Evaluation of Serum Selenium Levels in Hypo and Hyperthyroid Patients
Presenting to Khartoum Teaching Hospital
(September 2010 - February 2011)

By
Dr. Huda Abdel Malik Mohammed Abdel Rahman
M.B. B.S. (U of K) 2003
A thesis submitted in partial fulfillment for the requirements of the degree of
Clinical MD (Sudan) in Pathology, 2011

Supervisor
Dr. Abd Elhafiz Khattab
M.B. B.S., DCP, FRCPath
Associate Professor of Pathology
U of K

2011
Dedication

To all my family members

Who so tirelessly,

Cheered me on through the academic path.....
Acknowledgment

I would like to thank my supervisor Dr. A. H. Khattab, for being so thoughtful, patient and understanding.

I would also like to thank all the technical staff in clinical chemistry department at Khartoum teaching Hospital laboratory department, for their unlimited support.

Not to forget all patients whom I included in this study they were all cooperative and deserve a better health.
LIST OF ABBREVIATIONS

T4 : Thyroxine
T3 : Triiodothyronine
MIT : Monoiodotyrosine
DIT : Diiodotyrosine
TSH : Thyroid stimulating hormone
TRH : Thyrotrophin releasing hormone
TBG : thyroxine binding globulin
AntiTPO : Antiithyroid peroxidase
AntTg : Antithyroglobulin
TSI : Thyroid stimulating immunoglobulin
GD : Graves disease
ATD : Autoimmune thyroid disease
Gpx : Glutathione peroxidase
TxRs : Thioredoxin
D : Deiodinase
PPb : par per bilion
SD : standard deviation
ABSTRACT

Background:
Selenium deficiency has been implicated in the pathogenesis of many thyroid diseases, namely hypothyroidism, autoimmune thyroid disease (ATD), endemic cretinism and thyroid cancer.

Objectives:
The study is conducted to evaluate serum selenium status in hypo and hyperthyroid patients and to compare the pre and post treatment levels.

Methods:
This is a prospective hospital based case control study conducted in Khartoum Teaching Hospital (KTH) (September 2010 to February 2011). The studied subjects were divided into 3 groups. The 1st two groups were adult male and female patients diagnosed clinically and biochemically as having hypo or hyperthyroidism. The 3rd group was age and sex matched normal controls. Data about the disease were collected from patients through a questionnaire. Sera were tested for selenium by atomic absorption spectrophotometer AA6800 (Shimadzu), and for thyroid hormones (T3 and T4) and TSH using the principle of chemiluminescence by Elecsys 2010 (Roche).
Results:

The studied subjects were 60 (40 cases and 20 controls). The mean serum selenium level in the hypothyroid group of patients was 63.82 ppb (±26.2 SD), significantly low compared to controls (P value<0.05).

It was found that the mean serum selenium level in the newly diagnosed hypothyroid cases was 30.54ppb (±24.68SD), significantly lower compared to those on thyroxine replacement (74.71ppb ±17.57SD)( P value>0.05).

The mean serum TSH of the hypothyroid patients (20.06µu/ml) and of the hyperthyroid patients (2.36µu/ml) was inversely related to serum selenium. The relation was statistically significant (P value <0.05).

The mean serum T4 of the hypothyroid group (5.9µg/dl) was inversely related to serum selenium. The relation was statistically significant (P value<0.05).

Conclusion:

This is the first study conducted in Sudan to explore selenium status in hypothyroidism. Significantly low serum selenium levels in hypothyroid patients confirm the possible implication of selenium deficiency in the causation of hypothyroidism as reported in the literature.

Controlling hypothyroidism with thyroxine replacement may have a possible role in improving selenium deficiency; still this finding needs further exploration through longitudinal studies.
Inverse relationships were found between: (serum selenium and serum TSH) and (serum selenium and serum T4) confirming the role of selenium in thyroid hormone metabolism.

Further studies that determine the prevalence of autoimmune thyroid disease in Sudan and to specifically evaluate selenium status in autoimmune thyroid disease are more valuable and thus needed.

Protocols for introducing selenomethionine supplements to patients with autoimmune thyroid disease and monitoring the response by following the level of thyroid antibodies are needed.
مقدمة:
يرتبط نقص السيلينيوم بكثير من أمراض الغدة الدرقية وهي خمول الغدة الدرقية، أمراض المناعة الذاتية، سرطان الغدة الدرقية والتقدم.

أهداف البحث:
يهدف البحث إلى دراسة معدل السيلينيوم بالدم في مرضى الغدة الدرقية الخاملة والنشطة.
ودراسة تأثير علاج مرض الغدة على مستوى السيلينيوم في الدم.

طريقة البحث:
يعد هذا البحث بحثًا متبناً حالة ضابط، تم إجرااؤه بمستشفي الخرطوم التعليمي في الفترة من فبراير 2010 حتى فبراير 2011.

ينقسم المبحوثين إلى ثلاثة مجموعات:
المجموعة الأولى والثانية تتضمن الذكور والإناث البالغين من مرضى الغدة الدرقية الخاملة والنشطة على التوالي.
المجموعة الثالثة تمثل الاصحاب البالغين من الذكور والإناث.

تم اجراء التالى للمبحوثين:
• اخذ المعلومات اللازمة عن المرضى ونوع وفترة العلاج عن طريق الاستبيان.
• اخذ عينات الدم ومن ثم استخلاص السيرورة لعمل الفحوصات التالية:
  1. فحص هرمونات الغدة الدرقية T3، T4 بالإضافة لهرمون الغدة النخامية TSH.
  2. فحص معدل السيلينيوم.

نتائج البحث:
• يتضمن البحث 60 مبحوثاً (40 مريض و20 من الأصحاء).
وجد أن متوسط مستوى السيلينيوم في مرضى الغدة الدرقية الخاملة هو 63.82 ppb (الانحراف المعياري 26.2) ويعتبر هذا انخفاضاً ملحوظاً مقارنة بالضوابط.
• متوسط مستوى السيلينيوم في مرضى الغدة الدرقية الخاملة الذين تلقوا علاج الغدة هو 74.71 ppb (الانحراف المعياري 17.57) وهذا يعد مرتفعاً ارتفاعاً ملحوظاً مقارنة بمتوسط مستوى السيلينيوم في المرضى الذين لم يتلقوا علاج الغدة (30.45 ppb) (الانحراف المعياري 24.36).
• وجد ان هناك علاقة عكسية ملحوظة بين متوسط مستوى السيلنيوم في الدم وهرموني TSH & T4

الاستنتاج:

تعد هذه أول دراسة في السودان لبحث مستوى السيلنيوم في مرضى الغدة الدرقية. بينت الدراسة انخفاض مستوى السيلنيوم في الدم في مرضى الغدة الدرقية الخاملة ووجدت علاقة عكسية بين مستوى السيلنيوم في الدم وهرموني T4&TSH مؤكدة دور السيلنيوم في ايض هرمونات الغدة الدرقية.
# LIST OF FIGURES

<table>
<thead>
<tr>
<th>Fig.</th>
<th>Description</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fig. 1</td>
<td>Gender distribution of the studied subjects</td>
<td>38</td>
</tr>
<tr>
<td>Fig. 2</td>
<td>Age distribution of the studied subjects</td>
<td>39</td>
</tr>
<tr>
<td>Fig. 3</td>
<td>Serum selenium level in the euthyroid group</td>
<td>40</td>
</tr>
<tr>
<td>Fig. 4</td>
<td>Serum selenium levels in hypothyroid patients</td>
<td>41</td>
</tr>
<tr>
<td>Fig. 5</td>
<td>Serum selenium levels in hyperthyroid patients</td>
<td>42</td>
</tr>
</tbody>
</table>
### LIST OF TABLES

| Table .1 | Correlation between mean serum selenium levels in the hypothyroid group and controls | 43 |
| Table .2 | Correlations between mean serum selenium levels in the hyperthyroid group and controls | 44 |
| Table .3 | Correlations between mean serum selenium levels in the hypothyroid patients pre and post treatment with thyroxine | 45 |
| Table .4 | Correlations between mean selenium level and TSH in thyroid disease | 46 |
| Table .5 | Relations between thyroid hormones T3 and T4 and serum selenium in thyroid disease | 47 |
1. INTRODUCTION AND LITERATURE REVIEW

1.1. Introduction

1.1.1. Selenium’s protective powers for the thyroid:

In March 2003 French researchers looked at the connection between selenium intake, size of the thyroid and its structure. What they found was that in women lower selenium levels were associated with increased thyroid volume (goiter) and finally they concluded that selenium may protect against both goiter and autoimmune thyroid disease. Patients with all forms of autoimmune thyroid disease are known to have low selenium levels. Whether low selenium contributes to the condition or is caused by thyroid dysfunction is uncertain. However, recent studies show that patients with Graves' disease using selenium and the antioxidant vitamins beta carotene and vitamin C along with the anti-thyroid drug methimazole showed a better response than patients using the anti-thyroid drug methimazole alone. Patients improving from or in remission from autoimmune thyroid disease have higher selenium levels compared to their initial levels at the time of diagnosis\(^{(1)}\).
1.2. Literature review

1.2.1. The thyroid

1.2.1.1. Structure and development

The thyroid is a brownish-red and highly vascular gland located anteriorly in the lower neck, extending from the level of the fifth cervical vertebra down to the first thoracic. The gland varies from an H to a U shape and is formed by 2 elongated lateral lobes with superior and inferior poles connected by a median isthmus (with an average height of 12-15 mm) overlying the second to fourth tracheal ring \(^{(2)}\).

Under the middle layer of deep cervical fascia, the thyroid has an inner true capsule, which is thin and adheres closely to the gland. Extensions of this capsule within the substance of the gland form numerous septae, which divide it into lobes and lobules. The lobules are composed of follicles; the structural units of the gland, consisting of a layer of simple epithelium enclosing a colloid-filled cavity. This colloid (pink on hematoxylin and eosin [H&E] stain) contains an iodinated glycoprotein, iodothyroglobulin, a precursor of thyroid hormones. Follicles vary in size, depending upon the degree of distention, and they are surrounded by dense plexuses of fenestrated capillaries, lymphatic vessels, and sympathetic nerves \(^{(2)}\).

1.2.1.2. Function of the thyroid:

The thyroid gland secretes three hormones: thyroxin (T4) and triiodothyronine (T3), both of which are iodinated derivatives of tyrosine, and calcitonin, a polypeptide hormone. T4 and T3 are produced by the follicular cells (principle cells) but calcitonin is produced by the Para follicular cells (c cells or clear cells) and has a minor role in calcium homeostasis \(^{(3)}\).
1.2.1.3. Thyroid hormones

There are two biologically active thyroid hormones: thyroxin (T4) and 3, 5, 3'-triiodothyronine (T3). They are composed of a phenyl ring attached via an ether linkage to a tyrosine molecule. Both have two iodine atoms on their tyrosine (inner) ring. They differ in that T4 has two iodine atoms on its phenyl (outer) ring, whereas T3 has only one. The compound formed if an iodine atom is removed from the inner ring of T4 is 3, 3', 5'-triiodothyronine (reverse T3, rT3), which has no biological activity but may be found in significant concentrations in the sera of patients with the sick euthyroid syndrome (4).

Thyroid hormone synthesis involve a number of specific enzyme catalyzed reactions, beginning with the uptake of iodide by the gland and culminating in the iodination of tyrosine residues in the protein thyroglobulin (3).

1.2.1.3.1. Thyroid hormones synthesis

Iodine intake: The normal European daily dietary intake of iodine is about 150 µg, of which approximately 125 µg is taken up by the thyroid gland and used for hormone synthesis.

Iodine (as the iodide, I⁻) is relatively abundant in seawater, and seafood is a rich dietary source. Fruit and vegetables also contain significant concentrations of iodine, although the amount depends on the soil and growing region. Areas of iodine deficiency tend to be inland, at high altitude and isolated and daily iodine intake may be as low as 25 µg.

The synthesis in the thyroid gland takes place in the following way:

A. Dietary iodine is reduced to iodide (I⁻) in the gut, absorbed and circulates as iodide (5).
B. Iodide trapping:

The active uptake of iodide ($\Gamma$) by the follicular cells involves an energy-requiring (ATPase-dependent) transport mechanism which allows $\Gamma$ to be taken up from capillary blood against both a concentration and an electrical gradient in exchange for $\text{Na}^+$. This enables the thyroid gland to concentrate iodide 30–50 times that of the circulating concentration. Other ions such as bromide, chlorate or pertechnetate (though not fluoride) may also compete with $\Gamma$ for this uptake process and this has important clinical uses. For example perchlorate is employed clinically as a competitive inhibitor and is useful when studying the kinetics of thyroid hormone secretion, it is also used as a prophylactic measure to inhibit uptake of radioactive iodide in cases of contamination or ingestion. The pertechnetate is used in radioisotope scanning of the thyroid. Thiocyanate and selenothiocyanate ($\text{SeCN}^-$) can also inhibit the thyroidal iodide pump by competitive inhibition. On a normal daily iodide intake the thyroid gland clears approximately 20 ml of plasma iodide per minute\(^{(5)}\).

In the follicular cell, iodide passes down its electrochemical gradient through the apical membrane and into the follicular colloid. Iodide is instantly oxidized – with hydrogen peroxide as oxidant - by a thyroid peroxidase to atomic or molecular iodine at the colloid surface of the apical membrane. Thiouracil and sulfonamides block this peroxidase.

C. The rough endoplasmic reticulum synthesizes a large storage molecule called thyroglobulin. This compound is build up by a long peptide chain with tyrosine units and a carbohydrate unit completed by the Golgi apparatus. Iodide-free thyroglobulin is transported in vesicles to the apical membrane, where they fuse with the membrane and finally release thyroglobulin at the apical membrane.
D. At the apical membrane the oxidized iodide is attached to the tyrosine units (L-tyrosine) in thyroglobulin at one or two positions, forming the hormone precursors mono-iodotyrosine (MIT) and di-iodotyrosine (DIT), respectively. This and the following reactions are dependent on thyroid peroxidase in the presence of hydrogen peroxide – both located at the apical membrane. As MIT couples to DIT it produces tri-iodothyronine (3, 5, 3’-T3), whereas two DIT molecules form tetra-iodothyronine (T4), or thyroxine. These two molecules are the two thyroid hormones. Small amounts of the inactive reverse T3 (3, 3’, 5’-T3) is also synthesized (5).

E. Each thyroglobulin molecule contains up to 4 residues of T4 and zero to one T3. Thyroglobulin is retrieved back into the follicular cell as colloid droplets by pinocytosis. Pseudo pods engulf a pocket of colloid. These colloid droplets pass towards the basal membrane and fuse with lysosomes forming phagolysosomes.

F. Lysosomal exopeptidases break the binding between thyroglobulin and T4 (or T3). Large quantities of T4 are released to the capillary blood. Only minor quantities of T3 are secreted from the thyroid gland.

G. The proteolysis of thyroglobulin also releases MIT and DIT. These molecules are deiodinated by the enzyme deiodinase, whereby iodide can be reused into T4 or T3. Normally, only few intact thyroglobulin molecules leave the follicular cells.

H. TSH stimulates almost all processes involved in thyroid hormone synthesis and secretion (4).

1.2.1.3.2. Thyroid hormones in the circulation

The normal plasma concentrations of T4 and T3 are 60-150 nmol/l and 1.0-2.9 nmol/l respectively. Both hormones are extensively protein bound: some 99.98% of T4
and 99.66% of T3 are bound principally to a specific thyroxine binding globulin (TBG) and to a lesser extent, to prealbumin and albumin. TBG is approximately one third saturated at normal concentrations of thyroid hormones. The major product of the thyroid gland is T4. Ten times less T3 is produced. Most T3 (approximately 80%) being derived from T4 by deiodination in the peripheral tissues, so that T4 is essentially a prohormone. Only the free (unbound) hormones are physiologically active. Although the total T4 concentration is 50 times that of T3, the different extents to which these hormones are bound to protein mean that the free T4 concentration is only 2 to 3 times that of free T3\(^3\).

Changes in the TBG concentration affect the total hormones concentration (not the free):

**Increased TBG (increases total T4 and T3 measurements)**

- Pregnancy
- Newborn state
- Chronic Active Hepatitis
- Biliary Cirrhosis
- Infection
- Acute intermittent porphyria
- Familial hyperthyroxinemia
- Drugs
- Oral Contraceptives
- Estrogens
- Tamoxifen
- Methadone
- Heroin
• Perphenazine

**Decreased TBG (reduces total T4 and T3 measurements)**

• Chronic Liver Disease
• Obstructive Jaundice or Cirrhosis
• Severe systemic illness
• Active Acromegaly
• Nephrotic Syndrome
• Surgical Stress
• Medications
• Androgens
• Salicylates
• L-asparaginase
• Large doses of glucocorticoids (Prednisone) \(^6\).

**Kinetics:** studies on the kinetics of release and metabolism of iodothyronine show that T3 has a shorter half life (about 1-3 days) than T4 (about 5-7 days), and reverse T3 is cleared very rapidly, with a half life of about 5 hours. However, T3 is 10 times more potent than T4 \(^5\).

**1.2.1.3.3. Thyroid hormones Peripheral metabolism:**

Although some T3 is produced in the thyroid, approximately 80-85 percent is generated outside the thyroid, primarily by conversion of T4 in the liver and kidneys. The pituitary and nervous system are capable of converting T4 to T3, so are not reliant on T3 produced in the liver or kidney. Within the liver and kidney, the enzyme responsible for peripheral production of T3 is a selenium-dependent enzyme called 5'-deiodinase. This enzyme removes iodine from T4's outer phenolic ring \(^7\). Peripheral deiodination can also result in the formation of reverse T3 (rT3) and account for 95
percent of all rT3 produced (small amounts are produced within the thyroid. The enzyme responsible for this conversion is 5-deiodinase and is not believed to be dependent on selenium for its activity. This enzyme acts on the nonphenolic ring of T4 (the inner tyrosyl ring) to produce the hormonally inactive rT3. Under normal conditions, 45-50 percent of the daily production of T4 is transformed into rT3. Substantial individual variation in these percentages can be found secondary to a range of environmental, lifestyle, and physiological influences. Production also increases in cases of starvation & in many non-thyroidal illnesses. Although an adequate understanding of the metabolic role of rT3 is somewhat limited, it is thought to be devoid of hormonal activity and to act as the major competitive inhibitor of T3 activity at the cellular level. Experimental data also suggests rT3 has inhibitory activity on 5'-deiodinase. Suggesting it might also directly interfere with the generation of T3 from T4\(^7\).

Further degradation of rT3 and T3 results in the formation of several distinct diiodothyroxines: 3,5-diiodothyronine(3,5-T2), 3,3'-diiodothyronine(3,3'-T2), and 3', 5'-diiodothyronine (3',5'-T2). The metabolic role of the T2 isomers is poorly understood and the absolute contribution of these hormones to physiological function in humans is unclear.

A second pathway of thyroid hormone metabolism involves the conjugation of the phenolic hydroxyl group of the outer phenolic ring with sulfate or glucuronic acid. These conjugation reactions occur primarily in the liver, and to a lesser degree in the kidney, and result in biotransformation of T4 and T3. The resultant metabolites are primed for elimination and are considered relatively inactive. It is thought that partially deiodinated thyroid hormone metabolites are preferred substrates for these conjugation reactions. Thyroid hormones can also undergo deamination and
decarboxylase reactions in the liver, resulting in the formation of so-called acetic acid analogues. These reactions occur at the alanine side-chain of the inner tyrosyl ring. Although these analogues are thought to be metabolically active, little is known about the quantities produced or their contribution to hormone activity in animals or humans \(^7\).

1.2.1.3.4. Mechanism of action

Thyroid hormones have many important biological effects. A major function is their control of the basal metabolic rate and calorigenesis through increased oxygen consumption in tissue via the effects of thyroid hormone on membrane transport (Cycling of Na\(^+\)/K\(^+\)-ATPase with increased synthesis and consumption of adenosine triphosphate) and enhanced mitochondrial metabolism (stimulation of mitochondrial respiration and oxidative phosphorylation). Thyroid hormones are known to stimulate neural development and normal growth, promote sexual maturation, stimulate adrenergic activity with increased heart rate and myocardial contractility, stimulate protein synthesis and carbohydrate metabolism, increase the synthesis and degradation of cholesterol and triglycerides, increase the requirement for vitamins, increase the calcium and phosphorus metabolism, and enhance the sensitivity of adrenergic receptors to catecholamines. These effects are typically magnified in patients with either an overactive thyroid gland, such as in hyperthyroidism or reduced in patients with a sluggish thyroid such as in hypothyroidism \(^8\).

1.2.1.3.5. Regulation of the thyroid gland

Regulation of thyroid hormones occur from regions external to the gland (extra thyroidal pathway) and from within the thyroid itself (auto regulation).
1.2.1.3.5.1. Extra thyroidal pathway (hypothalamic-pituitary-thyroid axis)

The activity of the thyroid gland is controlled by thyroid stimulating hormone (TSH) from the anterior pituitary, the secretion of this is controlled by thyrotrophin releasing hormone (TRH) from the hypothalamus. TSH stimulates the secretion and synthesis of thyroid hormones and these (mainly T3) suppress TSH secretion and modulate the response of the pituitary to TRH (-ve feed back mechanism). TSH production is also blunted by somatostatin, rising levels of glucocorticoids, sex hormones and excessively high blood iodine concentration. 

1.2.1.3.5.2. The effect of dietary iodide in hormone synthesis (autoregulation)

The amount of iodine incorporated into thyroglobulin is directly related to the concentration of iodide reaching the thyroid gland from the circulation. Thus when dietary iodide is limited or deficient there will be little iodine incorporated into thyroglobulin, this will yield mainly MIT and few DIT residues in thyroglobulin and so, T3 is secreted in preference for T4, TSH secretion will increase and induces thyroid enlargement (endemic goiter).

Under conditions of iodine sufficiency (dietary iodine intake >50 µg/day), a lot of DIT will be produced and thyroglobulin will contain more T4 than T3.

Iodine excess: large doses of iodine will initially inhibit the synthesis and release of thyroid hormones, this paradoxical effect arises from inhibition of two intrathyroidal reactions; the adenylate cyclase response to the TSH & the organification of iodine (the Wolff-Chaikoff effect).
1.2.1.4. Disorders of the thyroid

1.2.1.4.1. Hypothyroidism: Hypothyroidism is defined as deficiency in thyroid hormone secretion and action; it is a common disorder that occurs in both mild and severe forms in 2% to 15% of the population \(^{(8)}\).

Women are afflicted more than men, and both sexes are affected more frequently with increasing age. Clinical symptoms vary from the obvious and easy to recognize lethargy, fatigue and cold intolerance to more subtle, subclinical disease with generalized symptoms that escape detection \(^{(9)}\).

Myxedema is a severe form of hypothyroidism in which there is accumulation of mucopolysaccharides in the skin and other tissues, leading to thickening of facial features and doughy induration of the skin \(^{(8)}\). The term cretinism, used to define the severe impairment of physical and neurological development resulting from iodine deficiency during fetal and post-natal development, derived from ‘cretin’, a term first used in the Swiss Alps. The word cretin entered the vernacular as a term of abuse indicating severe mental retardation. In 1994, nearly 30% of the world population was at risk of iodine deficiency. The commitment from the World Health Organization in 1990 to eliminate iodine deficiency disorders by the year 2000 (with a policy of iodide supplementation of salt) had reduced the population at risk to less than 15% by 1997 \(^{(5)}\). Symptoms of hypothyroidism include cold intolerance, weight gain, menstrual disturbances and impaired fertility, par aesthesia and fatigue. Physical signs include pallor, dull coarse facial features, hoarseness, bradycardia, and hyporeflexia \(^{(9)}\).

Metabolic abnormalities associated with hypothyroidism include anemia, dilutional hyponatremia, hyperlipidemia, and reversible increase in creatinine.

Worldwide, iodine deficiency remains the foremost cause of hypothyroidism. In the United States and other areas of adequate iodine intake, autoimmune thyroid disease
is most common. The prevalence of antibodies is higher in women, and increases with age\textsuperscript{(9)}.

1.2.1.4.1.1. Primary hypothyroidism

Autoimmune: The most frequent cause of acquired hypothyroidism is autoimmune thyroiditis (Hashimoto thyroiditis), it is a histologic diagnosis first described by Hakaru Hashimoto, a Japanese surgeon working in Berlin. The incidence of Hashimoto's thyroiditis seen in practice is unknown but is roughly equal to that of Graves' disease (on the order of 0.3 - 1.5 cases per 1,000 population per year). The disease is 15 - 20 times as frequent in women as in men. It occurs especially during the decades from 30 to 50, but may be seen in any age group, including children. The body recognizes the thyroid antigens as foreign, and a chronic immune reaction ensues, resulting in lymphocytic infiltration of the gland and progressive destruction of functional thyroid tissue. Up to 95\% of affected individuals have circulating antibodies to thyroid tissue. Antimicrosomal or antithyroid peroxidase (anti-TPO) antibodies are found more commonly than antithyroglobulin antibodies(95\% vs 60\%). These antibodies may not be present early in the disease process and usually disappear over time\textsuperscript{(8)}. Hashimoto's thyroiditis is reported to occur in two varieties, an atrophic variety, perhaps associated with HLA-DR3 gene inheritance, and a goitrous form associated with HLA-DR5. Concerning susceptibility genes for Hashimoto's thyroiditis, non-MHC class II genes have been recently investigated. Regarding environmental factors, high iodine intake, selenium deficiency, pollutants such as tobacco smoke, infectious diseases such as chronic hepatitis C, and certain drugs are implicated in the development of autoimmune thyroiditis. The disease usually begins as gradual enlargement of the gland with gradual development of hypothyroidism (subclinical to overt hypothyroidism and may progress to myxoedema).
Occasionally the course may be marked by alternating hyper & hypothyroidism. Pregnant ladies with Hashimoto's may develop post partum painless thyrotoxicosis \(^{(10)}\).

Hashimoto's thyroiditis and hypothyroidism are associated with Addison's disease, diabetes mellitus, hypogonadism, hypopara-thyroidism, and pernicious anemia. Such combinations are described as the polyglandular failure syndrome \(^{(10)}\).

Other causes of hypothyroidism include postpartum thyroiditis, subacute granulomatous thyroiditis, drugs like amiodarone and lithium, post thyroidectomy, post radioactive iodine, following external neck irradiation, rare inborn errors of thyroid hormones metabolism \(^{(10)}\).

1.2.1.4.1.2. Central hypothyroidism

Central hypothyroidism (secondary or tertiary) results when the hypothalamic-pituitary axis is damaged. Various causes should be considered:

- Pituitary adenoma
- Tumors impinging on the hypothalamus
- History of brain irradiation
- Drugs (eg, dopamine, lithium)
- Sheehan syndrome
- Genetic disorders

1.2.1.4.2. Laboratory Studies

Third-generation TSH assays are readily available and are generally the most sensitive screening tool for primary hypothyroidism. The generally accepted reference range for normal serum TSH is 0.40-4.2 mIU/L. In NHANES III (1988-1994), of 17,353 people evaluated, 80.8% had a serum TSH below 2.5 mIU/L; TSH concentrations rose with advancing age. TSH levels peak in the evening and are
lowest in the afternoon, with marked variations due to physiologic conditions such as illness, psychiatric disorders, and low energy intake. If TSH levels are above the reference range, the next step would be to measure total T4 with a measure of binding proteins. The levels of these binding proteins can vary by hormonal status, inheritance, and in various disease states. Hence, free T4 assays are becoming popular as they measure unbound (i.e., free hormone). However, free T4 assays can be unreliable in the setting of severe illness. No currently available kit actually measures unbound T4 directly. Free thyroid hormone levels can be estimated by calculating the percentage of available thyroid hormone-binding sites (T3 resin uptake) or by measuring the concentration of TBG. A free thyroxine index (FTI) serves as a surrogate of the free hormone level. The FTI is the product of the T3 resin uptake and total T4 levels. Patients with primary hypothyroidism have elevated TSH levels and decreased free hormone levels. Patients with elevated TSH levels but normal free hormone levels or estimates are considered to have mild or subclinical hypothyroidism. Increased conversion of T4 to T3 occurs this maintains T3 levels In early hypothyroidism. Evaluation of the presence of thyroid autoantibodies (antimicrosomal or anti-TPO antibodies) and antithyroglobulin (anti-Tg) may be helpful in determining the etiology of hypothyroidism or in predicting future hypothyroidism. In addition, anti-TPO antibodies have been associated with a higher risk of infertility and miscarriage. In patients with hypothalamic or pituitary dysfunction, TSH levels do not increase in appropriate relation to the low free T4 levels. The absolute levels may be in the normal or even slightly elevated range but inappropriately low for the severity of the hypothyroid state. Hence, when secondary or tertiary hypothyroidism is suspected, a serum TSH measurement alone is inadequate; a free T4 should be measured. The TRH stimulation test is rarely needed
currently because of improved TSH assays when performed; there will be no TSH response to exogenous TRH in cases of destructive lesions of the pituitary. In patients with hypothalamic disease, the peak TSH response to TRH may be normal, but is generally delayed until 45 or 60 minutes after the TRH administration rather than after usual time of 20 to 30 minutes.

1.2.1.4.2.1. Histological findings

Autoimmune thyroiditis causes a decrease in intrathyroidal iodine stores, an increased iodine turnover, and defective organification. Chronic inflammation of the gland causes progressive destruction of the functional tissue with widespread infiltration by lymphocytes and plasma cells with epithelial cell abnormalities. In time, dense fibrosis and atrophic thyroid follicles replace the initial lymphocytic hyperplasia and vacuoles. Functional tissue destruction and infiltration may also be caused by previous administration of radioiodine, surgical fibrosis, metastasis, lymphomatous changes, sarcoidosis, tuberculosis, amyloidosis, cystinosis, thalassemia, and Riedel thyroiditis.

1.2.1.4.3. Treatment

The treatment goals for hypothyroidism are the reversal of clinical progression and the corrections of metabolic derangements, hypothyroidism can be adequately treated with a constant daily dose of levothyroxine (LT4). Clinical benefits begin in 3-five days and level off after 4-6 weeks. In elderly patients or those with known ischemic heart disease, treatment should begin with one fourth to one half the expected doses. Achieving a TSH level within the reference range may be slowed because of delay of hypothalamic-pituitary axis readaptation and may take several months. After dose stabilization, patients can be monitored with annual clinical evaluations and TSH monitoring. Patients should be monitored for symptoms and signs of overtreatment,
which include tachycardia, palpitations, nervousness, tiredness, headache, increased excitability, sleeplessness, tremors, and possible angina\(^9\).

1.2.1.4.1.3. Subclinical hypothyroidism

Significant controversy persists regarding the treatment of patients with mild hypothyroidism. Some have argued that treatment of these patients improves symptoms, prevents progression to overt hypothyroidism, and may have cardioprotective benefits. The American Association of Clinical Endocrinologists (AACE) guidelines state that treatment is indicated in patients with TSH levels above ten mIU/mL or in patients with TSH levels between 5 and 10 mIU/mL in conjunction with goiter and/or positive antithyroid peroxidase antibodies, as these patients have the highest rates of progression to overt hypothyroidism. An initial dose of 25-50 mcg/d of LT4 can be used and can be titrated every 6-8 weeks, to achieve a target TSH of between 0.3 and 3 mIU/mL\(^9\).

1.2.1.4.1.4. Myxedema coma

An effective approach is to use intravenous LT4 at a dose of 4 mcg/kg of lean body weight, or approximately 200-250 mcg as a bolus in a single or divided dose, depending on the patient's risk of cardiac disease followed by 100 mcg 24 hours later and then 50 mcg daily IV or PO along with stress doses of intravenous glucocorticoids. Use of intravenous triiodothyronine is controversial and based on expert opinion. It has a higher frequency of adverse cardiac events and is generally reserved for patients who are not improving clinically on LT4. LT3 can be given initially as a 10 mcg IV bolus and repeated every 8-12 hours until the patient can take maintenance oral doses of T4. Advanced age, high dose T4 therapy, and cardiac complications had the highest associations with mortality. Suspected myxedema
coma is a medical emergency with a high risk of mortality that requires initiation of parenteral (intravenous) LT4 and glucocorticoids prior to laboratory confirmation. **Follow up:** once appropriate therapeutic dose is maintained, patients can be monitored annually or semi-annually with lab tests ((TSH,T4,T3),TpoAbs) and physical examination \(^{(9)}\).

1.2.1.4.2. Hyperthyroidism

1.2.1.4.2.1. Thyrotoxicosis

Is the hypermetabolic condition associated with elevated levels of free thyroxine (FT\(_4\)) and/or free triiodothyronine (FT\(_3\)). Hyperthyroidism includes diseases that are a subset of thyrotoxicosis, that are caused by excess synthesis and secretion of thyroid hormone by the thyroid; they are not associated with exogenous thyroid hormone intake and subacute thyroiditis the most common forms of hyperthyroidism include diffuse toxic goiter (Graves disease), toxic multinodular goiter (Plummer disease), and toxic adenoma. Together with the thyrototoxic phase of subacute thyroiditis, these conditions constitute 85-90% of all causes of thyrotoxicosis. Less common causes are iodine induced thyrotoxicosis and thyrotoxicosis factitia. Uncommon causes are TSH secreting pituitary tumor, excess HCG (molar pregnancy, choriocarcinoma), thyroid hormone resistance, metastatic thyroid cancer, struma ovarii with thyrotoxicosis \(^{(9)}\).

1.2.1.4.2.1.1. Symptoms and signs

Nonspecific changes due to excessive thyroid hormone include weight loss, nervousness, fatigue, heat intolerance, and rapid heartbeat or palpitations sometimes associated with atrial fibrillation and high-output congestive heart failure (CHF) and increase in the rate of bone resorption. Thyroid hormone excess causes left ventricular thickening, which is associated with an increased risk of CHF. Thyrotoxicosis has been associated with dilated cardiomyopathy, right heart failure with pulmonary
hypertension. Ophthalmopathy and dermopathy specifically associated with Graves disease include periorbital edema, chemosis, and proptosis with extraocular muscle dysfunction and diplopia. The dermopathy, a painless swelling of the pretibial area may occur in patients with severe opthalmopathy (9).

1.2.1.4.2.2. Grave's disease

The most common cause of thyrotoxicosis is Graves disease (50-60%). Graves disease is an organ-specific autoimmune disorder characterized by a variety of circulating antibodies, including common autoimmune antibodies, as well as anti-thyroid peroxidase (anti-TPO) and antithyroglobulin (anti-TG) antibodies. The most important autoantibody is thyroid-stimulating immunoglobulin (TSI). TSI is directed toward epitopes of the thyroid-stimulating hormone (TSH) receptor and acts as a TSH-receptor agonist. Similar to TSH, TSI binds to the TSH receptor on the thyroid follicular cells to activate thyroid hormone synthesis and release and thyroid growth (hypertrophy). This results in the characteristic picture of Graves thyrotoxicosis, with a diffusely enlarged thyroid, very high radioactive iodine uptake, and excessive thyroid hormone levels compared with a healthy thyroid. The disease has got a peak occurrence in people aged 20 to 40 years, and a male: female ratio of 1.5 – 10. Usually there is a strong family history past medical history of other autoimmune diseases like rheumatoid arthritis, vitiligo and pernicious anaemia. The disease has higher prevalence in patients with HLA-DRw3 and HLA-B89. (11).

1.2.1.4.2.2.1. Laboratory Studies

TSH levels usually are suppressed to immeasurable levels (<0.05 µIU/mL) in thyrotoxicosis. Third-generation TSH assays are recommended for screening purposes. The degree of thyrotoxicosis cannot be estimated easily by the TSH level and must be measured using an assay of thyroid hormone levels in the plasma.
Therefore, measuring free T₄ (and T₃ if T₄ levels are normal) is recommended in patients with suspected thyrotoxicosis when TSH is low.(¹¹)

Subclinical hyperthyroidism is defined as a suppressed TSH level (<0.5 µU/mL in many laboratories) in combination with serum concentrations of T₃ and T₄ that are within the reference range.(¹¹)

**Thyroid autoantibodies:**

The most specific autoantibody for autoimmune thyroiditis is an enzyme-linked immunosorbent assay (ELISA) for anti-TPO antibody. The titers usually are significantly elevated in the most common type of hyperthyroidism, Graves thyrotoxicosis, and usually are low or absent in toxic multinodular goiter and toxic adenoma. A significant number of healthy people without active thyroid disease have mildly positive TPO antibodies; thus, the test should not be performed for screening purposes. TSI, if elevated, helps establish the diagnosis of Graves disease. A positive anti-TG antibody test does not predict the development of thyroid dysfunction and should not be measured.(¹¹)

**Radioisotope scanning:** helps differentiate different types of hyperthyroidism (¹¹).

1.2.1.4.2.3. Euthyroid Goiter

**Definition:** diffuse or nodular, is noncancerous hypertrophy of the thyroid without hyperthyroidism, hypothyroidism, or inflammation. The most common type of thyroid enlargement during puberty, pregnancy, and menopause.

1.2.1.4.2.3.1. Etiology

a) Intrinsic thyroid hormone production defects:

- Iodine deficiency (endemic goiter)
- Goitrogens.
b) Regular use of medications that decrease thyroid hormone production e.g. anti-thyroid drugs & iodine containing compounds.

c) Familial (12).

1.2.1.4.2.4. Thyroid cancer

Is the cancerous enlargement of the thyroid gland.

Risk factors include: previous irradiation of the neck, positive family history and chronic goiter.

Histological types include: papillary, medullary, follicular and anaplastic.

Clinical presentation include: neck swelling, difficulty in swallowing, hoarseness of the voice and thyroid lump.

1.2.1.4.2.4.1. Tests for thyroid cancer

I. Elevated serum calcitonin (for medullary cancer) or serum thyroglobulin (for papillary or follicular cancer)

II. Laryngoscopy showing paralyzed vocal cords

III. Thyroid biopsy showing anaplastic, follicular, medullary, or papillary cancer cells

IV. Thyroid scan showing a nodule that does not light up on the scan (cold nodule)

1.2.1.4.2.4.2. Treatment: surgery, radiotherapy (13).

1.2.1.4.2.5. Euthyroid sick syndrome (low T3 syndrome)

Can be described as abnormal findings on thyroid function tests that occur in the setting of a no thyroidal illness (NTI), without preexisting hypothalamic-pituitary and thyroid gland dysfunction (14).

TSH secretion is normal or decreased, total T4 levels are decreased, and total T3 levels are markedly decreased. This can be confused with secondary hypothyroidism. In these patients, the primary abnormality is the decreased peripheral production of T3 from T4. They have an increased reverse T3, which can be measured (14).
1.2.2. Selenium and the thyroid

1.2.2.1. Selenium and selenoproteins

The essential trace element, selenium (atomic number 34, atomic mass 78.96, a non-metal chemically related to sulfur and tellurium), and which we largely obtain from bread and cereals, fish, poultry and meat, plays a vital part in many metabolic functions. While new research increasingly suggests its relevance to disease prevention, evidence that dietary intake is falling in some parts of the world is giving cause for concern (15).

The amount of selenium available in the soil varies and this affects the amount of selenium found in local produce. High levels of selenium are found in the high plains of northern Nebraska and the Dakotas whereas the soil in some parts of China and Russia has scant amounts of selenium. In areas where the diet primarily consists of locally grown food, selenium levels in the body correlate well to soil levels. (16)

The daily value recommended for selenium by the FDA is 70 mcg. When supplements are used, 100-200 mcg daily of selenomethionine is recommended. Amounts greater than 200 mcg should be avoided. Results of the National Health and Nutrition Examination Survey indicate that most Americans obtain adequate selenium from diet. However, studies also show a lower rate of cancer, heart disease and autoimmune thyroid disease in patients with higher selenium levels. Surveys also show that patients with rheumatoid arthritis with lower selenium levels have more swelling, pain, stiffness and loss of function in their joints (16).

1.2.2.2. Selenium Deficiency

Selenium deficiency is thought to contribute to autoimmune disease by making the body more susceptible to nutritional and biochemical stresses as well as infectious diseases. Three diseases caused directly by selenium deficiency include Keshan
Disease, which causes an enlarged heart, Kashin-Beck Disease, which causes osteoarthropathy, and Myxedematous Endemic Cretinism, a form of hypothyroidism which results in mental retardation \(^{(17)}\).

1.2.2.3. Selenium toxicity

Occurs when doses higher than 400 mcg daily are ingested over time, causes a condition of selenosis. Symptoms include garlic breath odor, hair loss, white blotchy nails, irritability, fatigue, gastrointestinal upset and mild nerve damage. Selenium toxicity is rare in the United States and primarily is related to industrial accidents \(^{(17)}\).

Selenium was discovered by the Swedish chemist Berzelius in 1817, but a biological role for this trace element remained unknown until 1957 when Schwarz and Foltz showed that selenium deficiency could cause necrotic liver degeneration. However, the first real understanding of the physiological basis for a selenium nutritional requirement did not occur until 1973, when it was shown that selenium was an essential component of mammalian enzymes like glutathione peroxidases (GPx). It is now well established that selenium plays an important biological role in living organisms, mostly through its incorporation in a family of proteins called selenoproteins. The main biological form of selenium is selenocysteine (Sec), a cysteine analog that is synthesized from a serine bound to tRNA. Sec is identical to cysteine except for the fact that, in place of sulfur, it contains a selenium atom, which is typically ionized at physiological pH. In several instances, replacement of Sec by cysteine in a selenoprotein has been shown to result in a dramatic decrease of enzymatic activity, supporting the concept that the ionized selenium atom is critical for proper protein function \(^{(18)}\).

The single unifying, and defining, feature of selenoproteins is the fact that they all include one or more Sec residues in their primary structure. To date, all selenoproteins
with known functions, with the exception of selenoprotein P appear to have enzymatic activities in which the Sec residue is located at the catalytic site, where it likely participates in redox reactions. However, the amino acid sequences, enzymatic activities, tissue distribution of expression, and other molecular features of the different family members are extremely varied. Similarly, at the physiological level, these enzymes are involved in diverse metabolic and physiological functions ranging from antioxidant defense to fertility, muscle development and function, thyroid hormone metabolism, and immune function. Consequently, the range of pathologies associated with primary or secondary defects of selenoprotein function is enormous, with no easily definable unifying feature to tie together this disparate group of phenotypes at the pathophysiological level.

1.2.2.4. Selenoprotein Biosynthesis

The incorporation of Sec, which is considered to be the 21st amino acid, occurs in a unique and peculiar way; in fact, the Sec codon is an in-frame UGA, which normally corresponds to a termination codon. The recognition of UGA as a Sec codon, instead of a translational stop signal, requires the presence of a stem loop sequence called SECIS (SEC insertion sequence), which typically resides several hundred to several thousand base pairs downstream of the UGA codon in the 3’ untranslated regions of eukaryotic selenoprotein transcripts. Interestingly, the targeted deletion of tRNA\textsuperscript{Sec} gene (Trsp) results in an embryonic lethal phenotype in mice. Now 25 human selenoproteins were identified\textsuperscript{(18)}.

1.2.2.5. Glutathione peroxidase (GPx) and Disease

Selenium containing enzyme that protects tissues from oxidative damage by removing peroxides resulting from free radical action, linked to oxidation of glutathione; part of the body’s antioxidant protection\textsuperscript{(18)}. 
Decreased antioxidant activity occurs in selenium deficiency and renders patients susceptible to viral infections, cancers and autoimmunity. An example is the dilated cardiomyopathy in Keshan disease patients is likely related to decreased activity of GPx-1, secondary to selenium deficiency, which allows the accumulation of coxsakievirus mutations, hence converting benign strains into cardio toxic ones (18). To date, all selenoproteins with known functions have enzymatic activity. However, it is noteworthy that GPx-4, in addition to its enzymatic function, can also play a structural role in mature spermatozoa, impairment of GPx-4 is highly suspected to result in male infertility (18).

1.2.2.6. TrxRs and Disease
As their name implies, TrxRs reduce thioredoxins (Trxs), which are small, ubiquitous, redox-active peptides with a conserved catalytic site that undergoes reversible oxidation/reduction at two Cys residues, TrxRs are involved in a broad range of physiological and pathological pathways ranging from cancer to sperm maturation and male fertility (18).

1.2.2.7. Selenoprotein P and Selenium Transport
Selenoprotein P (SEPP1) is unique among selenoproteins because, in humans, it incorporates 10 Sec residues. It is a secreted protein that contains about 50% of the selenium content of human plasma, and it appears to be important for transporting selenium to the brain. Other selenoprotiens are selenoprotien s involved in inflammation, selenoprotien 15kda related to cancer and tumor progression, selenoprotien n related to muscle diseases. An important selenoprotiens involved in thyroid hormone metabolism are the iodothyronine deiodinases (18).
1.2.3. Selenium, selenoproteins and thyroid disease

The thyroid gland has an exceptionally high selenium content, even during selenium deficiency. At least 11 selenoproteins are expressed, which may be involved in the protection of the gland against the high amounts of H2O2 produced during thyroid hormone biosynthesis. As determined here by in situ hybridization and Northern blotting experiments, glutathione peroxidases (GPx) 1 and 4 and selenoprotein P were moderately expressed, occurring selectively in the follicular cells and in leukocytes of germinal follicles of thyroids affected by Hashimoto's thyroiditis. Selenoprotein 15 was only marginally expressed and distributed over all cell types. GPx3 mRNA was exclusively localized to the thyrocytes, showed the highest expression levels and was down-regulated in 5 of 6 thyroid cancer samples as compared to matched normal controls (19).

Selenium deficiency (reducing selenoproteins activity), and disturbed thyroid function may develop under conditions of special dietary regimens, such as long term total parenteral nutrition TPN, or after inadequate nutrition in children (20).

1.2.3.1. The role of selenium in thyroid autoimmunity and cancer

The selenoenzyme families of glutathione peroxidases (GPx) and thioredoxin reductases (TRx) possess powerful antioxidant properties and form a complex defense system that protects thyrocytes from oxidative damage. Se supplementation in patients with autoimmune thyroiditis seems to modify the immune response, probably by enhancing plasma GPx activity and decreasing excess levels of hydrogen peroxide. However, the enhancement of immunocompetence may also be the result of the synergistic action of various selenoproteins and not exclusively of GPx. There is evidence supporting considerable oxidative stress in Graves' disease where Se supplementation, because of its free radical scavenging properties, may increase the
enzymatic antioxidant activity. TRx has been found significantly elevated in GD revealing its involvement in the pathogenesis of this condition and representing a potential future target for therapeutical intervention \(^{(21)}\).

Selenium deficiency has been implicated in the etiology of Hashimoto's thyroiditis, and just by supplementing with selenium, there will usually be a decrease in antibody levels. This was shown in an elegant study from Crete published in 2007. This study reported a 21 \% reduction in TPO antibodies after one year of selenomethionine supplements(200 mcg per day) \(^{(22)}\).

Low Se serum levels have also been associated with increased risk of thyroid cancer and may play a role in carcinogenesis. It is noteworthy, that the Food and Drug Administration has recently determined that there is sufficient evidence to warrant a qualified health claim for Se and cancer. Furthermore, the recent discovery that defects in the SECIS-binding protein 2 (SBP2), which is an indispensable protein for the incorporation of Se into the selenoproteins, result in thyroid dysfunction, together with the recognition of the many roles of selenoprotein P in Se distribution and storage in the human body, reveal not only the indispensability of Se and the selenoproteins as essential factors in thyroid metabolism and pathogenesis, but open up new prospects for enhanced treatment \(^{(21)}\).

### 1.2.3.2. Selenium & peripheral thyroid hormone metabolism

The iodothyronine deiodinases types I, II, and III (D1, D2, and D3, respectively) regulate the activity of thyroid hormone via removal of specific iodine moieties from the precursor molecule T4. These 3 enzymes constitute a group of dimeric integral membrane thioredoxin fold–containing proteins that can activate or inactivate thyroid hormone, depending on whether they act on the phenolic or tyrosil rings of the iodothyronines, respectively. D2 generates the active form of thyroid hormone T3 via
deiodination of T4. In contrast, D3 inactivates T3 and, to a lesser extent, prevents T4 from being activated. Finally, D1 is a kinetically inefficient enzyme that activates or inactivates T4 on an equimolar basis, and its role in health remains to be clarified. In general, a given cell type will express only 1 type of deiodinase at a given time, though some tissues express none, and some express all the 3. For example, D1 is expressed mainly in the liver, kidney, and muscle. D2 is expressed mainly in the pituitary and brain. Thyroid hormone signaling results from the interaction of nuclear thyroid hormone receptors (TRs) with specific target gene promoters, a process that can either enhance or repress transcription \(^{(23)}\).

This process is modulated via binding of thyroid hormone, the ligand, to the TRs, which results in alterations in the composition of the transcriptional complex. Signaling through this pathway is, of course, sensitive to changes in serum thyroid hormone concentrations, the modern paradigm of thyroid hormone action also recognizes that thyroid hormone signaling in individual tissues can change even as serum hormone concentrations remain normal, thanks to local activation or inactivation of thyroid hormone. The underlying mechanism of these phenomena is deiodination. The deiodinases are critical determinants of the cytoplasmic T3 pool (80% of T3 is formed from peripheral deiodination of T4) and therefore modulate nuclear T3 concentration and TR saturation \(^{(23)}\).

In the pituitary thyrotroph, TSH is subject to negative feedback by T3, which is largely obtained by deiodination by pituitary D2. The actions of the deiodinases are integrated and thus promote the maintenance of serum T3 concentrations. Fluctuations in serum T4 and T3 concentrations lead to homeostatic, reciprocal changes in the activity of D2 and D3. As serum T3 concentrations increase, expression of type III iodothyronine deiodinase (Dio3), which encodes D3, is
upregulated, increasing T3 clearance, while expression of Dio2, which encodes D2, is modestly downregulated, decreasing T3 production. Conversely, if serum T3 concentrations were to fall, downregulation of the D3 pathway would decrease the clearance of T3 (23).

The increase in plasma T4 and decreases in T3 in the selenium deficient rats (given selenium deficient diets compared to rats given selenium adequate diets) indicated that the conversion of T4 to T3 by the D1 in the liver and kidney was diminished possibly as a consequence of the enzyme being a selenoprotein. The decrease in D2 in selenium deficiency also suggested that D2 may also be a selenoenzyme; however, D2 activity may also be modified by plasma T4 concentration, with high T4 inhibiting D2 activity (24).

Under normal circumstances an increase in plasma free T4 decreases plasma TSH, but in the hyperthyroxinaemia of selenium deficiency, plasma TSH is unchanged or increased, indicating an inability of the pituitary to recognize the high free T4 due to the impaired intrapituitary conversion of T4 to T3.

Selenium deficiency either through a direct effect or through elevated plasma TSH causes an increase in thyroidal T3 and T4 synthesis at the expense of a decrease in the total thyroidal iodine even when there is an adequate supply of iodine in the diet (24).

The effects of combined selenium and iodine deficiencies have been evaluated in different fluids and tissues, such as plasma (25), central nervous system (26), brown adipose tissue, liver and thyroid (27) reported that combined selenium and iodine deficiencies significantly lowered thyroidal T3 and plasma T4 compared with rats with single iodine deficiency, also plasma TSH concentration and thyroid weight are significantly greater (28).
In African countries like Zaire, there are areas where both iodine and selenium are very scarce in the soil (these deficiencies seem to run parallel in most areas). Consequently a high percentage of the people have goiters and hypothyroidism (24).

Studies have shown that if iodine is low, selenium must also be kept low to prevent the hypothyroidism from becoming worse (from increased D-I and T4 depletion, as explained above.) So if both minerals are low, then the person is hypo and gets a goiter, but the damage to the thyroid is kept to a minimum. More severe problems happen when either selenium or iodine is high and the other is low. If selenium is high and iodine low, then T4 to T3 to T2 conversion is accelerated without T4 being replenished, leading to a worsening of the hypothyroidism. If iodine is high and selenium is low, then H2O2 is not degraded by GPX. Since H2O2 drives the thyroid hormone production, then the thyroid over-produces thyroid hormone (Grave's hyperthyroidism), the thyroid is damaged from the oxidation by the H2O2, and the end result is that the damaged thyroid ultimately decreases activity and hypothyroidism results (Hashimoto's thyroiditis). This could explain the observed progression of Grave's to Hashimoto’s (29).

Selenium deficiency is common in the critically ill patients and this could play a role in the pathogenesis of the low T3 syndrome. The syndrome also occurs after major surgery and life threatening traumas (e.g. head injuries). After severe injury, T4 deiodination is decreased, leading to the low T3 syndrome. Injury increases free radical production, selenium is redistributed in the tissues, and the iodothyronine deiododinases are inactivated (30).
1.3. JUSTIFICATION

1- Selenium is one of the essential trace elements that guard our bodies against autoimmunity and cancer.

2- It is decided to study selenium status in thyroid diseases (hypo and hyperthyroidism), since thyroid disorders are very common in our country.

3- Many studies documented that the soil in Sudan, especially in Darfur is deficient in both selenium and iodine rendering Sudanese people more susceptible to goiter and thyroid diseases.

4- It is also documented from many studies that supplementing these patients with selenium helps to improve the disease outcome, thereby reducing morbidity and mortality, and this is especially for autoimmune thyroid diseases where selenium plays a major role in reducing auto antibodies levels.
1.4. OBJECTIVES

1.4.1. General objectives:
To evaluate serum selenium level in thyroid disease (hypo and hyper thyroid patients).

1.4.2. Specific objectives:
1- To measure serum selenium level in hypothyroid, hyperthyroid patients and normal controls (age and sex matched groups).
2- To compare serum selenium level in hypo and hyperthyroid patients’ pre and post treatment of their thyroid disease.
2. MATERIALS AND METHODS

2.1. Study design:
Prospective hospital based case control study.

2.2. Study area:
The study was conducted in Khartoum teaching hospital. It was chosen since it is a central large federal hospital and most of the patients are seen there. The study was conducted in the clinical chemistry department of Khartoum teaching hospital laboratories.

2.3. Study duration:
The study was conducted during the period (September 2010 to February 2011).

2.4. Study population:
2.4.1. Cases:
**Inclusion criteria:** male and female adults (age 16 years and above) who were diagnosed as having hypothyroidism (group 1) and hyperthyroidism (group 2) on the basis of clinical presentation and laboratory investigations (performing thyroid function tests) and who were voluntarily accepting to participate in the study.

**Exclusion criteria:** subjects known to have low selenium like asthmatics and cancer patients.

2.4.2. Controls: male and female adults who were found to be euthyroid both clinically and biochemically (performing thyroid function tests), and have no evidence of thyroid disease (e.g. goiter).

2.5. Sampling technique:

2.5.1. Ethical consideration:
Ethical approval was given by the director of Khartoum Teaching Hospital laboratory. Consents were obtained from the study subjects before entry into the study.
2.5.2. Sample size:
Forty cases (20 hypothyroid and 20 hyperthyroid) Twenty age and sex matched normal controls.

2.6. Data collection:
Data were collected from patients by questionnaire (appendix 1). The questionnaire included demographic characteristics, disease identification data, treatment details, and results of thyroid function tests and serum selenium levels were also included.

2.7. Collection of blood samples:
Five ml of venous blood withdrawn in a plain container, centrifuged for 15 minutes, two ml of clear serum were obtained and divided equally for thyroid function tests (TSH, total T4 & total T3) and for selenium measurements.

2.8. Technique of biochemical analysis:
Thyroid function tests were measured by using the principle of chemiluminescence by Elecsys 2010 (Roche).
Serum selenium was measured in diluted samples (5 times dilutions with distilled water & nitric acid) by using the principle of atomic absorption by AA 6800 atomic absorption spectrophotometer (Shimadzu) (appendix 2).

2.9. Quality control: serial dilutions of a commercially prepared selenium standard (thousand ppb concentration) were used to prepare the standard (absorbance vs. concentration) calibration curve. Sample concentration was obtained from the curve. Each sample was read three times and the mean of the closest two readings was derived.

2.10. Data analysis:
Data were analyzed by computer system using SPSS program.
2.11. Data interpretation:

Thyroid hormones and TSH were determined as high, low or normal according to the laboratory reference ranges.

Serum selenium was also determined as low, high or normal (adult reference range between 75 and 120 ppb).

Final results were presented in tables and figures.
3. RESULTS

3.1. Demographic characteristics of the study group

3.1.1. Gender distribution:
The studied subjects were 60 (40 cases and 20 controls). Fifty six of them were females (93.3%), with only 4 males included (6.7%). (Figure 1).

3.1.2. Age distribution:
Most of the studied subjects were in the age group from 31 to 50 years (53.3%). With (41.7%) in the age group from 16 to 30 years and only 5% age >50 years. (Figure 2).

3.2. Disease identification:
Of the hypothyroid group 14 patients (70%) were on treatment with thyroxine. Six patients (30%) were not on treatment, of which three (15%) were not started on treatment (new cases) and the remaining 3 patients were on treatment but stopped. Regarding the hyperthyroid group 12 patients (60%) were on treatment, 7 patients were on treatment and stopped (35%). Only 1 patient (5%) was not started on treatment (new case).

3.3. Serum selenium level in the studied subjects:

3.3.1. Serum selenium level in the controls:
The mean serum selenium level in the normal controls was 78.39 ppb (±17.99SD). (Figure 3).

3.3.2. Serum selenium level in the hypothyroid group:
The mean serum selenium level in the hypothyroid group of patients was 63.82 ppb (±26.2SD). (Figure 4).
The mean serum selenium of the hypothryoid group was significantly low compared to controls (P value<0.05). (Table 1).

3.3.3. Serum selenium level in the hyperthyroid group:
The mean serum selenium level in the hyperthyroid group of patients was 81.89ppb (± 29.9SD). (Figure 5). The mean serum selenium was statistically insignificant compared to controls. (Table 2).

3.4. Serum selenium level in the studied cases pre & post treatment:
3.4.1. Serum selenium level in the hypothryoid group pre & post treatment with thyroxine:
The mean serum selenium level in the hypothryoid group who were not started on treatment (new cases) was 30.54ppb (± 24.68SD).
The mean serum selenium level in the hypothryoid patients who are on replacement with thyroxine was 74.71ppb (± 17.57SD).
The difference was statistically significant (P value<0.05). (Table3).
The mean serum selenium level in the hypothryoid patients who were on treatment but now stopped was 52.8ppb (± 27.1SD).

3.4.2. Serum selenium level in the hyperthyroid group pre & post treatment with anti thyroid drugs:
The mean serum selenium level in the hyperthyroid patients who are on treatment with ant thyroid drugs was 87.96ppb (± 35.05SD).
The mean serum selenium level in the hyperthyroid patients who were on treatment but now stopped was 73.07ppb (± 20.16SD).
Only one patient was never been on treatment (new case) with a level of 70.8ppb.
3.5. Effect of serum selenium level on TSH & thyroid hormones in thyroid disease:

3.5.1. Effect of serum selenium level on TSH:

In the hypothyroid group of patients the mean serum selenium level was 63.82ppb (reference range from 75 to 120 ppb) and the mean TSH was 20.06 (reference range from 0.5 to 5 µU/ml). The relation is statistically significant (P value < 0.05).

In the hyperthyroid group the mean selenium was 81.89ppb and the mean TSH was 2.36 µU/ml the relation is statistically significant (P value < 0.05). (Table 4).

3.5.2. Effect of serum selenium level on T4:

In the hypothyroid group mean selenium was 63.82ppb. The mean T4 was 5.9 µg/dl (reference range from 4.5 to 13 µg/dl). The relation is statistically significant (P value < 0.05).

In the hyperthyroid group of patients mean selenium was 81.89 and the mean T4 was 11.08. The relation is not statistically significant. (Table 5).

3.5.3. Effect of serum selenium level on T3:

In the hypothyroid group mean selenium was 63.8 ppb and the mean T3 was 1.4 µg/dl. In the hyperthyroid group mean selenium was 81.89 ppb and the mean T3 was 3.7 µg/dl. Both relations were statistically insignificant.
Figure 1: Gender distribution of the studied subjects
Figure 2: Age distribution of the studied subjects
Figure 3: Serum selenium level in the euthyroid group
Serum selenium levels in hypothyroid patients

Figure 4 Serum selenium levels in hypothyroid patients
Figure 5 Serum selenium levels in hyperthyroid patients

Mean = 81.892
Std. Dev. = 29.93843
N = 20
Table 1: Correlation between mean serum selenium levels in the hypothyroid group and controls.

P value<0.05

<table>
<thead>
<tr>
<th></th>
<th>Minimum Selenium value</th>
<th>Maximum Selenium value</th>
<th>Mean Selenium value</th>
<th>Std. Deviation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hypothyroid</td>
<td>10.9</td>
<td>101.4</td>
<td>63.82</td>
<td>26.29</td>
</tr>
<tr>
<td>Euthyroid</td>
<td>32.6</td>
<td>99.3</td>
<td>78.39</td>
<td>17.99</td>
</tr>
</tbody>
</table>
Table 2: Correlations between mean serum selenium levels in the hyperthyroid group and controls

<table>
<thead>
<tr>
<th></th>
<th>Minimum Selenium level</th>
<th>Maximum Selenium level</th>
<th>Mean Selenium level</th>
<th>Std. Deviation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hyperthyroid</td>
<td>45</td>
<td>160</td>
<td>81.89</td>
<td>29.93</td>
</tr>
<tr>
<td>Euthyroid</td>
<td>32.6</td>
<td>99.3</td>
<td>78.39</td>
<td>17.99</td>
</tr>
</tbody>
</table>
**Table 3: Correlations between mean serum selenium levels in the hypothyroid patients’ pre and post treatment with thyroxine**

P value <0.05

<table>
<thead>
<tr>
<th></th>
<th>Number of cases</th>
<th>Mean Selenium level</th>
<th>Std. Deviation</th>
</tr>
</thead>
<tbody>
<tr>
<td>New cases</td>
<td>3</td>
<td>30.54</td>
<td>24.6</td>
</tr>
<tr>
<td>(not started on treatment)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Patients on thyroxine</td>
<td>14</td>
<td>74.71</td>
<td>17.57</td>
</tr>
</tbody>
</table>
Table 4: Correlations between mean selenium level and TSH in thyroid disease

P value < 0.05

<table>
<thead>
<tr>
<th></th>
<th>Mean Selenium level</th>
<th>Mean TSH (0.5 - 5µU/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hypothyroid</td>
<td>63.82</td>
<td>20.06</td>
</tr>
<tr>
<td>Hyperthyroid</td>
<td>81.89</td>
<td>17.9</td>
</tr>
</tbody>
</table>
Table 5: Relations between thyroid hormones T3 and T4 and serum selenium in thyroid disease

<table>
<thead>
<tr>
<th></th>
<th>Mean Serum selenium</th>
<th>Mean Serum T4 Ref. 4.5 - 13µg/dl</th>
<th>P value</th>
<th>Mean Serum T3 Ref. 0.8 - 2µg/dl</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hypothyroid</td>
<td>63.82</td>
<td>5.9</td>
<td>&lt;0.05</td>
<td>1.4</td>
<td>&gt;0.05</td>
</tr>
<tr>
<td>Hyper thyroid</td>
<td>81.89</td>
<td>11.08</td>
<td>&gt;0.05</td>
<td>3.7</td>
<td>&gt;0.05</td>
</tr>
</tbody>
</table>
4.1. DISCUSSION

The thyroid gland has exceptionally high selenium content. Selenoproteins are involved in the protection of the gland against the high amounts of hydrogen peroxide produced during thyroid hormone biosynthesis. Selenium deficiency has been implicated in the etiology of many thyroid diseases, namely hypothyroidism, autoimmune thyroid disease (ATD), and endemic cretinism.

This study is a hospital based, case control, in which serum selenium levels were measured in hypo and hyperthyroid patients and compared to levels of healthy age and sex matched controls.

Selenium levels were determined as low, normal or high according to the international reference range from 75 to 120 ppb (equivalent to µg/dl), levels below 75 ppb were considered low, and those above 120 ppb were considered high.

In this study, it was found that the mean serum selenium level in the hypothyroid group of patients was significantly low compared to controls. The level was even lower significantly in the newly diagnosed cases, though they were only three in number. Interestingly, among those newly diagnosed one case presented with myxedema (generalized swelling, hoarseness, sleepiness and hypotension) and had got the lowest serum selenium level. The mean serum selenium level in the hypothyroid patients receiving l-thyroxine replacement was found to be significantly higher than the level in the new cases (not started on treatment), although both levels were still below the lower limit of the reference range.

Mean serum selenium level was intermediate in the hypothyroid patients who were on thyroxine replacement but stopped. The reasons for stopping treatment in most cases were pregnancy and lactation. Unfortunately, bad follow up was the cause in many others.
Similar findings were obtained by a group of researchers who had reported significantly low selenium levels in hypothyroid patients compared to controls (31).

Another study from Crete published in 2007, reported low selenium level in patients with Hashimoto’s thyroiditis (considered to be the commonest cause of hypothyroidism) and they also reported a 21% reduction in the TPO antibodies after one year of selenomethionine supplements (22).

In contrast, other group of researchers who studied serum selenium level in hypo and hyperthyroid patients (in three areas with different iodine intake) compared to normal controls found no difference in serum selenium levels between patients with subclinical hypothyroidism, clinical hypothyroidism and clinical hyperthyroidism and their normal controls (32).

Regarding the effect of treatment on the improved selenium levels, no solid data was found in the literature whether L-thyroxine helps correct selenium deficiency in hypothyroidism or not. But, it was found in the literature that hypothyroidism as a result of break down in the conversion of T4 to T3 (as could be in selenium deficiency) will benefit little from L-thyroxine as it is a prohormone and needs to be converted to the active form (T3) (29).

Putting in mind that the studied cases were not on selenium supplements or selenium rich diet so, this desired treatment effect needs to be further explored.

In the hyperthyroid group of patients, the mean serum selenium level was within the reference range and found to be higher than the level of the controls but this finding was statistically insignificant.

In the literature low selenium (but with adequate or high iodine) can lead to the development of Grave’s hyperthyroidism (24).
Studying the relationships between serum selenium and thyroid hormones (total T4 and T3) and TSH, it was concluded that serum selenium was inversely correlated with TSH and T4. No significant relationship was found between serum selenium and T3. The insignificant P value may be justified by the small size of the sample population.

In the literature one study showed that serum selenium correlates directly with T3 and inversely with TSH \(^{(33)}\). Another group of researchers who studied serum selenium levels in animals fed with selenium deficient diet compared to animals fed with selenium adequate diet is that selenium deficiency resulted in an increase in plasma T4 and decrease in plasma T3 \(^{(34)}\).

**The finding of this study and other studies about the relationships between selenium and (T4, T3 and TSH) can be explained as follows:**

The iodothyronine deiodinases types I, II and III regulate the activity of thyroid hormones via removal of specific iodine moieties from the precursor molecule T4.

**Type 1** deiodinase in the liver and the kidney is responsible for the peripheral conversion of T4 to T3. It is a selenoenzyme and so its activity is reduced in selenium deficiency leading to increases in plasma T4 and decreases in plasma T3.

**Type 2** deiodinase is expressed mainly in the pituitary, it is doubtful whether its activity is selenium dependent or not, but the high plasma T4 (as in selenium deficiency) inhibits its activity with the resultant inability of the pituitary to recognize the high free T4 due to the impaired intra pituitary conversion of T4 to T3. So, TSH will be normal or increased.
4.2. CONCLUSION

This is the first study conducted in Sudan to explore selenium status in hypo and hyperthyroidism.

The study concluded that:

1- Significantly low serum selenium levels in hypothyroid patients confirm the possible implication of selenium deficiency in the causation of hypothyroidism as reported in the literature.

2- Controlling hypothyroidism with thyroxine replacement may have a possible role in improving selenium deficiency; still this finding needs further exploration.

3- Inverse relationships were found between: (serum selenium and serum TSH) and (serum selenium and serum T4) confirming the role of selenium in thyroid hormones metabolism.
4.3. RECOMMENDATIONS

1- Further studies spanning longer durations and involving other patients from different hospitals and different areas of Sudan are needed to consolidate the findings of this study.

2- Efforts should be put on identifying the reference range of serum selenium in our home population in Sudan.

3- Further studies that determine the prevalence of autoimmune thyroid disease in Sudan and to specifically evaluate selenium status in autoimmune thyroid disease are more valuable and thus needed.

4- Longitudinal studies to explore the effect of treatment with thyroxine on serum selenium levels are also needed.

5- Measurement of free thyroid hormones concentration should be performed in all samples submitted for thyroid function tests in KTH and should replace total hormones measurements.

6- Introduction of thyroid antibodies measurements as complementary tests to those who are at great suspicion of having autoimmune thyroid disease.

7- Protocols for introducing selenomethionine supplements to patients with autoimmune thyroid disease and monitoring the response by following the level of thyroid antibodies.

8- Efforts should be made from the government on enrichment of the soil with both selenium and iodine to guard against the conditions arising from deficiency of both elements.
REFERENCES


21. Duntas LH. The role of selenium in thyroid autoimmunity and cancer. Thyroid. 2006 May;16(5):455-60.


## Appendix I

**Selenium level in thyroid disease**

### Questionnaire

**Patients name:** ..................................................

1. Age:  
   (1) 16-30yrs  
   (2) >31-50yrs  
   (3) >50yrs

2. Sex:  
   (1) Male  
   (2) Female

### Disease identification:

3. Case type:  
   (a) Known case  
   (b) New case

4. If known case, the diagnosis is:  
   (a) Hypothyroidism  
   (b) Hyperthyroidism

5. **Regarding treatment:**  
   (a) On treatment  
   Specify: ..........................................................

   **Duration:**  
   (a) weeks  
   (b) Months  
   (c) years

   (b) Not on treatment

   (c) Was on treatment and stopped  
   Specify, ..........................................................

   **Duration**  
   (a) weeks  
   (b) Months  
   (c) years

6. If new case,  
   Complain: ..........................................................

### Final diagnosis:

   (a) Hypothyroid  
   (b) Hyperthyroid  
   (c) Euthyroid

7. **Results of thyroid functions:**  
   (i) TSH value .....  
     a. high  
     b. low  
     c. normal

   (ii) T4 value .....  
     a. high  
     b. low  
     c. normal

   (iii) T3 value .....  
     a. high  
     b. low  
     c. normal

8. **Results of plasma selenium level**  
   Value: .....  
   a. high  
   b. low  
   c. normal
Appendix II

Principle of atomic absorption

Flame atomic absorption spectroscopy (F AAS) is a very common technique for detecting metals and metalloids in solid and aqueous samples. It is very reliable and simple to use. The technique is based on the fact that ground state metals absorb light at specific wavelengths. Metal ions in a solution are converted to atomic state by means of a flame. Light of the appropriate wavelength is supplied and the amount of light absorbed can be measured against a standard curve.

The technique of (F AAS) requires a liquid sample to be aspirated, aerosolized, and mixed with combustible gases, such as; tcetylene and air or acetylene and nitrous oxide. The mixture is ignited in a flame whose temperature ranges from 2100 to 2800 Qc. During combustion, atoms of the element of interest in the sample are reduced to free, unexcited ground state atoms, which absorb light at characteristic wavelengths.

The characteristic wavelengths are element specific and accurate to 0.0 1-0.1nm. To provide element specific wavelengths, a light beam from a lamp whose cathode is made of the element being determined is passed through the flame. A device such as a photon multiplier can detect the amount of reduction of the light intensity due to absorption by the analyte, and this can be directly related to the amount of the element in the solution.