysfunction was higher in older populations in all epidemiological surveys conducted so far [83-89]. The highest age- and sex-specific rates are in women older than 60 years of age, approaching 20 percent in some reports [10,84]. In a recent survey, the prevalence of subclinical hypothyroidism in men over the age of 74 years (16 percent) was almost as high as it was in women of the same age (21 percent) [10]. Up to 75 percent of patients have only mildly elevated serum thyrotropin values (5 to 10 mU per liter), and 50 to 80 percent of patients have positive tests for antibodies against thyroperoxidase, depending on the age, sex, and serum thyrotropin levels [10,84].

1.25. Assessment of thyroid function test

In this study it is found that Most (74%) had serum TSH levels between 5 and 10 mIU/L and some (26%) had serum TSH level >10 mIU/L.

In other studies most patients were diagnosed in primary care after investigation of typical symptoms of hypothyroidism or following screening in asymptomatic subjects. Most (74–87%) will have serum TSH levels between 5 and 10 mIU/L [10,89].

In this study the mean TSH of subclinical hypothyroid female patients whose age ≥40 differ significantly from that of subclinical hypothyroid female patients whose age <40 and it showed increment. However, there was no significant difference, between male whose age ≥40 and age < 40, in TSH, TT4, TT3.Where as, the mean TT4 decrease with age in both female and male.

In other studies consistent with this study subclinical hypoyhroidism is more common in women, and the prevalence increases with age and is associated with the presence of antithyroid antibodies. In the Whickham survey , serum TSH concentrations did not change as a function of age among adult men, whereas in women over 40 years the concentrations increased markedly[84].

For example, virtually all studies reported that higher TSH and prevalence rates for hypothyroidism in women and with advancing age, [90-95] with rates as high as 24% among women older than 60 years recruited from several senior citizens' centers and ambulatory clinics [92]. Dietary iodine is another factor. The Framingham Study showed that 13.6% of US women older than 60 years had TSH levels greater than 5 mIU/L [93].

1.26. Hypertension

Thyroid hormone has profound effects on cardiovascular haemodynamics and function, including effects on arterial blood pressure[96].(1)Overt hypothyroidism is associated with diastolic hypertension, which may contribute to an increased risk of atherosclerosis[97-101].The effects of overt hyperthyroidism on blood pressure are variable, reflecting the net effect of increased cardiac output and reduced systemic vascular resistance, but some subjects develop systolic hypertension which may contribute to an increased cardiovascular mortality[99,101].

It was found in this study that, there is a non significant, very weak correlation between the thyroid stimulating hormone and SBP and DBP. A similar finding was observed on the community based study[102].

In this study it was found that there was no association between subclinical hypothyroidism and hypertension. This finding is consistent with some previous cross-sectional and population based studies which indicates that there was no significant difference in the mean blood pressure and hypertension in subjects with subclinical hypothyroidism compared with euthyroid subjects[103,104-107].

1.27. BMI

It was found in this study that, there is a non significant, very weak positive correlation between the thyroid stimulating hormone and body mass index (p= 0.93, r = 0.004) and for mildly obese (p= 0.32, r = 0.09) too. Raised serum TSH (Subclinical hypothyroidism) was not associated with significant differences in BMI compared with euthyroid subjects. When obesity was examined as a (BMI $\geq$ 30Kg/m^2), there was no association between subclinical hypothyroidism and Obesity.

A cross-sectional study found a higher BMI among women with subclinical hypothyroidism of borderline statistical significance, but the opposite association was found among men[108-110] . A major study of subclinical hypothyroidism found no such association [111], but multivariate statistics were not used for this association. There is an established association between overt thyroid dysfunction and weight changes, because weight loss is a relatively constant phenomenon in hyperthyroidism [112].

Conflicting results have also been reported about the association between thyroid function and the BMI in individuals with TSH and FT4 within normal range (34 –37). A cross-sectional population study examined the association between the category of serum TSH or serum thyroid hormones and BMI or obesity [113]. There was a positive association between obesity (BMI > 30 kg/m2) and serum TSH levels (P<0.001). Moreover, there was a negative association between BMI and serum FT4 (P< 0.001) and no association between BMI and serum FT3 levels. The difference in BMI between the groups with the highest and lowest serum TSH levels was 1.9 kg/m2, which corresponds to a difference in body weight of 5.5 kg among women. The results of

this study suggest that even slightly elevated serum TSH levels are important in determining body weight in the population [113]. Among 87 obese women (BMI > 30 kg/m2), serum TSH Concentrations were positively associated with increasing BMI, but there was no relationship between serum FT4 and BMI [114].

Furthermore, in 6164 subjects living in Tromso, TSH concentrations were positively associated with BMI in women and men who did not smoke [115]. However, in 401 euthyroid subjects there was no association between thyroid status within the normal range and BMI and no difference in BMI when subjects were stratified according to serum TSH or FT4. Lastly, there was no difference in serum TSH or FT4 between lean and obese euthyroid subjects [116].

1.28. Thyroid function test of subclinical hypothyroid patients follow up for one year

1.28.1. Progression

It was found that three female patients (3%) with subclinical hypothyroidism were reverted to overt hypothyroidism and their TSH levels remarkably increased during the first year of follow-up. These patients had a TSH value greater than 10 and with family history of thyroid disease. The Wickham survey involved approximately 2800 randomly selected adults in whom thyroid function was assessed. After 20 years of follow up , a high risk of overt hypothyroidism was found in woman who had elevated serum levels of TSH and antithyroid antibodies at baseline (4.3% per year)[89].Moreover a high serum TSH alone or antithyroid antibodies alone at baseline also conferred an increased risk of overt hypothyroidism (2.6% per year and 2.1% per year) [84].

In a study evaluating patients aged over 60 years, the risk of progression to overt hypothyroidism is related to initial laboratory findings. Among these, overt hypothyroidism developed in all subjects with initial TSH levels above 20 mIU/liter, and 80% of those with high-titer thyroid antimicrosomal antibodies regardless of initial TSH level became overtly hypothyroid [117].

In another prospective study, serum TSH was the most powerful predictor of the outcome of spontaneous Subclinical Hypothyroidism in 107 subjects over the age of 55 yr with Subclinical Hypothyroidism and no history of thyroid disease .The incidence rate of overt hypothyroidism was 9.91 cases per 100 patient-years in the whole population, and 1.76, 19.67, and 73.47 cases

per 100 patient-years in subjects with initial TSH values between 5.0 –9.9, 10.0 –14.9, and 15.0 –19.9 mIU/liter, respectively [118].

1.28.2. Normalization

In this survey study 5% of subclinical hypothyroid patients normalized their TSH level and normalization was more frequent in patients with moderate TSH elevation (5.0 –9.9 mU/liter) without thyroid autoimmunity. The normalization of TSH levels in some patients with subclinical hypothyroidism is a well-known phenomenon, especially in those without previous history of thyroid disease and moderate elevations of TSH [119-121]. In a prospective survey including patients with previous thyroid disorders, it was reported that three of 82 patients (4%) normalized their TSH levels [122].In another study it was observed that normalization in 40 of 107 patients (37%) with spontaneous subclinical hypothyroidism [123].

In a prospective study of 82 women with increased TSH concentrations due to autoimmune thyroiditis, radioiodine treatment, or thyroidectomy, over a mean observation period of 9.2 yr with annual follow-up . After 10 yr, 28% of the women had developed overt hypothyroidism, and 68% remained in the subclinical stage. TSH value became normal in 4% (all from the group with TSH between 4 – 6 mIU/liter). [122]

In another prospective study, serum TSH was the most powerful predictor of the outcome of spontaneous subclinical hypothyroidism in 107 subjects over the age of 55 yr with subclinical hypothyroidism and no history of thyroid disease [118]. Twenty-eight patients (26.8%) developed overt hypothyroidism and 40 (37.4%) normalized their TSH values.

The same authors analyzed the time course of normalization of TSH levels in 40 patients (32 women, mean age 62.8 ± 8.2 yr) with spontaneous SCH during a mean (±sd) observation period of 38.3 ± 17.0 months (range, 12–72 months) (39). The rate of normalization was greater in patients who had lesser degrees of serum TSH elevations and negative antithyroid antibody titers. Thus, the rate of normalization was 52% for those with serum TSH values between 5.0 and 9.9 mIU/liter and only 13% for those with TSH values between 10 and 14.9 mIU/liter. Fifteen patients (37.5%) normalized their TSH levels during the first year of follow- up and 27 (67.5%) during the first 2 yr. Ten patients (25%) had decreased TSH values at the fourth or fifth years of

follow-up [124]. Finally, in another study, 11 of 21 octogenarians with SCH and a TSH value greater than 4.7 mIU/liter had normal thyroid function after 3 yr [125].

1.29. Symptoms

In this study subjects who were identified as subclinical hypothyroid more often reported having dry skin (24%), poor memory (21%), fatigue (15%), cold intolerance (12%), constipation (6%), hoarseness (4%) than did euthyroid subjects.

Mild thyroid failure is often asymptomatic; however, nearly 30% of patients with this condition may have symptoms that are suggestive of thyroid hormone deficiency [90,126]. Similar results were obtained from The Colorado Thyroid Disease Prevalence Study measured serum TSH levels and conducted symptom surveys in over 25,000 state residents. In response to a validated survey regarding symptoms of thyroid hormone deficiency, the 2,336 subjects who were identified as having mild thyroid failure significantly more often reported having dry skin (28%; $P < 0.001$), poor memory (24%; $P < 0.001$), slow thinking (22%; $P< 0.001$), muscle weakness (22%; $P< 0.001$), fatigue (18%; $P < 0.01$), muscle cramps (17%; $P < 0.001$), cold intolerance (15%; $P< 0.001$), puffy eyes (12%; $P < 0.05$), constipation (8%; $P< 0.05$), and hoarseness (7%; $P < 0.05$) than did euthyroid subjects [10].

Consistent with these findings, a Swiss study involving 332 women with hypothyroidism reported that 24% of the 93 subjects with mild thyroid failure exhibited typical symptoms of hypothyroidism [127].

These studies also emphasize the difficulty in making the diagnosis of primary hypothyroidism using clinical symptoms alone; euthyroid subjects and patients with mild or overt hypothyroidism all had similar constellations of symptoms. Despite statistical significance in large groups, it can be difficult in an individual patient to distinguish a euthyroid subject from one with either mild or overt thyroid disease.

The results of this study showed that the percentage of subjects with no symptom were 40% in subclinical hypothyroid patient vs. 44% in euthyroid patients, with 1 symptom were 16% in subclinical hypothyroid patient vs. 18% in euthyroid patients, with 2 symptom were 12% in

subclinical hypothyroid patient vs. 12% in euthyroid patients, with 3 symptom were 10% in subclinical hypothyroid patient vs. 6% in euthyroid patients, with 4 symptom were 22% in subclinical hypothyroid patient vs. 18% in euthyroid patients.

In the Colorado study, it was indicated as more symptoms were reported, the subject was more likely to be subclinical hypothyroid [10, 128-129]. A small increase in total symptoms was observed with progressive deterioration of thyroid function. Moreover, reporting more symptoms, in particular recently “changed symptoms” increased the likelihood of disease.

Limitation of the study

- First, this study is subject to limitations as the case is with other studies. The findings of this study may not be generalized because of small sample size. A well designed study with larger sample size should be carried out for substantiating the current findings for better care and clinical management.
- Second, there were no FT3,Tg Ab and TPO Ab measurement done but FT4 measurement done only if depending on the availability of the reagent.
- Third, many of the subclinical hypothyroid patients do not come as they are instructed for follow up every six month for thyroid function test.
- Fourth, this was a retrospective study, and a substantial proportion of patients were excluded for incomplete medical records.

Conclusion

This retrospective study has the following outcomes:

i. The prevalence of subclinical hypothyroidism evaluated to be 6.5% and 4.6% in female and 1.9% in male.

ii. There was no association of obesity and subclinical hypothyroidism.

iii. There was also no association of hypertension and subclinical hypothyroidism.

iv. The rate of progression of subclinical form to clinical (overt) was found to be 3% with those whose TSH >10MIU/L.

v. Some of the subclinical hypothyroid (5%) patients showed normalization of their TSH after a soild year follow up.

vi. Subclinical hypothyroid more often reported having dry skin , poor memory , fatigue , cold intolerance , constipation , hoarseness than did euthyroid subjects it could be difficult in an individual patient to distinguish a euthyroid subject from one with either mild or overt thyroid disease because all had similar constellations of symptoms.

vii. As more symptoms were reported, the subject was more likely to be subclinical hypothyroid than euthyroid. A small increase in total symptoms was observed with progressive deterioration of thyroid function.

Recommendation

Since the sample size of this study was small, large scale studies should be done to obtain clear information about the thyroid function of subclinical hypothyroid patients in the study area and in the Ethiopian population. In addition, it would be important if assessment of autoimmunity is done because thyroid dysfunction due to autoimmunity is common in subclinical hypothyroidism. It is also important to note that subclinical hypothyroidism can have repercussion on the cardiovascular system so that the lipid profile, other related tests are recommended to examine the effect. Further study on subclinical hypothyroidism on neuromuscular, subclinical hypothyroidism in children and during pregnancy is also important to observe the occult nature.

References

1. De La Vieja A, Dohan O, Levy O, et al. Molecular analysis of the sodium/iodide symporter: impact on thyroid and extrathyroid pathophysiology. Physiol Rev 2000; **80**:1083-1105.

2. Taurog A, Dorris ML, Doerge DR. Mechanism of simultaneous iodination and coupling catalyzed by thyroid peroxidase. Arch Biochem Biophys 1996; **330**:24-32.

3. Ohmiya Y, Hayashi H, Kondo T, et al. Location of dehydroalanine residues in the amino acid sequence of bovine thyroglobulin: identification of "donor" tyrosine sites for hormonogenesis in thyroglobulin. J Biol Chem 1990; **265**:9066-9071.

4. Dunn AD, Corsi CM, Myers HE, et al. Tyrosine 130 is an important outer ring donor for thyroxine formation in thyroglobulin. J Biol Chem 1998; **273**:25223-25229.

5. Ericson LE. Exocytosis and endocytosis in the thyroid follicle cell. Mol Cell Endocrinol 1981; **22**:1-24.

6. Salvatore D, Tu H, Harney JW, et al. Type 2 iodothyronine deiodinase is highly expressed in human thyroid. J Clin Invest 1996; **98**:962-968.

7. Bastenie PA, Bonnyns M, Neve P, Vanhaelst L, Chailly M. Clinical and pathological significance of asymptomatic atrophic thyroiditis: a condition of latent hypothyroidism. Lancet. 1967;**1**:915-918.

8. Cooper DS. Clinical practice: subclinical hypothyroidism. N Engl J Med. 2001;**345** :260-265.

9. Ayala AR, Danese MD, Ladenson PW. When to treat mild hypothyroidism. Endocrinol Metab Clin North Am 2000; **29**:399–415.

10. Canaris GJ, Manowitz NR, Mayor G, Ridgway EC. The Colorado thyroid disease prevalence study. Arch Intern Med 2000; **160**:526–534.

11. Roberts CG, Ladenson PW. Hypothyroidism. Lancet 2004;**363**:793– 803.

12. RossD Subclinical hypothyroidism. In: Braverman LE, Utiger RD, eds. Werner, Ingbar's the thyroid: a fundamental and clinical text. 8th ed. Philadelphia: Lippincott, Williams & Wilkins.2005; pp.1070–1078.

13. Surks M, Ocampo E **.** Subclinical thyroid disease. Am J Med 1996;**100**:217–223.

14. Boyages SC. Clinical review 49: iodine deficiency disorders. J Clin Endocrinol Metab 1993; **77**:587-591.

15. Meyer JD, Gaitan E, Merino H, et al. Geologic implications in the distribution of endemic goiter in Colombia, South America. Int J Epidemiol 1978; **7**:25-30.

16. Molineux L,Ayele T. An endemic goiter survey in the Armacho woreda, Begemdir province. Ethiop Med J 1963;**1**:239-245.

17. Mekonen E. Prevalence of goiter in Sekota District, Ethiopia. East Afri Med J 1996;**73**:264-267.

18. Hovander Y. Endemic goiter among children in Ethiopia highlands. Ethiop Med J 1970;**8**:179.

19. Popov L. Medical survey of goiter in Ethiopia. Ethiop Med J 1967;**6**:5-13.

20. Wolde-gabriel Z, Demeke T, West CE, Van der Haar F. Goiter in Ethiopia. Brit J Nutr 1993;**69**:257-268.

21. Cherinet A, Gonfa A, Melku U, Hana NT, Bantirgu HM. Thyroid response to varying doses of oral iodized oil in school children in Awassa, Ethiopia. Ethiop J Health Dev 2000;**14**:49-55.

22. Cherinet A, Kelbessa U. Determinants of iodine deficiency in school children in different regions of Ethiopia. East Afri Med J 2000;**77**:133-137.

23. Cherinet A,Yemane B, Girma A, Zewditu G, Tessema E. Goiter prevalence in children age 6-12 years in Ethiopia. Food and Nutrition Bulletin 2007;**8**:11-16.

24. Cherinet A and Yemane B.The goiter rate, its association with reproductive failure, and the knowledge of iodine deficiency disorder among women in Ethiopia.BMC Public Health 2007;**7**:316.

25. Surks MI, Sievert R. Drugs and thyroid function. N Engl J Med 1995; **333**:1688-1694.

26. Zhang ZJ, Qiang Li, Kang WH, Tan QR, Gao CG, Zhang FG, Wang HH, Ma XC, Ce Chen, Wei Wang, Li Guo, Zhang YH, Yang XB, Zhang RG. Differences in hypothyroidism between lithium- free and -treated patients with bipolar disorders. Life Sci 2006; **78**:771–776.

27. Kleiner J, Altshuler L, Hendrick V, Hershman JM. Lithium induced subclinical hypothyroidism: review of the literature and guidelines for treatment. J Clin Psychiatry1999; **60**:249–255.

28. Singer P 2005 Primary hypothyroidism due to other causes. In: Braverman LE, Utiger RD, eds. Werner, Ingbar's the thyroid: a fundamental and clinical text. 8th ed. Philadelphia: Lippincott, Williams & Wilkins2005; pp. 745–754.

29. Cherinet A, Kelbessa U, Wolde-gabriel S. Health effects of cassava consumption in south Ethiopia. East Afri Med J 1998;**75**:166-170.

30. Lawrence JE, Lamm SH, Pino S, et al. The effect of short-term low-dose perchlorate on various aspects of thyroid function. Thyroid 2000; **10**:659-663.

31. Brucker-Davis F. Effects of environmental synthetic chemicals on thyroid function. Thyroid 1998; **8**:827-856.

32. Porterfield SP. Thyroidal dysfunction and environmental chemicals potential impact on brain development. Environ Health Perspect 2000; **108**:433-438.

33. Carella C, Mazziotti G, Amato G, Braverman LE, Roti E. Clinical review 169: Interferon-α-related thyroid disease: pathophysiological, epidemiological, and clinical aspects. J Clin Endocrinol Metab 2004;**89**:3656–3661.

34. Caraccio N, Dardano A, Manfredonia F, Manca L, Pasquali L, Iudice A, Murri L, Ferrannini E, Monzani F. Long-term follow- up of 106 multiple sclerosis patients undergoing interferon-β 1a or 1b therapy: predictive factors of thyroid disease development and duration. J Clin Endocrinol Metab 2005; **90**:4133–4137.

35. Hancock SL, Cox RS, McDougall IR . Thyroid diseases after treatment of Hodgkin's disease. N Engl J Med1991; **325**:599–605.

36. Anker GB, Lonning PE, Aakvaag A, Lien EA . Thyroid function in postmenopausal breast cancer patients treated with tamoxifen. Scand J Clin Lab Invest 1998; **58**:103–107.

37. Kumar N, Allen KA, Riccardi D, Bercu BB, Cantor A, Minton S, Balducci L, Jacobsen PB . Fatigue, weight gain, lethargy and amenorrhea in breast cancer patients on chemotherapy: is subclinical hypothyroidism the culprit? Breast Cancer Res Treat 2004; **83**:149–159.

38. Lester FT, Tesga E. The pattern of Adult medical admission in Addis Ababa, Ethiopia. East Afri Med J 1976;**53**:620-634.

39. Mengistu M. The pattern of thyroid disease in adult Ethiopians and experience in management. Ethiop Med J 1993;**31**:25-35.

40. Ali A, Abebe A, Mohamed A. Pattern of surgical admission at Tikur Anbessa Hospital , Addis Ababa. East and central Afr J Surg 2001;**6**:31-34.

41. Kahsay G. The pattern of surgical admission at Gondar teaching Hospital, Ethiopia. East Afri Med J 1997;**74**:62-65.

42. Berhanu K, Tessema E, Adem A, Alemayehu W. Thyroid disease in Tikur Anbessa Hospital. Ethiop Med J 2004;**42**:205-209.

43. LaFranchi S. Congenital hypothyroidism: etiologies, diagnosis, and management. Thyroid 1999; **9**:735-740.

44. Bogale W, Meaza T, Sara S. Pattern of hypothyroid in Ethio-Swedish children's hospital. Ethiop Med J 2002;**40**:1-9.

45. Bianco AC, Slavatore D, Gereben B, et al. Biochemistry, cellular and molecular biology, and physiological roles of the iodothyronine selenodeiodinases. Endocr Rev 2001; **23**:38-39.

46. Huang SA, Tu HM, Harney JW, et al. Severe hypothyroidism caused by type 3 iodothyronine deiodinase in infantile hemangiomas. N Engl J Med 2000; **343**:185-189.

47. Desai J, Yassa L, Marqusee E, George S, Frates MC, Chen MH, Morgan JA, Dychter SS, Larsen PR, Demetri GD, Alexander EK **.** Hypothyroidism after sunitinib treatment for patients with gastrointestinal stromal tumors. Ann Intern Med 2006 ;**145**:660–664.

48. Sawin CT, Chopra D, Azizi F, Mannix JE, Bacharach P. The aging thyroid. Increased prevalence of elevated serum thyrotropin levels in the elderly. JAMA 1979;**242**:247–250.

49. Sherman SI, Gopal J, Haugen BR, et al. Central hypothyroidism associated with retinoid X receptor-selective ligands. N Engl J Med 1999; **340**:1075-1079.

50. Tonacchera M, Agretti P, De Marco G, Perri A, Pinchera A, Vitti P, Chiovato L **.** Thyroid resistance to TSH complicated by autoimmune thyroiditis. J Clin Endocrinol Metab 2001;**86**:4543–4546.

51. Alberti L, Proverbio MC, Costagliola S, Romoli R, Boldrighini B, Vigone MC, Weber G, Chiumello G, Beck-Peccoz P, Persani L. Germline mutations of TSH receptor gene as cause of nonautoimmune subclinical hypothyroidism. J Clin Endocrinol Metab 2002; **87**:2549–2555.

52. Tonacchera M, Perri A, De Marco G, Agretti P, Banco ME, Di Cosmo C, Grasso L, Vitti P, Chiovato L, Pinchera A. Low prevalence of thyrotropin receptor mutations in a large series of subjects with sporadic and familial nonautoimmune subclinical hypothyroidism. J Clin Endocrinol Metab 2004; **89**:5787–5793.

53. Hollowell JG, Staehling NW, Flanders WD, Hannon WH, Gunter EW, Spencer CA, Braverman LE **.** Serum TSH, T(4), and thyroid antibodies in the United States population (1988 to 1994):National Health and Nutrition Examination Survey (NHANES III).J Clin Endocrinol Metab 2002; **87**:489–499.

54. TunbridgeWM,Evered DC, Hall R, Appleton D, Brewis M, Clark F, Evans JG, Young E, Bird T, Smith PA. The spectrum of thyroid disease in a community: the Whickham survey. Clin Endocrinol (Oxf)1977; **7**:481–493.

55. Parle JV, Franklyn JA, Cross KW, Jones SC, Sheppard MC. Prevalence and follow-up of abnormal thyrotrophin (TSH) concentrations in the elderly in the United Kingdom. Clin Endocrinol (Oxf) 1999;**34**:77–83.

56. Lindeman RD, Schade DS, LaRue A, Romero LJ, Liang HC, Baumgartner RN, Koehler KM, Garry PJ. Subclinical hypothyroidism in a biethnic, urban community. J Am Geriatr Soc1999; **47**: 703–709.

57. Bemben DA, Winn P, Hamm RM, Morgan L, Davis A, Barton E. Thyroid disease in the elderly. Part 1. Prevalence of undiagnosed hypothyroidism. J Fam Pract1994; **38**:577–582.

58. Bagchi N, Brown TR, Parish RF. Thyroid dysfunction in adults over age 55 years. A study in an urban US community. Arch Intern Med 1990; **150**:785–787.

59. Szabolcs I, Podoba J, Feldkamp J, Dohan O, Farkas I, Sajgo M, Takats KI, Goth M, Kovacs L, Kressinszky K, Hnilica P, Szilagyi G. Comparative screening for thyroid disorders in old age in areas of iodine deficiency, long-term iodine prophylaxis and abundant iodine intake. Clin Endocrinol (Oxf)1997; **47**:87–92.

60. Laurberg P, Pedersen KM, Hreidarsson A, Sigfusson N, Iversen E, Knudsen PR. Iodine intake and the pattern of thyroid disorders: a comparative epidemiological study of thyroid abnormalities in the elderly in Iceland and in Jutland, Denmark. J Clin Endocrinol Metab 1998;**83**:765–769.

61. Vanderpump MP,Tunbridge WM,French JM,et al. The incidence of thyroid disorders in the community: a twenty-year follow-up of the Whickham Survey. Clin Endocrinol (Oxf). 1995;**43**:55-68.

62. Díez JJ,Iglesias P. Spontaneous subclinical hypothyroidism in patients older than 55 years: an analysis of natural course and risk factors for the development of overt thyroid failure. J Clin Endocrinol Metab. 2004;**89**:4890-4897.

63. Kong WM, Sheikh MH,Lumb PJ,et al. A 6-month randomized trial of thyroxine treatment in women with mild subclinical hypothyroidism. Am J Med. 2002;**112**:348-354.

.

64. Roberts LM,Pattison H,Roalfe A, et al. Is subclinical thyroid dysfunction in the elderly associated with depression or cognitive dysfunction? Ann Intern Med. 2006;**145**:573-581.

65. Gussekloo J,van Exel E,de Craen AJ,Meinders AE,Frölich M,Westendorp RG. Thyroid status, disability and cognitive function, and survival in old age. JAMA. 2004;**292**:2591-2599.

66. Jorde R,Waterloo K, Storhaug H, Nyrnes A,Sundsfjord J,Jenssen TG. Neuropsychological function and symptoms in subjects with subclinical hypothyroidism and the effect of thyroxine treatment. J Clin Endocrinol Metab. 2006;**91**:145-153.

.

67. Bastenie, P.A. Hypothyroidism and coronary heart disease. Acta Cardiol.1982; **37**: 365–373.

68. Danese, M.D. et al. Effect of thyroxine therapy on serum lipoproteins in patients with mild thyroid failure: a quantitative review of the literature. J. Clin. Endocrinol. Metab.2000; **85**: 2993–3001.

69. Bindels, A.J.G.H. et al. The prevalence of subclinical hypothyroidism at different total plasma cholesterol levels in middle-aged men and women: a need for case finding? Clin. Endocrinol. (Oxf) 1999; **50**: 217–220.

70. Tanis, B.C. et al. Effect of thyroid substitution on hypercholesterolaemia in patients with subclinical hypothyroidism: a re-analysis of intervention studies. Clin. Endocrinol. (Oxf) 1996;**44**: 643–649.

71. Meier, C. et al. TSH-controlled L-hyroxine therapy reduces cholesterol levels and clinical symptoms in subclinical hypothyroidism: a double-blind placebo-controlled trial (Basel thyroid study). J. Clin. Endocrinol. Metab.2001; **86**: 4860–4866.

72. Perk,M. and O'Neill, B.J. The effect of thyroid hormone therapy on angiographic coronary artery disease progression. Can. J. Cardiol. 1997;**13**: 273–276.

73. Hak, E. et al. Subclinical hypothyroidism is an independent risk factor for atherosclerosis and myocardial infarction in elderly women: the Rotterdam study. Ann. Intern. Med.2000; **132**: 270–278.

74. Vanderpump, M.P.J. et al. The development of ischemic heart disease in relation to autoimmune thyroid disease in a 20-year follow up of an English community. Thyroid 1996; **6**: 155–160.

75. Biondi B. Cardiovascular effects of mild hypothyroidism. Thyroid. 2007;**17**:625-630.

76. Haddow, J.E. et al.Maternal thyroid deficiency during pregnancy and subsequent neuropsychological development of the child. N. Engl. J. Med. 1999;**341**: 549–555.

77. Morreale de Escobar, G. et al. Is neuropsychological development related to maternal hypothyroidism or maternal hypothyroxinaemia? J. Clin. Endocrinol. Metab. 2000;**85**, 3975–3987.

78. Christ-Crain M,Meier C,Huber PR,Staub J-J,Muller B. Effect of l-thyroxine replacement therapy on surrogate markers of skeletal and cardiac function in subclinical hypothyroidism. Endocrinologist. 2004;**14**:161-166.

79. Haggerty JJ, Jr,Garbutt JC, Evans DL,et al. Subclinical hypothyroidism: a review of neuropsychiatric aspects. Int J Psychiatry Med. 1990;**20**:193-208.

80. Roberts LM, Pattison H,Roalfe A,et al. Is subclinical thyroid dysfunction in the elderly associated with depression or cognitive dysfunction? Ann Intern Med. 2006;**145**:573-581.

81. Biostatistics, Daniel,1995, 4th edition, John Wiley and Sons.

82. Luboshitzky R, Dgani Y, Atar S, et al. Thyroid dysfunction and goiter among immigrants from Ethiopia. Harefuah 1994; 127:289-293.

83. Hollowell JG, Staehling NW, Flanders WD, Hannon WH, Gunter EW, Spencer CA, Braverman LE **.** Serum TSH, T(4), and thyroid antibodies in the United States population (1988

to 1994):National Health and Nutrition Examination Survey (NHANES III).J Clin Endocrinol Metab 2002; **87**:489–499.

84. TunbridgeWM,Evered DC, Hall R, Appleton D, Brewis M, Clark F, Evans JG, Young E, Bird T, Smith PA. The spectrum of thyroid disease in a community: the Whickham survey. Clin Endocrinol (Oxf)1977; **7**:481–493.

85. Parle JV, Franklyn JA, Cross KW, Jones SC, Sheppard MC. Prevalence and follow-up of abnormal thyrotrophin (TSH) concentrations in the elderly in the United Kingdom. Clin Endocrinol (Oxf) 1999;**34**:77–83.

86. Lindeman RD, Schade DS, LaRue A, Romero LJ, Liang HC, Baumgartner RN, Koehler KM, Garry PJ. Subclinical hypothyroidism in a biethnic, urban community. J Am Geriatr Soc1999; **47**: 703–709.

87. Bemben DA, Winn P, Hamm RM, Morgan L, Davis A, Barton E. Thyroid disease in the elderly. Part 1. Prevalence of undiagnosed hypothyroidism. J Fam Pract1994; **38**:577–582.

88. Bagchi N, Brown TR, Parish RF. Thyroid dysfunction in adults over age 55 years. A study in an urban US community. Arch Intern Med 1990; **150**:785–787.

89. Vanderpump, M.P.J. et al. The incidence of thyroid disorders in the community: a twenty-year follow-up of the Whickham survey. Clin. Endocrinol. (Oxf) 1995; **43**: 55–69.

90. Vanderpump MPJ, Tunbridge WMG. The epidemiology of thyroid disease. In: Braverman LE, Utiger RD, eds. The Thyroid. 9th ed. Philadelphia, Pa: Lippincott- Raven Publishers; 1996:474-482.

91. Eggersten R, Petersen K, Lundberg PA, Nystrom E, Lindstedt G. Screening for thyroid disease in a primary care unit with a thyroid stimulating hormone assay with a low detection limit. BMJ. 1988;**297**:1586-1592.

92. Sawin CT, Chopra D, Azizi F, Mannix JE, Bacharach P. The aging thyroid: increased prevalence of serum thyrotropin levels in the elderly. JAMA. 1979;**242**: 247-250.

93. Sawin CT, Castelli WP, Hershman JM, McNamara P, Bacharach P. The aging thyroid: thyroid deficiency in the Framingham study. Arch Intern Med. 1985;**145**: 1386-1388.

94. Okamura K, Ueda K, Sone H, et al. A sensitive thyroid stimulating hormone assay for screening of thyroid functional disorder in elderly Japanese. J Am Geriatr Soc. 1989;**37**:317-322.

95. Livingston EH, Hershman JM, Sawin CT, Yoshikawa TT. Prevalence of thyroid disease and abnormal thyroid tests in older hospitalized and ambulatory persons. J Am Geriatr Soc. 1987;**35**:109-114.

96. Klein, I. & Ojamaa, K.Thyroid hormone and the cardiovascular system. New England Journal of Medicine 2001;**344:** 501–509.

97. Saito, I. & Saruta, T.Hypertension in thyroid disorders. Endocrinology and Metabolism Clinics of North America 1994; **23**: 379– 386.

98. Fletcher, A.K. & Weetman, A.P. Hypertension and hypothyroidism. Journal of Human Hypertension 1998; **12**: 79–82.

99. Danzi, S. & Klein, I. Thyroid hormone and blood pressure regulation. Current Hypertension Reports 2003; **5**: 513–520.

100. Cappola, A.R. & Ladenson, P.W. Hypothyroidism and atherosclerosis. Journal of Clinical Endocrinology and Metabolism. 2003;**88:** 2438– 2444.

101. Ching, G.W.M., Franklyn, J.A., Stallard, T.J., Daykin, J., Sheppard, M.C. & Gammage, M.D. Cardiac hypertrophy as a result of long term thyroxine therapy and thyrotoxicosis. Heart 1996; **75:** 363–368.

102. John P., Alexandra P., Max K., Peter O., Peter J., Peter F. and Valdo M. Subclinical thyroid dysfunction and blood pressure: a community-based study . Clinical Endocrinology 2006 **;65**: 486–491.

103. Cappola, A.R., Fried, L.P., Arnold, A.M., Danese, M.D., Kuller, L.H., Burke, G.L., Tracy, R.P. & Ladenson, P.W. Thyroid status, cardiovascular risk, and mortality in older adults. JAMA 2006; **295**, 1033– 1041.

104. Dõrr, M., Wolff, B., Robinson, D.M., John, U., Lûdemann, J., Meng, W., Felix, S.B. & Võlzke, H.The association of thyroid function with cardiac mass and left ventricular hypertrophy. Journal of Clinical Endocrinology and Metabolism 2005; **90:** 673–677.

105. Lindemann, R.D., Schade, D.S., LaRue, A., Romero, L.J., Liang, H.C., Baumgartner, R.N., Koehler, K.M. & Garry, P.J.Subclinical hypothyroidism in a biethnic, urban community. Journal of the American Geriatrics Society 1999; **47**: 703–709.

106. Kvetny, J., Heldgaard, P.E., Bladbjerg, E.M. & Gram, J. Subclinical hypothyroidism is associated with a low-grade inflammation, increased triglyceride levels and predicts cardiovascular disease in males below 50 years. Clinical Endocrinology 2004; **61**: 232–238.

107. Hak, A.E., Pols, H.A., Visser, T.J., Drexhage, H.A., Hofman, A. & Witteman, J.C. Subclinical hypothyroidism is an independent risk factor for atherosclerosis and myocardial infarction in elderly women: the Rotterdam study. Annals of International Medicine 2000; **132**: 270–278.

108. Tagliaferri M, Berselli ME, Calo G, et al.Subclinical hypothyroidism in obese patients: relation to resting energy expenditure, serum leptin, body composition, and lipid profile.

Obes Res. 2001;**9(3)**:196–201.

109. Brunilda Figueroa, Himilce Ve´lez, Margarita Irizarry-Ramı´rez .Association of thyroid stimulating hormone levels and body mass index over weight Hispanic in Puerto Rico. Ethn Dis. 2008;**18[Suppl 2]:**S2-151–S2-154.

110. Lindeman RD, Romero LJ, Schade DS, Wayne S, Baumgartner RN, Garry PJ.Impact of subclinical hypothyroidism on serum total homocysteine concentrations, the prevalence of coronary heart disease (CHD), and CHD risk factors in the New Mexico Elder Health Survey. Thyroid 2003;**13**:595–600.

111. Hak AE, Pols HA, Visser TJ, Drexhage HA, Hofman A, Witteman JC.Subclinical hypothyroidism is an independent risk factor for atherosclerosis and myocardial infarction in elderly women: the Rotterdam Study. Ann Intern Med 2000; **132**:270–278.

112. Krotkiewski M.Thyroid hormones in the pathogenesis and treatment of obesity. Eur J Pharmacol 2002;**440**:85–98.

113. Knudsen N, Laurberg P, Rasmussen LB, Bulow I, Perrild H, Ovesen L, Jorgensen T.Small differences in thyroid function may be important for body mass index and the occurrence of obesity in the population. J Clin Endocrinol Metab 2005;**90**:4019–4024.

114. Iacobellis G, Ribaudo MC, Zappaterreno A, Iannucci CV, Leonetti F. Relationship of thyroid function with body mass index, leptin, insulin sensitivity and adiponectin in euthyroid obese women. Clin Endocrinol (Oxf) 2005;**62**:487–491.

115. Nyrnes A, Jorde R, Sundsfjord J. Serum TSH is positively associated with BMI. Int J Obes (Lond) 2006;**30**:100–105.

116. Manji N, Boelaert K, Sheppard MC, Holder RL, Gough SC, Franklyn JA.Lack of association between serum TSH or free T4 and body mass index in euthyroid subjects. Clin Endocrinol (Oxf) 2006;**64**:125–128.

117. Rosenthal MJ, Hunt WC, Garry PJ, Goodwin JS.Thyroid failure in the elderly. Microsomal antibodies as discriminant for therapy. JAMA 1987; **258**:209–213.

118. Diez JJ, Iglesias P. Spontaneous subclinical hypothyroidism in patients older than 55 years: an analysis of natural course and risk factors for the development of overt thyroid failure. J Clin Endocrinol Metab 2004; **89**:4890–4897.

119. TunbridgeWMG,Brewis M, French JM, Appleton D, Bird T, Clark F, Evered DC, Grimley Evans J, Hall R, Smith P, Stephenson J, Young E.Natural history of autoimmune thyroiditis. BMJ 1981; **282**:258–262.

120. Gordin A, Lamberg BA.Spontaneous hypothyroidism in symptomless autoimmune thyroiditis. A long-term follow-up study. Clin Endocrinol (Oxf) 1981;**15**:537–543.

121. Parle JV, Franklyn JA, Cross KW, Jones SC, Sheppard MC.Prevalence and follow-up of abnormal thyrotrophin (TSH) concentrations in the elderly in the United Kingdom. Clin Endocrinol (Oxf) 1991;**34**:77–83.

122. Huber G, Staub JJ, Meier C, Mitrache C, Guglielmetti M, Huber P, Braverman LE. Prospective study of the spontaneous course of subclinical hypothyroidism: prognostic value of thyrotropin, thyroid reserve, and thyroid antibodies. J Clin Endocrinol Metab 2002; **87**:3221–3226.

123. Dı´ez JJ, Iglesias P. Spontaneous subclinical hypothyroidism in patients older than 55 years: an analysis of natural course and risk factors for the development of overt thyroid failure. J Clin Endocrinol Metab 2004;**89**:4890–4897.

124. Dı´ez JJ, Iglesias P,Kenneth DB. Spontaneous normalization of thyrotropin concentration in patients with subclinical hypothyroidism. J Clin Endocrinol Metab 2005; **90**:4124–4127.

125. Gussekloo J, van Exel E, de Craen AJ, Meinders AE, Frolich M, WestendorpRG.Thyroid status, disability and cognitive function, and survival in old age. JAMA 2004; **292**:2591–2599.

126. Kutty KM, Bryant DG, Farid NR. Serum lipids in hypothyroidism: a re-evaluation.
J Clin Endocrinol Metab. 1978;**46**:55-56.

.

127. Fatourechi V, Klee GG, Grebe SK, Bahn RS, Brennan MD, Hay ID, McIver B, Morris JC III.Effects of reducing the upper limit of normal TSH values. JAMA 2003;**290**:3195–3196.

128. Canaris GJ, Steiner JF, Ridgway EC.Do traditional symptoms of hypothyroidism correlate with biochemical disease? J Gen Intern Med 1997;**12**:544–550.

129. Zulewski H, Muller B, Exer P, Miserez AR, Staub JJ.Estimation of tissue hypothyroidism by a new clinical score: evaluation of patients with various grades of hypothyroidism and controls. J Clin Endocrinol Metab 1997;**82**:771–776.

Annex

Annex 1: Consent from (English version)

Name _____ Sex _____ Age _____

Phone _____ (If available)

NMC serial no _____ Hospital/Clinic _____

Doctor's Name _____ Card No. _____

The undersigned, being tested for the levels of the hormone TT3, TT4, FT4, TSH in my blood, volunteered for the blood sample taken from me to be used for a research on thyroid function assessment. I understand that the study is related to the original purpose of the test I came to Nuclear medicine department for.

It is clear to me that the information provided by my doctor will be kept confidential and that my name will not appear on any publication. I have the right to withdraw from the study anytime and for any reason

Name and signature

_____

Date

_____

Annex 2: Consent from (Amharic version)

ስም _____ጾ _____ ዕድሜ_____

የስልክ ቁጥር (ካለ) _____

የላቦራቶሪ ተራ ቁጥር_____ ሆስፒ ል/ክሊኒክ_____

የዶክተር ስም_____ የካርድ ቁጥር _____

ኔ ስሜ ከላይ የተጠቀሰው በአዲስ አበባ ጥቁር አንበሳ ስፔሻላይዝድ ሆስፒ ል በመገኘው በኒውክሌር ሜዲስን ማዕከል ውስጥ በደሜ ውስጥ ያለውን FT4,TT3,TT4 **ና** TSH **ንጥረ ነገር መጠን ለማወቅ ምርመራ ለማድረግ መጥቻለሁ፡፡ ከዚሁ ምርመራ ጋር ለተያያዘ** FT4,TT3,TT4 **ና** TSH **መጠን ምርምር በፈቃደኝነት ደሜን ሰጥቻለሁ፡፡**

የምሰጠው መረጃ መስጢራዊነት ንደሚጠበቅና ስሜ በማንኛውም የምርምር ጽሁፍ ላይ ንደማይሰፋር ተረድቻለሁ፡፡ በማንኛውም ጊዜ ና ምክንያት ራሴን ከምርምሩ የማግለል መብት ንዳለኝ አውቃለሁ፡፡

ስም ና ፊርማ

_____

ቀን

_____

Annex 3. Data collection form

1. Personal identification
    a) Full name of subject _____
    b) Subject identification number _____
    c) Card No. _____
2. Demographic details
    a) Date of birth _____
    b) Gender 1. Male ☐ 2. Female ☐
    c) Address: City/region _____
    Woreda: _____ Kebele: _____ House number: _____
3. Group Study ☐ Control ☐
4. Date of diagnosis:_____
5. Duration (years) of SCH: _____
6. Physical examination

    Symptoms

    __Tiredness, weakness

    __Dry skin

    __Feeling cold

    __Hair loss

    __Difficulty concentrating and poor memory

    __Constipation

    __Weight gain with poor appetite

    __Dyspnea

    __Hoarse voice

    __Menorrhagia (later __oligomenorrhea or amenorrhea)

    __Paresthesia Impaired hearing

7. Blood pressure
    - Systolic /diastolic blood pressure (mmHg)_____
8. Chemistry

    TSH(mU/L) _____

    TT4(nmoles/L) _____

    FT4(pmoles/L)_____

    TT3(nmoles/L)_____
9. Current SCH therapy _____
10. Other medical therapies _____
11. Family history of Throid disease _____
12. Family history of hypertension _____