



Sudan University of Science and Technology



Collage of Graduate Studies

**Evaluation of Serum Lipid Profile among Sudanese  
Patients with Hyper or Hypothyroidism  
In Khartoum State**

تقويم مستوي الدهون في مصل المرضي السودانيين المصابين بفرط نشاط  
الغدة الدرقية أو قصور الغدة الدرقية في ولاية الخرطوم

A dissertation submitted in partial fulfillment for requirements of M.Sc. degree  
in medical laboratory science (Clinical Chemistry).

**By:-**

Moneer Abd El-bagi Ali Ibrahim

**Supervisor:**Dr. Nuha Eljaili Abubker

Assistant professor of Clinical Biochemistry

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# الايه

بسم الله الرحمن الرحيم

قال تعالى:

( قل لو كان البحر مدادا لكلمات ربي  
لنفد البحر قبل ان تنفد كلمات ربي ولو  
جئنا بمثله مددا)

سورة الكهف الايه (109)

## **Dedication**

*To .....*

*Memories of my father, sisters and brothers*

*To .....*

*Whom may God bless me because of her prayers...My  
mother!!*

*To .....*

*The compassionate souls which make my dreams come to*

*Reality .... My father*

*To .....*

*The person who devoted her time and energy helping us to*

*Achieving this study ... my respectful supervisor*

*Ustaz: Nuha Elj-ailiAbubker*

*To .....*

*The peoples, whom I love, respect and appreciate.*

*To.....*

*All these dedicated my study.*

## **Acknowledgments**

Thank you **My God**, for giving me the ability and courage to bring this research to light.

Thank you my family, my father, my mother, my sisters and my brothers for helping, encouragement, and supporting me.

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My supervisor, who started with me this thesis from the zero level. He was the one who directed me to this thesis important topic, so I am really grateful to him.

I feel indebted to many people who participated fully and helped me a lot to achieve this work.

I would like to thank all people in Clinical Chemistry department Sudan university who have been a positive influence, those who helped me to seek my way to a solid ground & stand on it, also thanks to all the people who seemed to be negative influences, they taught me how to be patient, & how to be a better person.

## Abstract

A case control study conducted during the period from April to October 2016, to evaluate serum lipid profile among Sudanese patients with thyroid diseases (hyperthyroidism and hypothyroidism) in Khartoum state.

The study group included 60 patients 30 hyperthyroidism, and 30 were hypothyroidism and 30 were apparently healthy individuals as control group (match age and sex).

Aspectrophotometer was used for measurement of lipid profile (total cholesterol, triglycerides, LDL-c and HDL-c). ELISA reader was used for hormones measurements (total T4, T3) and TSH and program software SPSS was used for analysis the results.

The results showed that thyroid disorders are common among female (85%) while male was (15%). Also most common among age (36-45) years (in hyperthyroidism (53%), hypothyroidism (57%).

In hyperthyroidism total cholesterol, triglycerides and LDL-c were significantly increased when compared to a control group while HDL-c was significantly decreased, as in table (4-2) total cholesterol (mean± SD: 187 ±39 mg/dl versus 136.8±20.9mg/dl

p.value = 0.00), triglycerides (mean± SD:164.6 ±69.6 mg/dl versus 118±37.4mg/dl, p.value =0.00), LDL-c (mean± SD:126.7 ±43.9 mg/dl versus

80.4±18.9 mg/dl, p.value = 0.00) and HDL-c (mean± SD: 27.9 ±10.1mg/dl versus 32.7±6.7 mg/dl, p.value =0.00).

In hypothyroidism the level of total cholesterol, triglycerides, and LDL-c were significant increased (mean± SD: 190.6 ±48.1mg/dl versus 136.8 ± 20.9 mg/dl p.value 0.00) (mean± SD: 182 ±92.5 mg/dl versus 118 ±37.4mg/dl, p.value = 0.00) LDL-c (mean± SD: 129.2 ±49.9 mg/dl versus 80.4±18.9mg/dl, p.value = 0.00), HDL-c was significant decreased (mean± SD: 24.9± 8.3mg/dl versus 32.7 ±6.7mg/dl, p.value = 0.00).

Correlation studies were showed significant positive correlation between TSH and total cholesterol, triglycerides and LDL-c (r=0.434, 0.252, and 0.404. p.value= 0.00, 0.025, 0.00) respectively. While a significant negative correlation with HDL-c (r=-0.252, p.value=0.024).

The study concluded that there were significant increased in lipid profile (total cholesterol, LDL-c and triglycerides) in both hyperthyroidism and hypothyroidism in compared to control subject, while HDL-c was significant decreased in both hyperthyroidism and hypothyroidism in compared to control.

Also there were significant positive correlation between TSH level and total cholesterol, triglycerides, LDL-c and significant negative correlation with HDL-c.

## مستخلص الدراسة

أجريت هذه الدراسة في الفترة من ابريل وحتى اكتوبر ٢٠١٦م لتقويم مستوى مصلى الدهون لدى المرضى السودانيين المصابين بفرط نشاط الغدة الرقية او قصور نشاط الغدة الرقية في ولاية الخرطوم.

مجموعة الدراسة تشمل المصابين بامراض الغده الرقية 60(٣٠مريض لديهم فرط نشاط الغدة الرقية 30 لديهم قصور في نشاط الغدة الرقية) 30 من الاصحاء كمجموعة تحكم, وتم التوفيق بين مجموعة المرضى ومجموعة التحكم من حيث العمر والنوع.

جهاز قياس الطيف الضوئي استخدم لعمل التحاليل الخاصة لمستوى الدهون في المصل (الكلسترول الكلي ثلاثى جليسيراييد, البروتينات الدهنية ذات الكثافة العالية والمنخفضة ) بينما استخدمت طريقة الاليزا لقياس مستوى هرمونات الغدة الرقية فى المصل (ثيروكسين الرباعى الكلى, ثيروكسين الثلاثى الكلي, والهرمون المنشط للغده الرقية) وتم تحليل البيانات بواسطة برنامج الحزمة الاحصائية للعلوم الاجتماعية.

اظهرت الدراسة ان امراض الغدة الرقيه اكثر شيوع لدى الاناث (85% ) من الذكور(15%) وكذلك اكثر انتشارا لدى المرضى في الاعمار ( 36-45) سنة (53% فرط نشاط الغدة الرقية و 57% لديهم قصور في نشاط الغدة الرقية).

عند مقارنة متوسط مستوى الدهون لدى المرضى بفرط نشاط الغدة الرقية بمجموعة التحكم وجد ان هناك زيادة ذات دلالة احصائية في مستوى (الكلسترول الكلي ثلاثى جليسيراييد, والبروتينات الدهنية ذات الكثافة المنخفضة) بينما هناك انخفاض ذو دلالة احصائية في مستوى البروتينات ذات الكثافة العالية.

كما في الجدول (2-4): للكستروال الكلي (المتوسط±الانحراف المعياري: 39±187 مقابل 136.8± 20.9 ملجم/100 مل, وكان الاحتمال الاحصائي للمقارنة 0.00) للثلاثي الجلسيريد (164±69.6 مقابل 118±37.4 ملجم/100 مل, وكان الاحتمال الاحصائي للمقارنة 0.00) للبروتينات ذات الكثافة المنخفضة (126.7±43.9 مقابل 80.4±18.9 ملجم/100 مل, وكان الاحتمال الاحصائي للمقارنة 0.00) اما البروتينات ذات الكثافة العالية (27.9±10.9 مقابل 32.7±6.7 ملجم/100 مل, وكان الاحتمال الاحصائي للمقارنة 0.00).

تحليل معامل بيرسون للارتباط اظهر ان هناك علاقة ايجابية ذات دلالة احصائية بين الهرمون المنشط للغدة الرقية و مستوى الكستروال الكلي, ثلاثي جلسرايد , وكستروال البروتينات منخفضة الكثافة (معامل ارتباط بيرسون (0.404,0.2520.434) الاحتمال الاحصائي للمقارنة (0.00, 0.02) 0.00) علي التوالي.

بينما هناك علاقة سلبية ذات دلالة احصائية بين الهرمون المنشط للغدة الرقية ومستوى كستروال البروتينات عالية الكثافة (معامل ارتباط بيرسون (-0.252) الاحتمال الاحصائي للمقارنة (0.024).

خلصت هذه الدراسة الي ان هناك زيادة ذات دلالة احصائية في مستوى حزمة الدهون الكلية في كل من مرضى المصابين بفرط نشاط الغدة الرقية وبقصور نشاط الغدة الرقية عند المقارنة بمستوى الدهون لدى الاصحاء ,بينما كستروال البروتينات ذات الكثافة العالية انخفض في كل من المصابين بفرط نشاط و قصور الغدة الرقية عند المقارنة بمستوي كستروال البروتينات ذات الكثافة المنخفضة لدى الاصحاء. ايضا هناك علاقة ايجابية ذات دلالة احصائية بين الهرمون المنشط للغدة الرقية ومستوى الكستروال الكلي, ثلاثي جلسريد, وكستروال البروتينات منخفضة الكثافة. بينما هناك علاقة سلبية ذات دلالة ايجابية بين الهرمون المنشط للغدة الرقية وكستروال البروتينات ذات الكثافة العالية.



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### **Abbreviations**

Abbreviations

Full term

CETP

Cholesteryl ester transfer protein

DIT

Diiodothyronine

ELISA	Enzyme linkage immunosorbent assay
HDL-c	High density lipoprotein cholesterol
LDL-c	Low density lipoprotein cholesterol
HMG-CoA	Hydroxy-methylglutaryl coenzyme A
MIT	Monoiodothyronine
TPO	Thyroid peroxidase
TR	Thyroid hormones receptor
TRH	Thyrotrophic releasing hormones
TSH	Thyroid stimulating hormones

# **CHAPTER ONE**

## **Introduction**



# 1. Introduction

## 1.1 Introduction:

Thyroid problems are one of the most common endocrine problems encountered in laboratory and clinical practice. Thyroid hormones are important regulators of many metabolic processes through their effect on protein, carbohydrate and lipid metabolism and also affect the basal metabolic rate. They have significant effect on synthesis, mobilization and metabolism of lipids. Hypothyroidism means under functioning of the thyroid gland while the over-functioning of thyroid gland is known as hyperthyroidism, hypothyroidism is more prevalent and incidence of hypothyroidism is twice to that of hyperthyroidism (Mohsin *et al.*, 2013).

Thyroid dysfunction is more common in women and epidemiological rate of prevalence rise with age (Sayed *et al.*, 2015). Diseases of thyroid gland are amongst the most abundant endocrine disorder in the world second only to diabetes mellitus (Shrestha, 2011).

Thyroid hormones are important modulator of intermediary metabolism, affect synthesis, mobilization and degradation of lipids although degradation is influenced more than synthesis, thyroid dysfunction associated with dyslipidemia which increased risk of endothelial dysfunction, hypertension, and cardiovascular diseases (Shrestha, 2011).

## **1.2 Rationale:-**

Thyroid disorders a global health problem with an increasing incidence. The thyroid disorders are spread in all regions of Sudan, especially in western Sudanese in some areas up to 70% of population, According to a national study conducted in 1997, the overall prevalence of all types of goiter was 22%and prevalence ranged from 5% in the city of Khartoum to 42% in the Upper Nile region. It has been estimated that every year more than 200 000 children born in the Sudan are at risk of iodine deficiency. That 3% of those children may develop cretinism, while 10% may experience severe intellectual impairment and 87% less severe intellectual disability, thyroid dysfunction associated with dyslipidemia, which increased endothelial cell dysfunction, hypertension and cardiovascular diseases.In Sudan there is few published data concerning thyroid disorders and its effects on serum lipid profile. Therefore the present study was conducted to evaluate serum lipid profile in Sudanese patients with thyroid dysfunction.

### **1.3 Objectives:**

#### **1.3.1 General objectives:**

To evaluate serum lipid profile among Sudanese patients with thyroid diseases (hyperthyroidism and hypothyroidism).

#### **1.3.2 Specific objectives:**

1-To estimate serum lipids profile (total cholesterol, triglycerides LDL-c and HDL-c) in study groups.

2- To estimate serum thyroid hormones (total T4 total T3 and TSH) in all participants.

3-To correlate between TSH level and (total cholesterol, triglycerides, LDL-c and HDL-c) in thyroid diseases (hyperthyroidism and hypothyroidism)

# **CHAPTER TWO**

## **Literature review**

## **2. Literature review**

### **2.1 Thyroid gland:-**

The thyroid gland is positioned in the lower anterior neck and is shaped like a butterfly made up of two lobes that rest on each side of trachea as wings, with a band of thyroid tissue called the isthmus, two parathyroid glands usually lie on each side at the back of thyroid gland is an endocrine gland(Marissa, 2010).

The thyroid gland is one of the largest of the endocrine organs weighing about 2-3grams in neonates and 25grams in adults, the normal thyroid gland is made up of two lobes Each lobe is about 5cm long, 3cm wide and 2cm thick joined by a thin band of tissue (isthmus), the gland is composed of closely packed spherical units (follicles), connected with a rich capillary network (Reed *et al.*, 2003).

#### **2.1.1 Thyroid gland functions:-**

The function of the thyroid gland is to generate the quantity of thyroid hormone necessary to meet the demands of the peripheral tissues, The thyroid also contains parafollicular cells (C cells) that are the source of the calcium-lowering hormone, called calcitonin(Reed *et al.*, 2003).

#### **2.1.2 Thyroid hormones synthesis:-**

Thyroid hormone is made primarily of the trace element iodine. Thyroid cells are organized into follicles. Follicles are spheres of

thyroid cells surrounding a core of a viscous substance termed colloid. The major component of colloids thyroglobulin, on the outer side of the follicle, iodine is actively transported into the thyroid cell by the Na<sup>+</sup>/I<sup>-</sup> symporter located on the basement membrane. Inside the thyroid cell, iodide diffuses across the cell to the apical side of the follicle, which abuts the core of colloid. Here, catalyzed by a membrane-bound enzyme called thyroid peroxidase (TPO). Concentrated iodide is oxidized and bound with tyrosyl residues on thyroglobulin. This results in production of monoiodothyronine (MIT) and diiodothyronine (DIT). This same enzyme also aids in the coupling of two tyrosyl residues to form triiodothyronine (T3) (one MIT residue\_ one DIT residue) or thyroxin (T4) (two DIT residues) (Marissa, 2010).

### **2.1.3 Thyroid hormones biochemistry:-**

Thyroid gland secretes two hormones, thyroxin (3, 5 3, 5-L-tetraiodothyronine) and triiodothyronine (3, 5 3-L-triiodothyronine) which are commonly known as T4 and T3 respectively. In addition, the thyroid gland secretes small amounts of biologically inactive (3,3,5-L-triiodothyronine) called reverse T3. Approximately 40% of the secreted T4 is deiodinated by peripheral tissue by enzymes deiodinase to yield T3 and 45% is deiodinated to yield r T3 (Laurence *et al.*, 2006).

### **2.1.4 Mechanism of Thyroid Hormone Action:-**

Thyroid hormones regulate a wide range of genes after its activation from prohormones, thyroxin (T4) to active form,

triiodothyroxine(T3) which is more active than T4 (Gregory *et al.*, 2012).

Thyroid hormone acts by binding to a specific nuclear DNA-bound thyroid hormone receptor (TR) T3 has a 15-fold higher binding affinity for TRs than does T4, which explains its function as the active thyroid hormone. In humans, two TR genes are found on different chromosomes (TR on chromosome 17, TR on chromosome 3)(Reed *et al.*, 2003).

Once released from the thyroid gland, thyroid hormone circulates in the bloodstream where free T4 and T3 are available to travel across the cell membrane. In the cytoplasm, T4 is deiodinated into T3, the active form of thyroid hormone. T3 combines with its nuclear receptor on thyroid hormone-responsive genes, leading to production of messenger RNA that, in turn, leads to production of proteins that influence metabolism and development. Effects of thyroid hormone include tissue growth, brain maturation, increased heat production, increased oxygen consumption, and an increased number of -adrenergic receptors (Marissa, 2010).

### **2.1.5 Regulation of thyroid function:-**

The thyroid gland participates with the hypothalamus and pituitary gland in a classic feedback control loop. In addition, there is an inverse relationship between the glandular organic iodine level and the rate of hormone formation. The parvocellular region of the paraventricular nuclei of the hypothalamus is the

source of the TRH that regulates TSH secretion, When secreted, this hormone stimulates cells in the anterior pituitary gland to manufacture and release thyrotropin (TSH). TSH, in turn, circulates to the thyroid gland and leads to increased production and release of thyroid hormone. When the hypothalamus and pituitary sense that there is an inadequate amount of thyroid hormone in circulation, TRH and TSH secretion increase and will lead to increased thyroid hormone production. If thyroid hormone levels are high, TRH and TSH release will be inhibited, leading to lower levels of thyroid hormone production and vice versa if thyroid hormone levels are low. This feedback loop requires a normally functioning hypothalamus, pituitary, and thyroid gland, as well as an absence of any interfering agents or agents that mimic TSH action (Marissa, 2010).

### **2.1.6 Thyroid dysfunction:-**

Hypothyroidism and hyperthyroidism are the two primary pathological conditions that involve the thyroid gland, hyperthyroidism term to describe over production of thyroid hormones, while hypothyroidism defect in production of thyroid hormones (Laurence *et al.*, 2006).

#### **2.1.6.1 Hyperthyroidism:-**

Hyperthyroidism is defined as a hypermetabolic condition caused by excessive production of thyroid hormones. This disorder is caused by a number of conditions resulting from excess availability of thyroid hormones. Some clinician prefer



the general term thyrotoxicosis rather than hyperthyroidism to define the hypermetabolic state associated with increased amounts of thyroid hormones in the circulation, the common cause of thyrotoxicosis due to hyperthyroidism is graves' disease, an autoimmune disorders (Laurence *et al.*, 2006).

Symptoms of hyperthyroidism:-

Weight loss, fatigue, nervousness, palpitation, rapid pulse, menstrual irregularities, heat intolerance, increased sweating, diarrhea, restlessness, tremor, muscle weakness, eye changes (grave's disease), and variable gland enlargement(Laurence *et al.*, 2006).

#### **2.1.6.2 Hypothyroidism:-**

Hypothyroidism is defined as a deficiency in thyroid hormones secretion and action. Myxedema is a severe form of hypothyroidism in which there is accumulation of mucopolysaccharides in the skin and other tissues, leading to a thickening of facial features and a doughy induration of the skin. Cretinism is the term used to describe severe hypothyroidism that develops in the newborn period (Laurence *et al.*, 2006).

**Symptoms of hypothyroidism:-**

Weight gain, easy fatigue, lethargy, cold intolerance, hair loss, constipation, depression, slow reflexes, slower heart beat, hoarseness/ Deeping voice, dry patchy skin, elevated cholesterol,

puffy eyes, menstrual irregularities and muscle weakness (Laurence *et al.*, 2006).

Types of hypothyroidism:-

1-Primary hypothyroidism; thyroid gland dysfunction, defect in production of thyroid hormones with normal stimulation of thyroid stimulating hormones TSH due to (iodine deficiency, thyroidectomy, and autoimmune diseases)

2-Secondary hypothyroidism; normal thyroid function with pituitary dysfunction, Deficiency or defect in TSH production or action

3-Tertiary hypothyroidism; hypothalamic dysfunction ,with normal thyroid gland and pituitary gland function, defect in production of thyrotropin releasing hormone (TRH)(Marissa, 2010).

### **2.1.7 Thyroid stimulating hormone(TSH):-**

Thyrotrophic is glycoprotein hormone, hetero dimer of two non covalently linked subunits,molecular weight of 26.6KDa,synthesized in the adenohypophysis that promotes the growth of, sustains, and stimulate the hormonal secretion of thyroid gland(Laurence *et al.*, 2006).

Thyrotrophic releasing hormone a tripeptide is produced in hypothalamus .TRH act on the pituitary thyrotrophic to stimulate both synthesis and release of TSH .TSH in turn control the

thyroid gland and the synthesis and release of the thyroid hormone. TSH also control size and number of thyroid follicular cells. Arise in thyroid hormone concentration elicit an inhibitory effect on the pituitary response to TRH conversely a fall in thyroid hormones concentration cause an increase in both TRH and TSH secretion (Laurence *et al.*, 2006).

TSH acts on the thyroid gland to induce thyroid hormone synthesis and release and to maintain trophic thyroid cell integrity. The TSH G protein-coupled (GPC) receptor is located on the thyrocyteplasma membrane and is encoded by a gene on chromosome 11q31, the TSH production rate is normally 100 to 400 mIU/day with a calculated circulating half-life of about 50 minutes. Secretion rates are enhanced up to 15-fold in hypothyroid subjects and are suppressed in states of hyperthyroidism (Reed *et al.*, 2003).

## **2.2 Lipids:**

Lipids are hydrophobic molecules that are insoluble or minimally soluble in water. They are found in cell membranes, which maintain cellular integrity and allow the cytoplasm to be compartmentalized into specific organelles. Lipids function as a major form of stored nutrients (triglycerides), as precursors of adrenal and gonadal steroids and bile acids (cholesterol), and as extracellular and intracellular messengers (e.g. prostaglandins, phosphatidylinositol). Lipoproteins provide a vehicle for transporting the complex lipids in the blood as water-soluble complexes and deliver lipids to cells throughout the body (Robert *et al.*, 2003).

### **2.2.1 Plasma lipid:**

#### **2.2.1.1 Cholesterol:-**

Cholesterol is an unsaturated steroid alcohol containing four rings (A, B, C, and D), and it has a single C-H side chain tail similar to a fatty acid in its physical properties. The only hydrophilic part of cholesterol is the hydroxyl group in the A-ring. Cholesterol is, therefore, also an amphipathic lipid and is found on the surface of lipid layers along with phospholipids. Cholesterol is oriented in lipid layers so that the four rings and the side chain tail are buried in the membrane in a parallel orientation to the fatty acid acyl chains on adjacent phospholipid molecules. The polar hydroxyl group on the cholesterol A-ring faces outward, away from the lipid layer,

allowing it to interact with water by monocovalent hydrogen bonding. Cholesterol can also exist in an esterified form called cholesteryl ester, with the hydroxyl group conjugated by an ester bond to a fatty acid, in the same way as in triglycerides. In contrast to freecholesterol, there are no polar groups on cholesteryl esters, making them very hydrophobic Cholesterol is also unique in that, unlike other lipids, it is not readily catabolized by most cells and, therefore, does not serve as a source of fuel. Cholesterol can, however, be converted in the liver to primary bile acids, such as cholic acid and chenodeoxycholic acid, which promote fat absorption in the intestine by acting as detergents. A small amount of cholesterol can also be converted by some tissue, such as the adrenal gland, testis, and ovary, to steroid hormones, such as glucocorticoids, mineralocorticoids, and estrogens.

Finally, a small amount of cholesterol, after first being converted to 7-dehydrocholesterol, can also be transformed to vitamin D3 in the skin by irradiation from sunlight (Amaret *al.*, 2010).

### **2.2.1.2 Fatty Acids**

Fatty acids, as seen in the structure shown in, are simply linear chains of C-H bonds that terminate with a carboxyl group (-COOH). In plasma, only a relatively small amount of fatty acids exists in the free or unsterilized form, most of which is bound to albumin(Amar *et al.*, 2010).

### **2.2.1.3 Triglycerides**

As can be inferred from the name, triglycerides contain three fatty acid molecules attached to one molecule of glycerol by two ester bonds. Each fatty acid in the triglyceride molecule can potentially be different in structure, thus producing many possible structural forms of triglycerides. Triglycerides containing saturated fatty acids (Amar *et al.*, 2010).

### **2.2.1.4 Phospholipids**

Phospholipids are similar in structure to triglycerides except that they only have two esterified fatty acids (The third position on the glycerol backbone instead contains a phospholipid head group. There are several types of phospholipid head groups, such as choline, inositol, serine, and ethanolamine, which are all hydrophilic in nature. The various types of phospholipids are named based on the type of phospholipid head group present. Phosphatidylcholine, for example, has a choline head group and is the most common phospholipid found on lipoproteins and in cell membranes (Amar *et al.*, 2010).

### **2.2.2 Plasma lipoproteins:**

Lipoproteins are synthesized by the liver and the intestines. Their main physiological function is that of transporting dietary and endogenously synthesized lipids in the blood. Lipoproteins are macromolecular complexes. Their constituents are arranged to form an outer shell or envelope, comprised of the amphipathic Apo lipoproteins, phospholipids and free cholesterol (polar

groups facing outwards), surrounding an inner core containing the non-polar cholesterol esters and triglycerides. In normal plasma, there are five main lipoprotein classes, defined according to their behavior upon ultracentrifugation (sedimentation rates), which depends on their hydrated densities, they also differ in their lipid and Apo lipoprotein content as well as in electrophoretic mobility (Evelyn *et al.*, 1999).

#### **2.2.2.1 Chylomicrons:**

These are large triglyceride-rich complexes, formed from dietary lipids and Apo lipoproteins in the microvillus of the small intestines. They are released into the lacteals of the lymphatics, reaching the systemic system via the thoracic duct. For a few hours after each meal, they serve to transport exogenous lipids from the intestines to all cells but are virtually absent from normal plasma after a 12-hour fast. Chylomicrons are the largest of the lipoproteins, and therefore have the lowest density and electrophoretic mobility. They contain very little protein (1-2%) but their Apo lipoprotein components, especially Apo B-48, are essential for their synthesis and secretion. This is illustrated by the complete absence of chylomicrons from the plasma of patients with beta lipoproteinemia, in whom there is an inherited defect of Apo B secretion (Evelyn *et al.*, 1999).

#### **2.2.2.2 Very low density lipoproteins (VLDL):**

These are moderately large particles, with triglycerides as the main lipid component, and Apo B-100, the main Apo

lipoprotein. They are formed mainly in the liver, and to a lesser extent in the intestinal mucosa. Their main function is to transport endogenously synthesized lipids from the liver to extra hepatic cells for utilization or storage. With a half-life of 2-4 hours, the rate of turnover of VLDL in humans is less rapid than that of chylomicrons. Two other classes of lipoproteins, the intermediate density and the low density lipoproteins, are derived from VLDL metabolism (Evelyn *et al.*, 1999).

**Intermediate density lipoproteins(IDL):**

The intermediate density lipoproteins (IDL), sometimes termed VLDL remnants, are transient intermediates formed during the conversion of VLDL to LDL. By virtue of their transient existence, IDL are not detected in normal plasma. However, in certain forms of hyperlipidemia, IDL excess in the plasma may produce a characteristic 'broad p' band during lipoprotein electrophoresis and thus can become a major determinant of both serum total cholesterol and triglyceride concentrations (Evelyn *et al.*, 1999).

**2.2.2.3 Low density lipoproteins (LDLc):**

These are relatively smaller, cholesterol-rich particles, derived mainly from the metabolism of VLDL; however, direct hepatic synthesis and secretion of LDLc has been demonstrated in patients with familial hypercholesterolemia and Type III hyperlipidemia. LDL serves as the main cholesterol carrier in the plasma. They differ from their precursors, the VLDL, in their



much lower triglyceride content and in retaining only one of the various Apo lipoproteins found in VLDL, namely, Apo B- 100 (Evelyn *et al.*, 1999).

#### **2.2.2.4 High density lipoproteins (HDLc):**

These are the smallest of the lipoproteins and the densest. Their lipid to protein ratio is 1: 1. Cholesterol (25%) and phospholipids (20%) make up the bulk of the lipid fraction, there being very little triglycerides(5%) in these particles. They are involved in mediating the reverse transport of cholesterol from cells in peripheral tissues to the liver, for excretion from the body. Several subgroups have been recognized, designated HDL- 1, HDL-2, HDL-3, and HDL-4, which differ in their protein and lipid content shape, structure, and density. There is progressive loss of phospholipids, cholesterol, and cholesterol esters when HDLc are transformed from HDL- 1 to HDL-4 (Evelyn *et al.*, 1999).

#### **2.2.3 Relation between thyroid hormones and plasma lipid:**

Thyroid function regulates a wide array of metabolic parameters, and significantly affects lipoprotein metabolism. Thyroid hormones induce the 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase, which is the first step in cholesterol biosynthesis. triiodothyronine (T3) upregulates LDL receptors by controlling the LDL-c receptor gene activation. This T3-mediated gene activation is done by the direct binding of T3 to specific thyroid hormone responsive elements. T3

controls the sterolregulatory element-binding protein-2 (SREBP-2), which inturn regulates LDL-c receptor's gene expression. T3has also been associated with protecting LDL-c from oxidation.Thyroid hormones can influence HDL-c metabolism by increasing cholesteryl ester transfer protein (CETP) activity,which exchanges cholesteryl esters from HDL2 to the verylow density lipoproteins (VLDL) and triglycerides to the oppositedirection. In Addition, thyroid hormones stimulate thelipoprotein lipase (LPL), which catabolizes the triglycerides-rich lipoproteins,and the hepatic lipase (HL), which hydrolyzesHDL2 to HDL3 and contributes to the conversion of intermediate-density lipoproteins (IDL) to LDL (Rizos, 2011).

# **CHAPTER THREE**

## **Materials and methods**

### **3. Materials and Methods:**

#### **3.1 Materials:**

##### **3.1.1 Study approach:**

Quantitative methods were used to estimate lipid profile and thyroid hormones in Sudanese patients with thyroid diseases (hyperthyroidism and hypothyroidism) in Khartoum state during the periods from April to October 2016.

##### **3.1.2 Study design:**

This was case -control study.

##### **3.1.3 Study area:**

This study was conducted in Khartoum state.

##### **3.1.4 Study populations:**

The study included patients with thyroid diseases (hyperthyroidism and hypothyroidism).

##### **3.1.5 Sample size:**

This study included 60 patients and 30 apparently healthy individual serve as control (age and sex were matched with test group).

### **3.1.6 Inclusion Criteria:**

Sudanese patients with hyperthyroidism and hypothyroidism and apparently healthy volunteers were included in this study.

**3.1.7 Exclusion criteria:** The criteria of exclusion based on excluding any patients with chronic diseases, familiar history, cardiovascular diseases, smoking, pregnant women any drug that affect lipids metabolism.

### **3.1.6 Ethical consideration:**

Oral Informed consent was obtained from all the participants at the start of the study to evaluate the serum lipid in patients with thyroid diseases (hyperthyroidism and hypothyroidism).

### **3.1.7 Data collection:**

The clinical data were obtained and recorded on questionnaire sheet Appendix I

### **3.1.8 Samples collection and processing:**

Local 70% antiseptic for the skin was used, 4ml fasting blood was collected by standard venipuncture method, directly in centrifuge tube (plain container) and the serum was separated, after centrifugation for 5 minutes at 5000 r.p.m at room temperature and the sera were used immediately for estimation of thyroid hormones.T3, T4 and TSH, and lipid profile (total cholesterol, triglycerides, LDL-c, and HDL-c).

## **3.2 Methods:**

### **3.2.1 Estimation of Thyroid stimulating hormones (thyrotropin) (TSH):**

**3.2.1.1. Principle of TSH method:** the TSH ELISA test is based on immune enzymatic reaction without competition, high affinity, and specificity monoclonal antibodies (antiTSH) immobilized (solid phase) on wells in excess, incubated against TSH molecules, and conjugated antiTSH antibodies, the test sample is allowed to react simultaneously with two antibodies, resulting in TSH molecules being sandwiched between two solid phase and enzyme linked antibodies, after incubation the wells wash with water to remove unbound antibodies, A solution of substrate is add and incubate resulting in development of colors, stop solution is add, the concentration of TSH is directly proportional to the color intensity of the sample. Absorbance is measured spectrophotometrically at 450nm Appendix (II).

Procedure and calculations of TSH: in Appendix (II)

### **3.2.2 Estimation of total thyroxin (T4):**

**3.2.2.1 Principle of method:** The essential reagents required for a solid phase enzyme immunoassay include immobilized antibody, enzyme antigen conjugate and native antigen (T4 in sample). Upon mixing immobilized antibody, enzyme-antigen conjugate and a serum containing the native antigen, a competition reaction results between the native antigen and the

enzymeantigen conjugate for a limited numberof insolubilized binding sites. The antibody-bound fractions separated from unbound antigen by decantation oraspiration. The enzyme activity in the antibody-boundfraction is inversely proportional to the native antigenconcentration. By utilizing several different serumreferences of known antigen concentration, a doseresponse curve can be generated from which theantigen concentration of an unknown can be ascertained Appendix (III).

Procedure and calculations of T4: in Appendix (III)

### **3.2.3 Estimation of Total triiodothyroxin(total T3):**

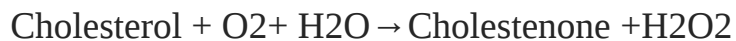
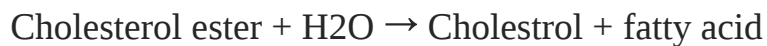
#### **3.2.3.1 Principle of T3 method:**

The essential reagents required for a solid phase enzyme immunoassay include immobilized antibody, enzyme antigen conjugate and native antigen (T3 in sample). Up on mixing immobilized antibody, enzyme-antigen conjugate and a serum containing the native antigen, a competition reaction results between the native antigen and the enzymeantigen conjugate for a limited numberof insolubilized binding sites, the antibody-bound fractions separated from unbound antigen by decantation. The enzyme activity in the antibody- or aspiration boundfraction is inversely proportional to the native antigenconcentration. By utilizing several different serumreferences of known antigen concentration, a doseresponse curve can be generated from which theantigen concentration of an unknown can beascertained Appendix (IV).

Procedure and calculations of T3: in Appendix (IV)

### 3.2.4 Estimation of total cholesterol:

**3.2.4.1 Principle of total cholesterol method:** Free and esterified cholesterol in sample originates, by means of the coupled reactions described below a colored complex that can be measured by spectrophotometry.

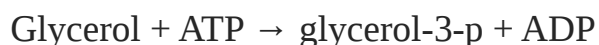
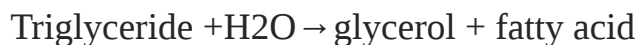


Appendix (V)

Procedure and calculations of total cholesterol: in Appendix (V).

### 3.2.5 Estimation of triglyceride:

**3.2.5.1 Principle of triglycerides method:** Triglycerides in the sample originates, by means of the coupled reactions described below a colored complex that can be measured by spectrophotometry.





$2\text{H}_2\text{O}_2 + 4\text{-aminoantipyrine} + \text{phenol} \rightarrow \text{Quinone mine} + 4\text{H}_2\text{O}$   
Appendix (VI).

Procedure and calculations of triglycerides: in Appendix (VI)

### **3.2.6 Estimation of high density lipoprotein (HDL-c):**

#### **3.2.6.1 Principle of method:**

Very low density lipoprotein (VLDL) and low density lipoprotein (LDL-c) in the sample precipitate by phosphotungstate and magnesium ions. The supernatant contain high density lipoprotein (HDL-c) the HDL-c is then spectrophotometrically measured by means of the coupled reactions describe below:

$\text{Cholesterol ester} + \text{H}_2\text{O} \rightarrow \text{Cholestrol} + \text{fatty acid}$

$\text{Cholesterol} + \text{O}_2 + \text{H}_2\text{O} \rightarrow \text{Cholestenone} + \text{H}_2\text{O}_2$

$2\text{H}_2\text{O}_2 + 4\text{-aminoantpyrine} + \text{phenol} \rightarrow \text{Quinone mine} + 4\text{H}_2\text{O}$ .  
(AppendixVII).

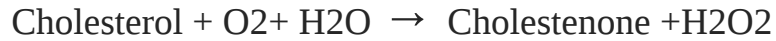
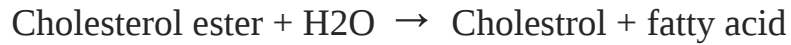
Procedure and calculations: in Appendix (VII).

### **3.2.7 Estimation of low density lipoprotein (LDL-c):**

#### **3.2.7.1 Principle of method:**

Low density lipoprotein (LDL-c) in sample precipitate with polyvinyl sulphate. Their concentration is calculated from difference between the serum total cholesterol and the

cholesterol in the supernatant after centrifugation. The cholesterol is spectrophotometric ally measured by mean of coupled reactions describe bellow:



LDL-c can be calculated by the following equation:

$$\text{LDL-c} = \text{total cholesterol} - (\text{triglycerides}/5 - \text{HDL-c})$$

### **3.3Quality control:**

The precision and accuracy of all methods used in this study were checked by commercially prepared control sample before application for the measurement of test and control samples.

### **3.4Data analysis:**

Data was analyzed using statistical package for the social science computer program. SPSS version 11

# **CHAPTER FOUR**

## **Results**

#### 4. Results

The results of the biomedical determinant serum lipid profile (total cholesterol, triglycerides, HDL-c and LDL-c) and thyroid hormones (T4, T3, and TSH) are given in a tables and figures.

**Table (4-1):** Show age and gender of patients with hyperthyroidism and hypothyroidism, the results showed that: the numbers of patients with hyperthyroidism whose age between (25-35)years were 7(23%), (36-45)years were 16(53%), and (46-65)years were 7(23%). While the numbers of patients with hypothyroidism whose age between (25-35) years were 7(23%), (36-45) years were 17(57%), and (46-65) years were 6(20%).

Numbers of male patients were 9(15%) (5 with hyperthyroidism,4 with hypothyroidism) while numbers of female patients were 51(85%)(25 with hyperthyroidism, 26 with hypothyroidism).

**Table (4-2):** Illustrate the mean of thyroid hormones and lipid profile in cases and control.

Lipid profile (total cholesterol (T.C), triglycerides(T.G), LDL-c and HDL-c) in hyperthyroidism T.C, T.G and LDL-c were significant increased in patients compared to control group while HDL-c decreased, as in table(4-2): T.C (mean± SD: 187 ±39 mg/dl versus 136.8±20.9 mg/dl, p.value = 0.00), T.G (mean± SD:164.6 ±69.6 versus 118±37.4mg/dl, p.value = 0.00), LDL-c

(mean± SD:126.7 ±43.9 versus 80.4±18.9mg/dl, p.value = 0.00), and HDL-c (mean± SD:27.9 ±10.1 versus 32.7±6.7mg/dl, P.value = 0.00), in hypothyroidism significant increase in T.C (mean± SD:190.6 ±48.1 versus 136.8 ± 20.9mg/dl, p.value= 0.00) T.G (mean± SD:182 ±92.5 versus 118 ±37.4mg/dl, P.value= 0.00) LDL-c(mean± SD: 129.2 ±49.9versus 80.4±18.9mg/dl, P.value = 0.00) and significant decreased in HDL-c (mean± SD: 24.9± 8.3 versus 32.7 ±6.7 mg/dl, P.value= 0.00).

**Figure (4-1):** Show correlation between the levels of TSH and total cholesterol, there was significant positive correlation (r=0.434, P.value = 0.00).

**Figure (4-2):** Show correlation between the levels of TSH and triglycerides, there was significant positive correlation (r=0.250, P. value = 0.025).

**Figure (4-3):** Show correlation between the levels of TSH and LDL-c, there was significant positive correlation (r=0.404, p.value= 0.00).

**Figure (4-4):** Show correlation between levels of TSH and HDL-c there was significant negative correlation (r=-0.252, p.value = 0.024).

**Table (4-1):-**Distribution of Age and gender of patients with hyperthyroidism and hypothyroidism.

Variables	Hyperthyroidism Number	Hypothyroidism Number
Age 25-35	23% (n=7)	23% (n=7)
Age36-45	53% (n=16)	57% (n=17)
Age46-65	23%(n=7)	20% (n=6)
Sex male	17% (n=5)	13% (n= 4)
Sex female	83% (n=25)	87% (n=26)

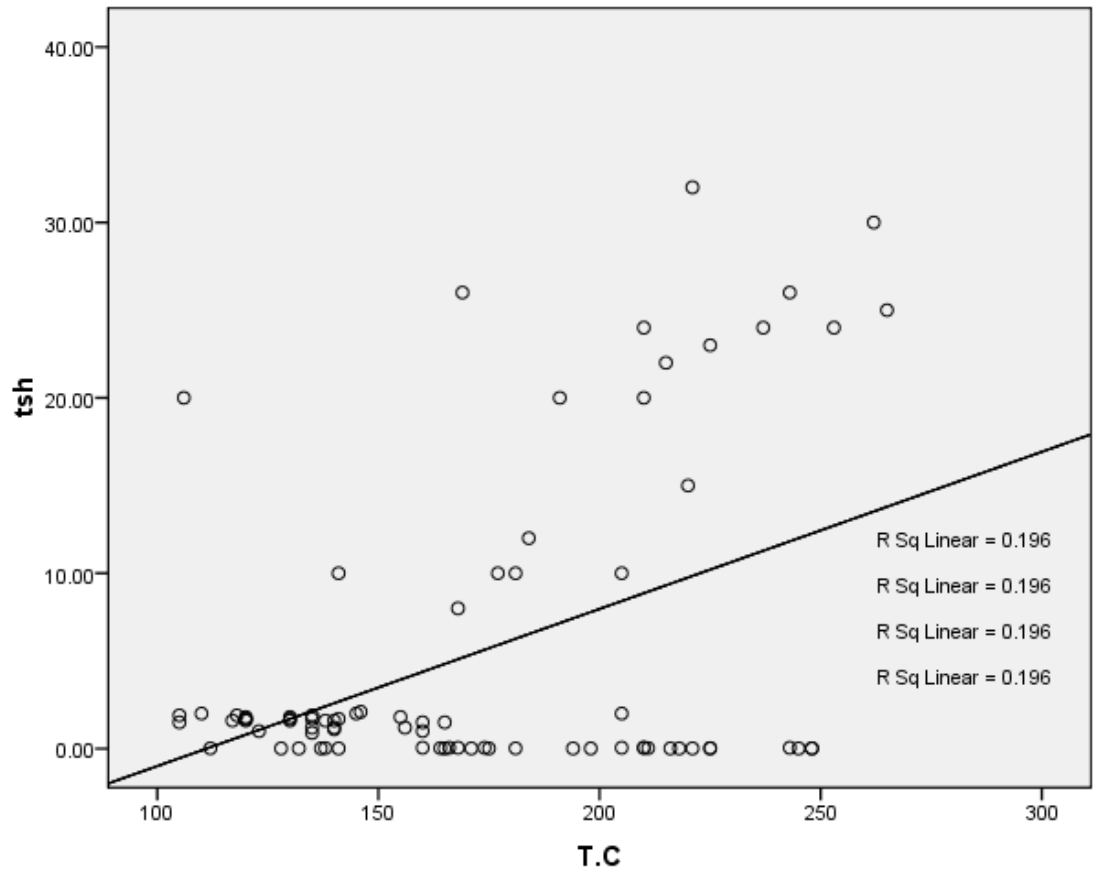
**Table 4-2:-**Comparison between means of lipids profile and thyroid hormones in thyroid diseases (hyperthyroidism and hypothyroidism) and control.

Parameter	Hyper	Hypo	Control	p.value
T4 µg/ml	197.6±10.5	39.5±26.1	85.8±13.9	0.00
T3 ng/ml	3.9±0.78	0.59±0.35	1.2±0.38	0.00
TSH µIU/ml	0.02±0.07	16.5±9.5	1.5±0.35	0.00
T.C mg/dl	187.6±39.0	190.6±48.1	136.8±20.9	0.00
T.G mg/dl	164.6±69.6	182±92.5	118±37.4	0.012
LDLc mg/dl	126.7±43.9	129.2±49.4	80.4±18.9	0.00
HDLc mg/dl	27.9±10.1	24.9±8.3	32.7±6.7	0.012

Data represent mean ± SD, p.value < 0.05 considered significant

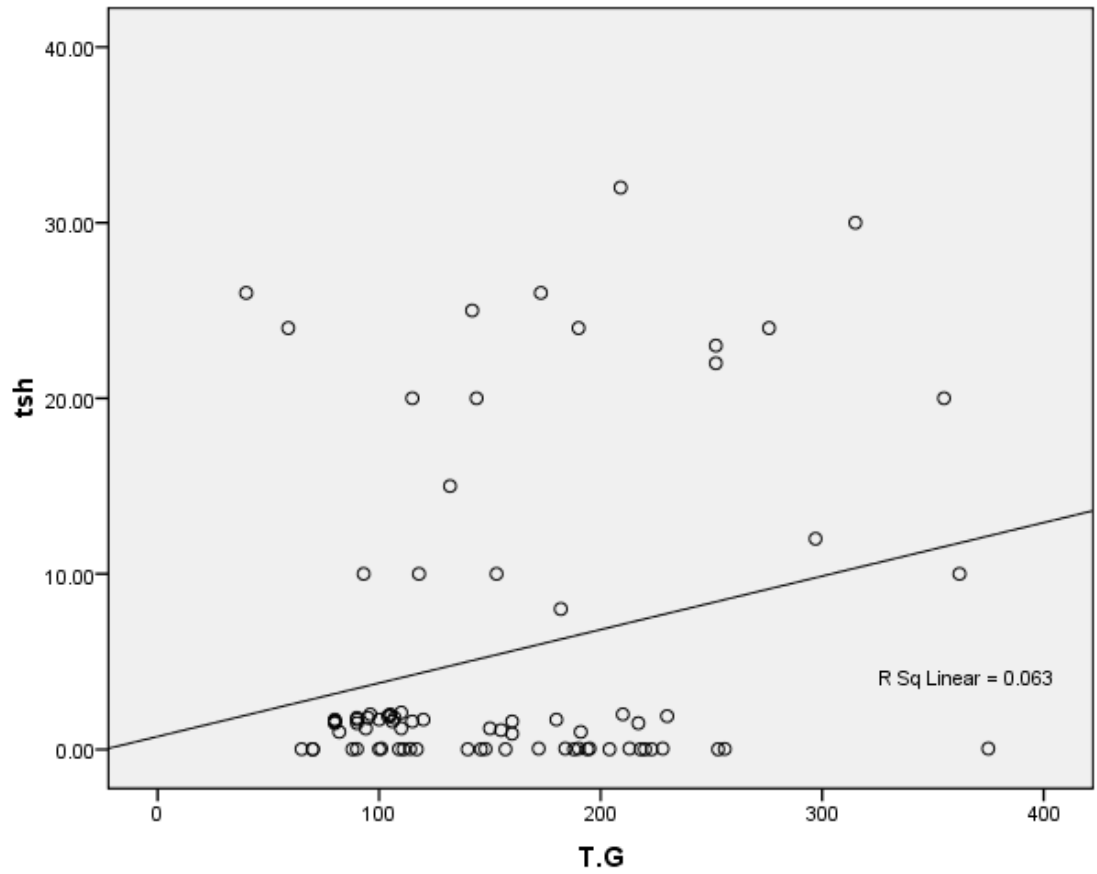
ANOVA test was used for comparison.



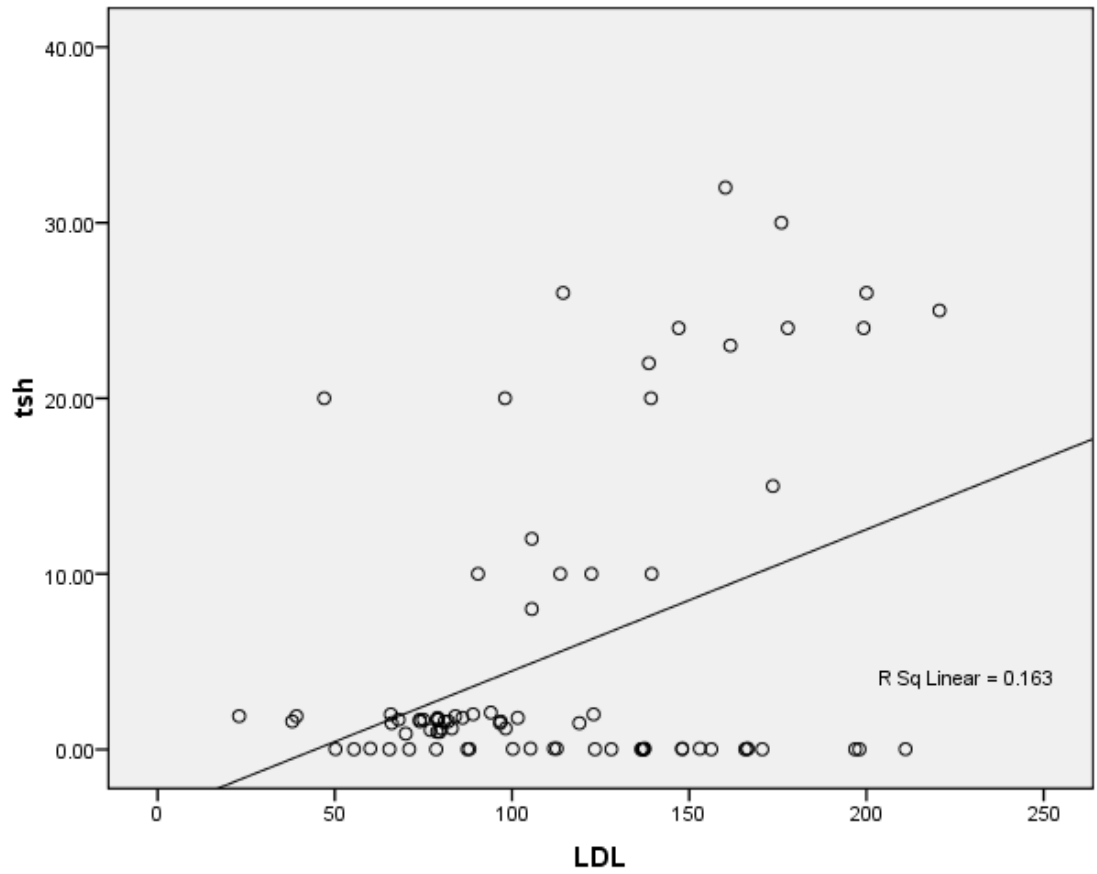


**Figure (4-1):** Correlation between total cholesterol level and TSH level

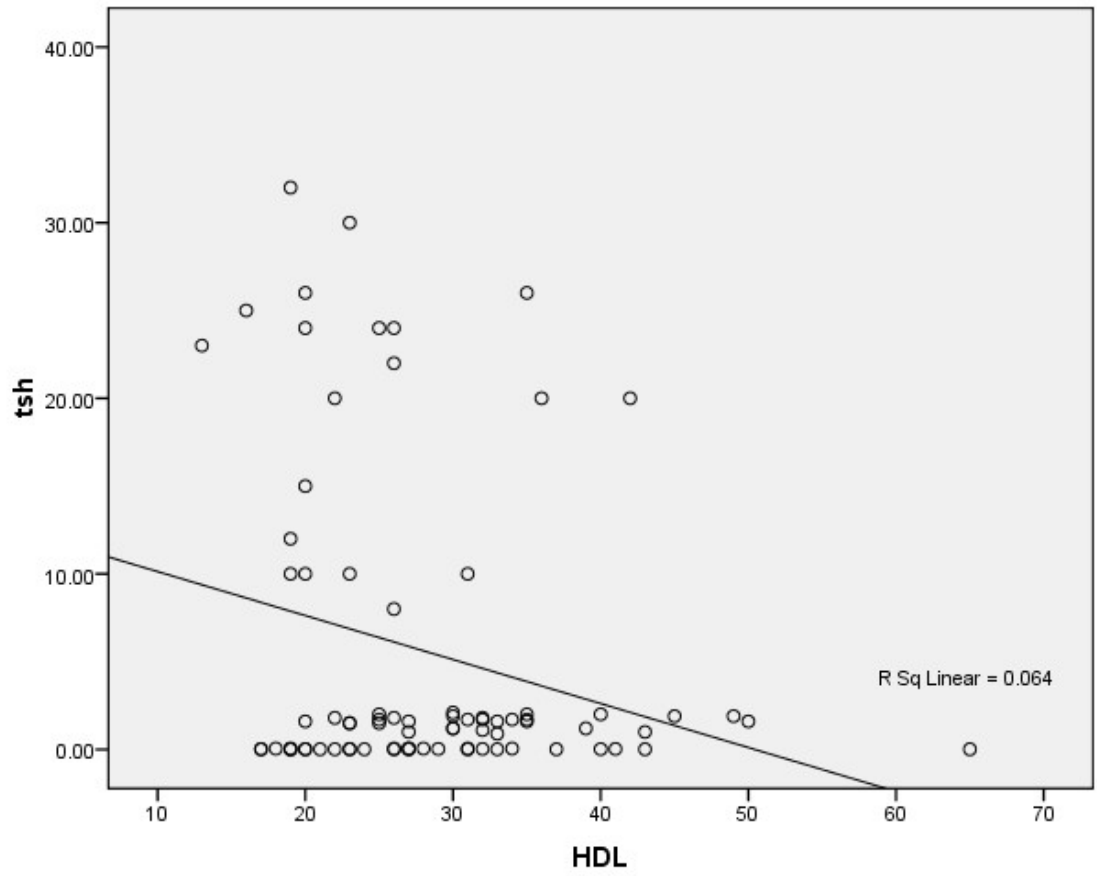
( $r = 0.434$ ,  $p\text{-value} = 0.00$ ).



**Figure (4-2):** Correlation between Triglycerides level and TSH level ( $r=0.250$ ,  $p.value=0.00$ ).



**Figure (4-3):** scatter plot of correlation between LDL-c level and TSH level ( $r=0.404$ ,  $p.value=0.00$ )



**Figure (4-4):** scatter plot of correlation between HDL-c level and TSH level ( $r=-0.252$  , $p.value=0.024$ )

# **CHAPTER FIVE**

## **Discussion, Conclusions, and Recommendations**

## 5.1 Discussion

Normal thyroid function is the most important for normal lipoprotein metabolism, so that alteration of the thyroid function leads to alteration in lipoprotein metabolism as well as lipid profile. Thyroid function affects lipoprotein metabolism as well as cardiovascular disease risk. Thyroid hormones have a significant effect on the synthesis, mobilization and metabolism of lipid factors (Sayed *et al.*, 2015).

The present study was carried out to evaluate the serum lipid profile among Sudanese patients with hyperthyroidism and hypothyroidism in Khartoum state.

The finding obtained from specially designed questionnaire revealed that, the majority of patients with hyper or hypothyroidism participate in this study were female (85%) while male was (15%). Also hyperthyroidism and hypothyroidism are most common among age (36-45) years (in hyperthyroidism 16(35%), hypothyroidism 17(57%)). These results in agreement with previous published results carried out by (Sayed *et al.*, 2015) which showed that thyroid disorders are common in female and old age this may be due to a sex difference in the prevalence of autoimmune diseases (Sayed *et al.*, 2015).

The result of present study showed there were significant increased in total cholesterol, triglycerides, and LDL-c (p.value = 0.00) in hyperthyroidism patients compared to control group, this

result agreed with another studies (Regmi *et al.*, 2010), which showed that total Cholesterol and LDL-c significantly increased. Also the result agreed with the result carried out by (Ferdos *et al.*, 2012) which found significant increased in total cholesterol, triglycerides and LDL-c while HDL-c was decreased.

This result may be due to thyroid hormones can influence HDL-c metabolism by increasing cholesteryl ester transfer protein (CETP) activity, which exchanges cholesteryl esters from HDL2 to the very low density lipoproteins (VLDL) and Triglycerides to the opposite direction.

This result disagreed with another study carried out by (Sayed *et al.*, 2015) which show, there were no significant differences in lipid profile in hyperthyroidism compared to control group.

In hypothyroidism the studies showed significant increased in levels of total cholesterol, triglycerides LDL-c (p.value = 0.00, 0.012 and 0.00) respectively while HDL-c was significant decreased (p.value= 0.021) this result in agreement with results (Sayed *et al.*, 2015), (Rizos *et al.*, 2011), (Khan *et al.*, 2013) and (Shrestha, 2011) which found significant increase in total cholesterol, triglycerides, LDL-c and HDL-c.

This results showed a significant positive correlation between TSH level and total cholesterol, triglycerides and LDL-c (r = 0.434, 0.250, 0.404 p.value = 0.00, 0.025 and 0.00) respectively and a significant negative correlation with HDL-c (r=-

0.252P.value =0.024).This result in agreement with the results carried out by (Shivaleela *et al.*, 2016; Waleed *et al.*, 2013).

Which found a significant positive correlation between TSH and total cholesterol, triglycerides, LDL-c and HDL-c, this relation may be due to thyroid hormones increases the expression and concentrations of apo A-I protein, major protein in HDL-c.

.



**5.2 Conclusion:** -According to the results of this study it is concluded that:

1- Total cholesterol, triglycerides, and LDL-c are significant increase in hyperthyroidism and hypothyroidism patients.

2- HDL-c is significantly decreased in hyperthyroidism and hypothyroidism patients.

3-There are significant positive correlation between level of TSH and level of total cholesterol, triglycerides and LDL-c. While significant negative correlation with HDL-c.

**5.3 Recommendations:** -From the finding of this study it is recommended that:

1- Investigation of thyroid hormones should be done in patients presenting with dyslipidemia.

2- Patients with thyroid dysfunction should be monitoring of lipid profile to prevent cardiovascular diseases.

3-Further studies should be done to estimate free thyroid hormones (FT4 and FT3) in dyslipidemic patients.

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