SUDAN ACADEMY OF SCIENCE (SAS)
Coordination Council of Sudan Atomic Energy
Commission (SAEC)

THE EFFECT OF HYPERTHYROIDISM ON SERUM
CHOLESTEROL IN SUDANESE FEMALES

ATHESIS SUBMITTED IN PARTIAL FULFILLMENT OF THE
REQUIREMENTS FOR THE DEGREE OF HIGHER DIPLOMA
IN NUCLEAR SCIENCE

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DEDICATION

To my Uncle and my
Friends

To
My brothers
With endless love
ACKNOWLEDGEMENTS

I am greatly indebted to my supervisor Dr Nabiela Mel-Bagir for her invaluable support, advice, motivation, and encouragement which have always remained a source of guidance.

Special thanks and appreciation go to RIA staff for their assistance.
This study was done, essentially to assess the effect of Hyperthyroidism on lipid metabolism, respectively on total cholesterol in Sudanese females.

Samples were collected from the referred patients to RIA lab in Sudan Atomic Energy Commission (SAEC). Ninety eight subjects were selected as study group. 48 hyperthyroid females age range (18-60) years In addition 50 Euthyroid specimens were collected from females (of the same ages range) and used as control.

Thyroid hormones, thyroxin ($T_4$) and Triiodothyronine ($T_3$), the Thyroid stimulating hormone (TSH), and serum total cholesterol were measured for all subjects.

Statistical analysis was done using SPSS computer program to compare the mean of cholesterol levels the control with the study group.

The result showed that the significantly ($P < 0.01$). High levels of thyroid hormones in patients were accompanied by significantly ($P < 0.01$) decreased cholesterol levels. When this finding was compared in the control group serum total cholesterol levels kept the normal range with the normal thyroid function.
Introduction

Hormones are group of chemical messengers manufactured by endocrine, autocrine and paracrine glands, regulates the functions of many vital organs. Chemically hormones fall into four categories peptides or proteins, secreted predominately by the anterior pituitary, steroids, from the adrenal cortex and the gonads, iodothyronines from the thyroid gland and catecholamines which are produced by the adrenal medulla.

The thyroid gland secretes thyroid hormones thyroxine $T_4$ and Triiodothyronine $T_3$ which are important for life.

The thyroid abnormality is prevalent through the world and includes hyperthyroidism which is clinical syndrome resulting from excessive thyroid hormone production due to Grave’s disease, Toxic multi nodular goiter (Plummer’s disease), Toxic uninodular goiter (toxic adenoma), chronic thyroiditis (Hashitoxicosis) and inappropriate TSH secretion.

Hypothyroidism is clinical syndrome caused by the deficiency of thyroid hormone secretion, its causes include, congenital absence of the thyroid gland enzymatic defects in thyroid hormones synthesis, chronic thyroiditis, and many other causes.

Cholesterol is present in tissues and plasma- lipoproteins either as free or compound with long-chain fatty acid, as cholesterol ester, it is synthesized in many tissues from acetyl-CoA and is ultimately eliminated from the body in the bile as bile salts or bile acids. Cholesterol is the precursor of all other steroids in the body such as cortisol, sex hormones, bile acids and vitamin D. It is a major component of cell membranes.

The objective of this study is to evaluate the effect of hyperthyroidism on serum cholesterol level. And compare the result with previous studies.
Chapter One

Literature review

1.1 Thyroid dysfunction

The thyroid dysfunction is one of the public health problems in Sudan. Iodine deficiency has been determined as one of the causes in Sudan. (Woodman et al., 1950) described the areas of deficiency i.e. Darfur zone in western Sudan, while the thyroid hyperactivities are in eastern Sudan. Several members of the family are usually affected. Iodine deficiency can be prevented by addition of iodide to table salt or cooking oil, the consequence of thyroid dysfunction (hypothyroidism) is associated with hypercholesterolemia and frequently with moderate hypertriglyceridemia reported by (Gyton and Hall, 2000).

Women are five times more likely than men to have thyroid disorders; this is due to several factors including age, family history, pregnancy or autoimmune conditions, such as Addison disease, pernicious anaemia, or rheumatoid arthritis.

Hyper thyroid females may experience a lighter menstrual flow, the flow cycle may extend beyond 28 days, or menstruation may completely stop.

Infrequently menstruation makes her more difficult to get pregnant, and if she get pregnant her body may not be able to support the pregnancy unless the thyroid disorder is treated.

Untreated hyperthyroidism during pregnancy can lead to variety of dangers that can lead to premature parturition or miscarriage. Antithyroid drugs or surgeries are the recommended treatment optioned for pregnant patients.
Postpartum thyroditis is a condition occurs after delivery and may include both hyper and hypothyroidism, postpartum thyroditis can be a temporary condition, or it can develop into a long-term thyroid disorder.

(Walton et al, 1965) demonstrated that patients with primary hypothyroidism had increased serum level of cholesterol while the opposite pattern was found in hyperthyroid patients. Thyroid status in human is an important factor in regulation of lipoprotein metabolism (Barbagallo et al, 1995).

1.2 The thyroid gland

1.2.1 Physiology of the thyroid gland

The thyroid gland secretes thyroid hormones T₄ thyroxine and triiodothyronine T₃, that play vital role in fetal development, and throughout life they influence metabolic processes in almost all tissues. The thyroid gland is the only significant source of T₄ and the unique among the endocrine organs in two important ways:

1) It maintains a large store of hormone
2) It requires iodide for hormone synthesis

1.2.2 Anatomy of the thyroid gland

The thyroid consists of a left and right lobes connected in human by an isthmus at the approximate level of cricoid cartilage. The adult thyroid weighing about 15-20g. The thyroid lobes are largely covered by the sternohyoid and sternothyroid muscles.

1.2.3 Histology of the thyroid tissue:

The basic functional unit of the thyroid is the follicle, hollow sphere of cells, the wall of the follicle is a single layer of the thyroid cells. A few adjacent parafollicular cells secrete (Calcitonin).
The follicular cells are cuboidal when quiescent and columnar when active. The follicular lumen contains colloid that is primary store of thyroglobulin secreted by the thyroid cells. This store is sufficient for about 100 days of normal thyroid hormone secretion. (Green and Forsham, 1983).

Fig-1

1.2.4 Iodide metabolism

Adequate ingestion of iodide is a prerequisite for the normal synthesis of thyroid hormones by the thyroid. The major sources of dietary iodide are water, iodated bread, iodinated salts and increasingly medication, disinfectants.
1.3 The synthesis of the thyroid hormones

1.3.1 Thyroglobulin

Thyroglobulin, is the precursor of all thyroid hormones, is a very large glycoprotein molecule. It is the major glycoprotein in the follicular lumen colloid with molecular weight of about 66000Da. Human thyroglobulin contains about 110 tyrosine residues. These residues are iodinated and ultimately form the thyroid hormones.

The degree of thyroglobulin iodination varies depending upon the availability of iodide and the efficiency of the iodination mechanism (Green and Forsham, 1983).

1.3.2 Iodide transport

Iodide is actively transported from the extra cellular fluids in to the thyroid follicular cell and incorporated into the protein “thyroglobulin”.

1.3.2.1 Iodide organification

Inorganic iodide is rapidly oxidized by the thyroid peroxidase and incorporated in to the tyrosine residues of the thyroglobulin (organified) forming monoiodothyrosine MIT and diiodothyrosine DIT.

1.3.2.2 Iodotyrosine coupling

Iodinated tyrosine residues in thyroglobulin combined to form triiodothyronines T₃ and tetraiodothyronine T₄. The active T₄ and T₃ secreted and transported through the plasma carried by thyroid binding globulin (TBG), albumin (TBA), and pre albumin (TBPA) to the target tissue.

1.3.3 Actions of thyroid hormones

Triiodothyronine T₃ is more potent and act faster than T₄ the former bind avidly to receptor on the cell nucleus, and promotes messenger
(mRNA), and ribosomal (rRNA) synthesis. The nuclear receptors for thyroid hormones are considered as nuclear transcription factors, and present almost in all tissues.

1.3.4 Functions of thyroid hormones

Thyroid hormones stimulate oxygen consumption and energy expenditure by all tissues. They are important for normal growth and development, congenital deficiency of thyroid hormones lead to thyroid dwarf (the cretin). They are essential for normal development and function of central nervous system, mental retardation is an important feature of cretinism.

1.4 Disorders of thyroid gland

1.4.1 Hyperthyroidism

Hyperthyroidism is the clinical syndrome resulting from excessive thyroid hormone production. It is useful to group the hyperthyroid syndrome into two categories

1. Those characterized by high radioiodine uptake, include:

A. Diffuse toxic goiter (Grave’s, disease)

This condition is marked by increased production of thyroid hormones by diffusely enlarged thyroid. Patients usually have eye manifestations.

B. Toxic multinodular goiter (Plummer’s disease)

This condition is manifested as thyroid hormone overproduction by one or several autonomous thyroid nodules, patients are usually older, and have more cardiovascular complications.

C. Chronic lymphocytic thyroditis (hashitoxicosis)

The symptoms of hyperthyroidism may develop in patients with preexisting thyroditis; few patients with autoimmune thyroditis and
long-standing hypothyroidism who develop thyrotoxicosis several years later have been reported.

D. Inappropriate TSH secretion
In this case, the hyperthyroidism is associated with elevated serum TSH, resistant to therapy, and tends to recur after thyroidectomy.

2. Those with low radioiodine uptake

A. Sub acute thyroiditis
This syndrome is caused by viral infiltration (mumps, coxsakie, influenza, echovirus, and adenovirus). Resulting in neck pain, which extend to jaw and neck or ears, fever, malaise, leukocytosis, and an increased sedimentation rate.

B. Silent thyroiditis
Also known as lymphocytic thyroditis with spontaneous resolving hyperthyroidism, painless. Graves's disease with iodine excess. In patients with Grave’s disease that have been treated with iodine-containing compounds.

C. Iodide induced hyperthyroidism (Jod-Basedows)
Excess iodide can leads to hyperthyroidism in individuals living in areas of iodine deficiency or in patients with autonomous multinodular goiter.

1.4.2 Factitious thyrotoxicosis
Ingestion of thyroid hormones usually by women with emotional problems, and by paramedical personnel. This case manifests no swelling, and suppressed radioiodine uptake.

1.4.3 Metastastic functioning thyroid carcinoma
Hyperthyroidism has been reported in afew patients with metastastic functioning thyroid carcinoma following removal of the primary
tumor. The metastatic lesion take up radioactive iodine, but uptake in the neck is decreased.

1.4.4 Signs and symptoms of hyperthyroidism

Symptoms related to the metabolic effects of thyroid hormones in the tissues include,

1. **metabolic effects**
   Weight loss, Muscular weakness, Hair loss, Thin skin and Oncholysis.

2. **Increased sympathetic activities**
   Nervousness, Palpitation, Heat intolerance, Sweating and Tremor.

3. **Unknown mechanism**
   Goiter and Ophthalmopathy.

Thyrotoxic crisis or storm represents an exaggeration of the signs and symptoms of hyperthyroidism.

1.4.5 The diagnosis of hyperthyroidism

Good history and physical examination should lead the clinician to suspect diagnosis of hyperthyroidism.

The screening test is the determination of serum thyroxine concentration.

The radioactive iodine-uptake is a very useful technique in some specific situations. Adose of 3 to 5 μCi of $^{125}$I or $^{131}$I is administered orally, and the amount taken by the thyroid gland is recorded 24 h later. Values are expressed as a percentage of the administered dose.

1.4.6 Treatment of Hyperthyroidism

1. Surgery
2. Antithyroid drugs
3. Radioactive iodine
1.5 Hypothyroidism

Deficiency of thyroid hormones results in constellation of symptoms and signs. The etiologic factors responsible for thyroid hypofunction, either present at birth.

Common causes of hypothyroidism includes congenital absence of thyroid gland, enzymatic defects in thyroid hormones synthesis, surgical removal of thyroid gland, therapy of thyrotoxicosis, chronic thyroditis, and many other causes.

1.5.1 Signs and symptoms of hypothyroidism

Symptoms includes generalised weakness, tiredness, and lthargy, intolerance to cold, dry skin, hair loss, memory impairment, and many other causes.

1.5.2 Diagnosis of hypothyroidism

History including presence of goiter, or hypothyroidism in other members of the family, drug ingestion previous thyroidectomy.

The screening laboratory test is the serum T₄ concentration. A low value in a patient with a characteristic history and physical finding is sufficient to confirm the diagnosis.

1.5.3 Regulation of thyroid hormone secretion

Secretion of thyroid hormones T₄ and T₃ is controlled by the hypothalamus and the anterior pituitary gland. The hypothalamus secretes the tripeptide thyrotropin releasing hormone (TRH), which stimulates the secretion of thyrotropin (TSH) by the anterior pituitary, and TSH stimulates thyroid hormones secretion by the thyroid gland. There is a feed back regulation on the (TRH), and (TSH) by the circulatory T₄ and T₃.
Thyroid hormones regulation of secretion (hypothalamic-pituitary-thyroidal axis).

1.6 Lipid metabolism

**Plasma lipids and lipoproteins**

Plasma lipid consist of triglycerol (16%), phospholipids(30%), cholesterol esters (14%), cholesterol(36%), and much of smaller fractions of unesterified fatty acids (4%) this later fraction is the metabolically active fatty acids.

1.6.1 Lipoproteins

Because fats is insoluble in water its transportation through the plasma as mixture of lipids and protein (lipoprotein) the major lipoproteins are chylomicron, which derived from intestinal absorption of tri glycerol and
other lipids, very low density lipoprotein (VLDL, or pre β-lipoprotein), low density lipoprotein (LDL or β-lipoprotein), and high density lipoprotein (HDL or α-lipoprotein) involved in VLDL and chylomicron and cholesterol metabolism (Robert et al, 2003).

1.6.2 Cholesterol

Cholesterol is an essential component of development. It is an integral part of cell membranes and consequently of various membrane micro domains, including lipid rafts. Cholesterol within membranes will affect the content of other lipids within membranes; specifically sphingomyelin (Patton. S et al, 1997). This could make cholesterol a fundamental mediator of metabolism through the propagation of signaling cascades (Smart. C.J et al, 1999). Cholesterol is also the precursor of steroid hormones such as progesterone and of metabolic mediators such as oxysterol. Finally, cholesterol is essential to both the activation and propagation of hedgehog signaling; sonic hedgehog (SHH) is responsible for patterning and development of the central nervous system (Porter, et al, 1996).

Within any given tissue, cholesterol originates from either de novo synthesis or exogenous sources.
Chapter Two

Materials and methods

2.1 Subjects

Patients and control samples have been collected from the referred patients to the Sudan Atomic Energy commission (RIA Lab) in Khartoum state.

Study group was 48 females with hyper thyroidism. Their ages were (18-50 years) and 50 females without any thyroid disorder and no history of endocrine abnormalities with the same ages to the patients were used as control group.

Five ml of blood sample have been collected from all patients and controls, serum samples were separated and kept frozen at (-20°C) till the time of analysis.

2.2 Radioimmunoassay (RIA) techniques for determination of T\textsubscript{4}, T\textsubscript{3} and TSH

2.2.1 Equipments and materials

- Adjustable Micropipettes (50, 100 and 200μl)
- Disposable polystyrene tubes
- Multitube vortex
- Distilled water
- Oven or water path
- Suitable gamma counter
- Colorimeter for cholesterol measurement

2.3.2 T\textsubscript{4} kit

- Magnetic racks and Magnetic separator
Principle
The (T4) radioimmunoassay method depends on the competition between iodine-125 labeled T4 and T4 contained in standard or in specimens to be assayed, for a fixed limited number of T4 antibody binding sites. After the incubation, the amount of iodine-125 labeled T4 bound to antibody inversely related to the amount of T4 present in the sample. In the antibody suspension of this kit the antibody is covalently bound to magnetisable particles. Separation of the antibody bound fraction is achieved by magnetic separator and decanting the supernatant. By measuring the proportion of iodine-125 labeled T4 bound in the presence of reference standards containing various known amounts of T4 concentrations, the concentrations of T4 present in the unknown samples can be interpolated.(Ellis&Mardell,1978).
-Contents of the kit (100 test pack)
-1 Bottle (148 k Bq, 4uCi) (125I) T4 solution (55ml, red) in barbitone buffer with bovine serum albumin and ANS (8- anillino-1-naphthalene sulfonic acid).
-1 Bottle T4 antibody suspension (55ml) with antimicrobial agent.
-6 vials T4 standard concentration as (0, 20, 40, 80, 160, 240 ng/ml) prepared by adding 1ml of distilled water into each vial and stand for 10 minutes.
Assay procedure
Sufficient (polystyrene) test tubes were labeled in duplicates and arranged in assay rack, then 25ul was pipetted into each tube of standards, quality control samples and patient’s samples. And 250ul of tracer (125I labeled T4), mixed and 250ul of T4 antibody added to each tube. Then incubated for 45 minutes at 37Co, then the rack was placed in the magnetic
base for 10 minutes, to separate the bound fraction from the free fractions by decant the supernatant. Then each tube was counted in the gamma counter to evaluate the gamma emission per minute, and binding percent was plotted vs. the concentration, to get standard calibration curve, and from the curve obtained the concentration of the $T_4$ in the patient samples was evaluated. This assay is bioassay method, (Radioimmunoassay), using radioactive isotope of iodine $I^{125}$ which is gamma emitter. (Ellis and Ekins, 1978).

**Calculations**

Results can be calculated using either lin-log or logit-log plotting.

1- Express the counts (B) for each of the standards and unknown as a percentage of the zero standard ($B_0$). Subtract background counts if significant.

\[ B\% = \frac{B\text{ of standard or unknown}}{B_0} \times 100\% \]

2- Plot the percentage values obtained for the $T_4$ standards against the $T_4$ concentration on lin-log graph paper and construct a standard curve.
The samples to be assayed (the unknowns) are run in parallel.

After determining the ratio of bound to free antigen in each unknown, the antigen concentrations can be read directly from the standard curve (as shown above).

2.3.3 Total triiodothyronine (T₃) Radioimmunoassay kit (PR)

2.3.3.1 The method

The radioimmunoassay depends on the competition between iodine-(¹²⁵I) labeled T₃ and T₃ contained in standard or in specimens to be assayed, for a fixed limited number of T₃ antibody binding sites. After the incubation, the amount of iodine-(¹²⁵I) labeled T₃ bound to antibody is inversely related to the amount of T₃ present in the sample. By measuring the proportion of iodine-125 labeled T₃ bound in the presence of reference standards containing various known amounts of T₃ concentrations, the concentrations of T₃ present in the unknown samples can be interpolated. (Oppenheimer, 1968).
a. Contents of the kit
- 1 Bottle (148kBq) $^{125}$T$_3$ solution (22ml red) in barbitone buffer with bovine serum albumin and ANS (8-anallino-1-naphthaline sulfuric acid).
- 6 vials of T$_3$ standards: pipette 1ml of distilled water into each vial for reconstitution and stand for 5 minutes. Their concentrations are 0.0, 0.5, 1.0, 2.0, 4.0 and 8.0 ng/ml.
- 1 Bottle of T$_3$ antibody (22ml blue).
- 1 Bottle precipitant, before used the reagents should be thoroughly mixed by gentle shaking and swirling to ensure a homogeneous suspension.

b. Assay principle
Sufficient number of test tubes were labeled and in duplicate arranged in assay rack. 25ul of standard solution, QC samples and patients sample were added to each tube and mixed the tubes and incubated at 37°C for one and half hour and then precipitating agent polyethylene glycol (PEG) was added and vortex well and then centrifuge to separate bound fraction, (liquid phase separation system) the supernatant was decanted and then each tube have been counted in the gamma counter. The principle of the assay is the same as that for T$_4$.

c. Calculation of results
Results can be calculated using either lin-log or logit-log plotting.
1. Express the counts (B) for each of the standards and unknown as a percentage of the zero standard ($B_0$), subtract background counts if significant (NSB).
   \[ B/B_0\% = B \text{ of standard or unknown} / B_0 \times 100\% \]
2. Plot the percentage values obtained for the T$_4$ standards against the T$_4$ concentration on lin-log graph paper and construct a standard curve.
The samples to be assayed (the unknowns) are run in parallel.

After determining the ratio of bound to free antigen in each unknown, the antigen concentrations can be read directly from the standard curve (as shown above).

### 2.3.4 TSH Radiometric assay kit

For the quantitative determination of human serum with magnetic separation reagent.

**a. Principle of the method**

CIAE TSH IRMA kit utilizes two-site sandwich immunoradiometric assay for the measurement of TSH in human. This involves the reaction of TSH present in specimen with monoclonal and polyclonal antibodies. The monoclonal antibody is labeled with $^{125}$I-McAb and the polyclonal antibody is coupled to magnetic iron oxide particles (Pc Ab<M>). The formed $^{125}$IMcAb-TSH-PcAb<M> complex (sandwich) is separated from the free fractions (tracer) by placing the assay tubes in magnetic separator
and decanting supernatants. The radioactivity of the tracer in the tubes is directly proportionate to the concentration of TSH in the sample (Wait, 1996).

b. Preparation for assay

1. Pipette 1.0ml of distilled water into each of the standard vials for reconstitution refers to the 1ST page for the exact concentrations. (18-25°C). (A-0.0, B-0.13, C-1.0, D-3.0, E-10., F-20, and G-80mIU/L).

2. Allow all reagents and serum to return to room temperature

3. Resuspend magnetic TSH antibody suspension by gentle mixing don’t magnetic stirrer for this purpose.

4. Makeup wash buffer by diluting concentrated wash buffer (20ml) to 180 ml distilled water.

c. Assay procedure

Sufficient test tubes were labeled and arranged in assay rack in duplicates, 100ul of STD and QC and samples was pipetted in corresponding tubes, and 25 ul tracer(anti TSH labeled by^{125}I), and vortexed then incubated at 37°C in incubator for one hour. The 250ul of anti TSH (anti TSH coupled to magnetic particles) (Pc Ab<M>) were added to each tube and mixed well and incubated at room temperature for one hour, the assay rack(s) were placed in the magnetic bases for 10 minutes and the supernatant was separated by decantation.

d. Wash step

In this step firstly we dilute the wash buffer by adding distilled water (1:15), and then 500ml of the diluent was added to each tube and mixed well and placed in the magnetic bases for 10 minutes and decanting again the supernatant, the wash step was repeated again and all the tubes were
counted in the gamma counter, to evaluate the concentration of TSH in the patients samples.

The quantitative analysis of TSH is achieved by the above method, which is immunoradiometric method, non competitive method in which the tracer is TSH antibody ($^{125}$IMcAb-TSH). We have two antibodies react with the TSH in the specimen to form sandwich complex ($^{125}$IMcAb-TSH-PcAb <M>).

e. Calculation of results

Manual calculation of results

1. Plot the count rates for each of standard tube against the TSH concentration on semi-log graph paper (or logit-log graph paper). Draw the standard curve through the mean of duplicate points, rejecting aberrant counts. Atypical curve is shown in figure 1.

2. Calculate the mean count rate for each unknown and read off the TSH concentration from the standard curve.

f. Computerized calculation of results

Good quality IRMA curve fit program should be suitable for calculating results.
IRMA standard curve
2.4 Enzymatic- colorimetric test for the determination of cholesterol concentration (Richmond, 1972)

**Principle**

Cholesterol and its esters are released from lipoproteins by detergent. Cholesterol esterase hydrolyzes the esters and \( \text{H}_2\text{O}_2 \) is formed in the subsequent enzymatic oxidase according to the following equation:

\[
\text{Esters} + \text{cholesterol} + \text{H}_2\text{O} \xrightarrow{\text{CHE}} \text{Cholesterol} + \text{Fatty acid}
\]

\[
\text{Cholesterol} + \text{O}_2 \xrightarrow{\text{CHOD}} \text{Cholesterol 4-enone} + \text{H}_2\text{O}_2
\]

\[
\text{H}_2\text{O}_2 + \text{4-AP} + \text{phenol} \xrightarrow{\text{POD}} \text{Quinonimine} + \text{H}_2\text{O}
\]

**Reagents**

**Table1.**

**Cholesterol reagents**

<table>
<thead>
<tr>
<th>Reagent1</th>
<th>Pipes pH 6.9</th>
<th>90 mmol/L</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Phenol</td>
<td>26 mmol/L</td>
</tr>
<tr>
<td>Reagent2</td>
<td>Peroxidase</td>
<td>1250 ul</td>
</tr>
<tr>
<td>Vial of enzyme</td>
<td>Cholesterol oxidase</td>
<td>300 ul</td>
</tr>
<tr>
<td></td>
<td>4-Aminophenazone</td>
<td>300 ul</td>
</tr>
<tr>
<td>Standard</td>
<td>Cholesterol solution</td>
<td>0.4 mmol/L</td>
</tr>
<tr>
<td></td>
<td></td>
<td>200 mg/dl</td>
</tr>
</tbody>
</table>

**Preparation and stability**

Dissolved the contents of one bottle R2 to the contents of one bottle buffer reagent R1. To prepare the working reagent which is stable for 4 months at 2-8\(^{\circ}\)C or 40 days at room temperature when stored in dark bottle.
**Table 2: Cholesterol procedure**

<table>
<thead>
<tr>
<th></th>
<th>Blank</th>
<th>Standard</th>
<th>Sample</th>
</tr>
</thead>
<tbody>
<tr>
<td>Standard</td>
<td>---</td>
<td>10ul</td>
<td>---</td>
</tr>
<tr>
<td>Sample</td>
<td>---</td>
<td>---</td>
<td>10ul</td>
</tr>
<tr>
<td>Working reagent</td>
<td>1.0 ml</td>
<td>1.0ml</td>
<td>1.0ml</td>
</tr>
</tbody>
</table>

Mix incubate for 5 minutes at 37°C measure the extinction (E) of standard and sample(s) against Blank reagent at 505nm (500-550).

**Calculation of results**

Cholesterol conc. = \(E_{\text{sample}} \times \text{con standard} = \text{Standard con} \times 200\text{mg/dl.}\)

\[E_{\text{standard}}\]
Chapter Three

Results and discussion

Table 3 shows the effect of hyperthyroidism on the levels of T3, T4, TSH and cholesterol. Mean ± SD.

<table>
<thead>
<tr>
<th>Parameters levels</th>
<th>T3 nmol/L</th>
<th>T4 nmol/L</th>
<th>TSH MIU/L</th>
<th>Cholesterol mg/L</th>
</tr>
</thead>
<tbody>
<tr>
<td>Euthyroid subjects N = 50</td>
<td>1.8 ±0.4</td>
<td>103 ±17</td>
<td>2.0 ± 0.8</td>
<td>185 ± 20</td>
</tr>
<tr>
<td>Hyperthyroid subjects N = 48</td>
<td>4.9 ± 1.2</td>
<td>204±18.4</td>
<td>0.16</td>
<td>112 ±13.7</td>
</tr>
</tbody>
</table>

This study included 98 Sudanese female subjects, 48 with hyperthyroidism, their blood samples collected from the referred patients to radioimmunoassay lab (RIA) at Sudan atomic energy commission (SAEC), and 50 euthyroid ones used as control.

For the two groups cholesterol concentration was determined using enzymatic method. The results from the all study groups is presented in tables (1), (2) and (3). The mean serum total cholesterol level showed significantly (P<0.05) lower level in the hyperthyroid group, of (112 ± 13.7) mg/dL, whereas the control group recorded (185 ± 20mg/dL).

The relation between serum concentration of T3, T4, and TSH for independent samples and the total serum cholesterol were performed. All hormones were found to be strongly correlated with total serum cholesterol concentration (P<0.01) Tables (4), (5) and (6).
$T_4$ and $T_3$ were inversely correlated to cholesterol as shown in figure (6). The increase in $T_4$ and $T_3$ concentrations was followed by decrease in serum total cholesterol levels.

Whereas TSH is directly correlated to cholesterol. The increase in TSH concentrations was followed by increase in serum total cholesterol levels.

Fig. 6

Cholesterol in normal (Euthyroid) and hyperthyroid patients with mean cholesterol 185 and 113.
Relation between cholesterol and thyroxin in normal (Euthyroid) and hyperthyroid patients.
Fig. 8

cholesterol

GROUP: 1 Euthyroid

Correlation between T4 and cholesterol in the Euthyroid group.
Correlation between thyroxin and cholesterol in the hyperthyroid group
### Table 5

**Correlations**

**Group = Control**

<table>
<thead>
<tr>
<th></th>
<th>T₄</th>
<th>T₃</th>
<th>TSH</th>
<th>Cholesterol</th>
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<td><strong>T₄</strong> Pearson Correlation</td>
<td>1.000</td>
<td>.014</td>
<td>.018</td>
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<td>Sig. (2-tailed)</td>
<td></td>
<td>.926</td>
<td>.899</td>
<td>.353</td>
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<td>1.000</td>
<td>.224</td>
<td>-.205</td>
</tr>
<tr>
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<td></td>
<td>.117</td>
<td>.154</td>
</tr>
<tr>
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<td>50</td>
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<td>50</td>
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<tr>
<td><strong>TSH</strong> Pearson Correlation</td>
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<td>1.000</td>
<td>-.136</td>
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<tr>
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<td>.346</td>
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<td>-.205</td>
<td>-.136</td>
<td>1.000</td>
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<tr>
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<td>.154</td>
<td>.346</td>
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A Group = Euthyroid
**Table -6**

**Group = Hyperthyroidism**

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<td>T3</td>
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<tr>
<td>Sig. (2-tailed)</td>
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<td>.987</td>
<td>.683</td>
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<tr>
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<td>48</td>
<td>48</td>
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</table>

**Correlation is significant at the 0.01 level (2-tailed).**

* Correlation is significant at the 0.05 level (2-tailed).

A Group = Hyperthyroidism
Findings in the present work showed that, serum total cholesterol levels of hyperthyroid females reported very low levels, when compared to the levels of euthyroid females.

In previous study, the total cholesterol level was studied in five patients with hyperthyroidism. The work reported that, hyperthyroidism is accompanied with decreased serum level of cholesterol, which increased by treatment (ÖBrien, et al (1993). Also Dunats, (2002) concluded that, the composition and transportation of lipoprotein are seriously disturbed in thyroid disease. The changes in lipoprotein are correlated with changes in free thyroxin (FT$_4$) levels; hyperthyroidism exhibits an enhanced excretion of cholesterol and an increased turnover of LDL resulting in decrease of total LDL cholesterol.

The result of this study again agreed with (Cachefo, et al (2001) who mentioned that hyperthyroid patients had decreased level of plasma cholesterol (P<0.01). Increased thyroid hormones decrease the level of cholesterol, phospholipids, and triglycerides in the plasma even though it increases the free fatty acids. Conversely, decreased thyroid hormones secretion greatly increases the plasma concentration of cholesterol, phospholipids and triglycerides. And almost always causes excessive deposition of fats in the liver as well. The large increase in circulating cholesterol in prolonged hypothyroidism is often associated with atherosclerosis.

Guyton and Hall, (2000) suggested that, one of the mechanisms by which thyroid hormones decreases the plasma level of cholesterol is to increase significantly the rate of cholesterol secretion in the bile and consequently loss in feces. other possible mechanism for the increased cholesterol secretion is that thyroid hormones induce increased number of low density
lipoprotein receptors on the liver cells, leading to rapid removal of low-density lipoproteins from the plasma by the liver and subsequent secretion of cholesterol in these lipoproteins by the liver cells.

The effect of increased thyroid hormones levels in hyperthyroid patients, which results in low serum total cholesterol levels was concluded in the present work. This result supported findings reported in studies abroad and proved that Sudanese female patients also suffer the same effects due to hyperthyroidism.
Conclusions

The thyroid dysfunction altered the level of total cholesterol in Sudanese females.

Increasing levels of T4 and T₃, correlated negatively with serum total cholesterol concentration.

Decreasing levels of TSH, correlated negatively with serum total cholesterol concentration.

Hyperthyroidism in the present work resulted in significantly (P<0.05) lower serum total cholesterol levels, in patients compared to euthyroid females.
Chapter Five

References

1. Barbagllo, C. M. Averna, M. R; Liotta, A; L. Gretta’s Plasma level of lipoproteins, and apolipoproteins in hyperthyroidism. Effects of L-thyroxine substitution therapy. Metabolism 44(10) 1283-7.


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