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LIPOPROTEIN (A) LEVELS AND RISK OF CARDIOVASCULAR DISEASES AMONG INDIVIDUALS WITH SUBCLINICAL HYPOTHYROIDISM

Noora Abduljaleel MOHAMMED

Master's Thesis

DEPARTMENT OF CHEMISTRY
Division of . Biochemistry.

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THESIS APPROVAL

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DECLARATION

I hereby declare that I wrote this Master's Thesis titled " Lipoprotein (a) Levels and Risk of Cardiovascular Diseases Among Individuals with Subclinical Hypothyroidism " in consistent with the thesis writing guide of the Graduate School of Natural and Applied Sciences, Firat University. I also declare that all information in it is correct, that I acted according to scientific ethics in producing and presenting the findings, cited all the references I used, express all institutions or organizations or persons who supported the thesis financially. I have never used the data and information I provide here in order to get a degree in any way.

10 January 2021

Noora Abduljaleel MOHAMMED

PREFACE

First, my deepest gratitude to God for all his mercy and blessings, and for giving me the strength and ability to complete this search in these difficult times.

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Finally, I would like to dedicate my research to my father's soul, and also my special thanks and appreciation to my dear mother for her constant invitations and encouragement. Many thanks to my brothers and sisters for their continuous support and encouragement

Noora Abduljaleel MOHAMMED

ELAZIĞ, 2021

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ABSTRACT

Lipoprotein (a) Levels and Risk of Cardiovascular Diseases Among Individuals with Subclinical Hypothyroidism

Noora Abduljaleel MOHAMMED

Master's Thesis

FIRAT UNIVERSITY
Graduate School of Natural and Applied Sciences

Department of Chemistry
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Background: Subclinical hypothyroidism (SCH) is the mildest type of thyroid hormone deficiency and can have adverse effects. It is important to identify patients with subclinical hypothyroidism that has an elevated cardiovascular risk (CVR). The aim of the study was to evaluate Lp (a) value as a cardiovascular risk factor among patients with subclinical hypothyroidism.

Materials and Methods: The study included 50 patients newly diagnosed with SCH and 50 healthy controls matched by age and gender. Cobas 6000 was used to analyze the parameters.

Results: In subclinical hypothyroidism patients, serum Lp (a), total cholesterol (TC) 152.98 (26.25), and low-density lipoprotein cholesterol (LDL-C) levels 96.54 (24.72) were significantly higher compared to healthy control. In addition, a significant positive association was found for subjects with subclinical hypothyroidism between TSH and anti-TPO (0.489), TC (0.311). However, Lp (a)(0.073), LDL-C (0.278), TG (0.28) levels, showed no significant positive correlation with the levels of TSH. Further more Lp (a) in patient with subclinical hypothyroidism has a significant correlation with APOB (0.407), LDL (0.309), TC (0.314).

Conclusion: We concluded that there was an increased concentration of certain CVR variables in SCH patients. The possible benefits could be preventing progression to overt hypothyroidism and initiating adequate therapy to improve lipid parameters.

Keywords: Hypothyroidism, thyroid stimulated hormone lipoprotein a, cardiovascular disease

ÖZET

Tez Başlığı Her Kelimenin İlk Harfi Büyük Olarak Buraya Yazılmalıdır

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Yüksek Lisans Tezi

FIRAT ÜNİVERSİTESİ
Fen Bilimleri Enstitüsü

Kimya Mühendisliği Anabilim Dalı
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Bu alanda yazı stili değişikliği yapılmamalıdır. Gerek duyulması halinde Stil Galerisinden "Özet Metni" stili kullanılmalıdır. Yazı büyüklüğü 10 punto ve 1,25 aralık ayarlıdır. Paragraf başları (ilk satır) 1 cm sol girinti ile başlar. Paragraflar arasında ayrıca boşluk bulunmaz.

İlk 1-2 cümlede konunun önemi ve tezin amacı tanımlanmalı, 1-2 cümle ile tezin hipotezleri açıkça verilmeli, 1-2 cümlede örneklem ve örneklem büyüklüğü belirtilmeli, 1-2 cümle ile yöntem hakkında bilgilendirme yapılmalı, birkaç cümle ile bulgular hakkında kısa bilgiler sunulmalıdır. Son olarak üretilen bilgi kısaca ifade edilmelidir. Özetin altında, konuyu tanımlayan anahtar kelimeler bulunmalıdır.

En az 3 (üç) en fazla 6 (altı) anahtar kelime kullanılır.

Özet ve Abstract sayfası 250 kelime ile sınırlandırılmalı, kaynak gösterimi, şekil, tablo ve denklem gibi öğeler bulundurmamalıdır. Mümkün oldukça üst ya da alt simge ve Yunan harfleri gibi semboller kullanılmamalıdır. Özetin 250 kelimeyi aşan kısımları Ulusal Tez Veri Merkezinde saklanmaz. Her hâlükârda özet ve anahtar kelimeler bir sayfa ile sınırlandırılmalıdır. Bir sayfayı aşan özet ve anahtar kelimeler Enstitü tarafından kabul edilmez.

Anahtar Kelimeler: Hipotiroidizm, tiroid ile uyarılan hormon lipoprotein a, kardiyovasküler hastalık

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ABBREVİATİONS

Abbreviations

TSH : Thyroid Stimulate Hormone
T3 : Triiodothyronine
T4 : Thyroxine
FT3 : Free Triiodothyronine
FT4 : Free Thyroxine
SCH : SubClinical Hypothyroidism
Tg : Thyroglobulin
TPO : Thyroid Peroxidase
TSHR : Thyroid Stimulate Hormone Receptor
TREs : Thyroid hormone Reaction Elements
RT3 : Reverse Triiodothyronine
TBG : Thyroid Binding Globulin
TSAb : Thyroid Stimulating Antibodies
TRAbs : Thyroid Receptor Antibodies
TSI : Thyroid Stimulate Immunoglobulin
TRH : Thyrotropin-Releasing Hormone
GH : Growth Hormone
TC : Total Cholesterol
TG : Tri Glyceride
HDL : High-Density Lipoprotein
LDL : Low-Density Lipoprotein
VLDL : Very Low-Density Lipoprotein
IDL : Intermediate-Density Lipoproteins
Lp (a) : Lipoprotein (a)
APO A : Apolipo Protein A
APO B : Apolipo Protein B
CVD : Cardiovascular Diseases
CVH : Coronary Heart Disease
IYD : Iodotyrosine Dehalogenase
MCT8 : Mono Carboxylate Transporter 8
MIT : 3-Monoiodo-L-Tyrosine
NIS : Sodium Iodide Symporter
DIT : Diiodotyrosine
I- : Iodide
H2O2 : Hydrogen peroxide

CRP : C - Reactive Protein

LCAT : Lecithin Cholesterol Acyl Transferase

CM : Chylo Microns

CE : cholesteryl Ester

TAVI : Trans catheter Aortic Valve Implantation

AC : Adenylate Cyclase

CAMP : Cyclic Adenosine Mono Phosphate

ATP : Adenosine Tri Phosphate

CVR : cardioVascular Risk

B.P : Blood Pressure

BMI : Boody Mass Index

WC : Waist Circum ference

1. INTRODUCTION

At any stage of life, thyroid disorders can appear. In mid-age and adulthood, they are more commonly experienced. Almost all significant metabolic pathways are influenced by thyroid hormones. In growth, differentiation, and metabolism, thyroid hormones (THs) play crucial roles. They are essential for the optimal functioning of nearly all tissues, with a significant impact on the metabolic rate and the consumption of oxygen. Hence it is not shocking that the most common endocrine disorders are thyroid gland disorders. Thyroid dysfunction involves several hundred individuals throughout the world. It is thus important to see that its functions are clinically correlated with conditions that cause hormone deficiency or excess [1]. Thyroid disorder refers to a subset of the most common medical disorders that are prevalent diseases. These diseases affect about 6.6 percent of people worldwide, with women having some form of thyroid gland abnormality (5-10) times greater than males. Thyroid disease epidemiology differs considerably across various subpopulations; residing in regions deficient in iodine, which constitutes a third of the population of the world, is a significant risk factor The incidence in goiter and hypothyroidism. The prevalence of hyperthyroidism is 0.5-2 percent in women and 0.1-0.2 percent in men, while hypothyroidism is 0.06-1.2 percent of the total in women and 0.1-0.4 percent in men, respectively [2]. The elevated serum hormone stimulating thyroid (TSH) Triiodothyronine (T3) concentrations and regular serum total and free thyroxine (T4) concentrations are associated with little to no symptoms of hypothyroidism. can be best expressed as subclinical hypothyroidism (SCH). This is known as a moderate case of thyroid failure and is a lab diagnosis [3, 4]. There is much more frequent subclinical hypothyroidism than overt hypothyroidism. Hypothyroidism is associated with a higher risk of cardiovascular disease (CVD), possibly due to the often associated hyperlipidemia marked by hypercholesterolemia in most cases. The therapeutic importance of lipoprotein (a)[Lp(a)] has received a great deal of attention. There is significant evidence to suggest that increased serum Lp(a) levels contribute significantly to the growth of CHD [5]. High levels of low-density lipoprotein cholesterol (LDL-C) have been consistently associated with an increased risk of developing cardiovascular disease The current research was conducted in subclinical hypothyroid patients to identify lipoprotein (a), lipid levels, and levels of non-HDL-C and find a substantial correlation between Lp(a) and non-HDL-C and thyroid hormones. Hemodynamic although heart abnormalities can develop because of deficient or excessive thyroid hormone concentration [3]. It is well known that by biochemical studies, overt hypothyroidism is characterized by cardiovascular symptoms. Dyslipidemia is a popular hypothyroidism characteristic, induced by multiple types of evidence [6, 7], But due to contradictory results over the years, not for the SCH. The variety of the outcome raised questions of whether or not SCH is characterized by cardiovascular symptoms.

The C-Reactive Protein (CRP) phase reactant is an effective and useful method for cardiovascular risk assessment[8]. It is a major predictor of multiple inflammatory conditions such as rheumatoid arthritis, myocardial infarction, etc. It is known that CRP plays an important role in the growth of atherosclerosis., a cardiovascular disorder caused by a modified low-density cholesterol lipoprotein pathway (LDL-C)[9]

1.1. The thyroids gland anatomy and histology

The thyroid gland comprises 2 lobes linked by a narrow, median isthmus which forms a "butterfly" shape (left and right). it is found in the neck, next To the trachea, just below the larynx, weighing 15-20g in adults. It is a strongly vascularized organ that has a tissue blood flow of around 5mL/g/min. While the thyroid is only 0.4 percent of body weight, it accounts for 2 percent of total blood flow [10] (Figure 1-1).

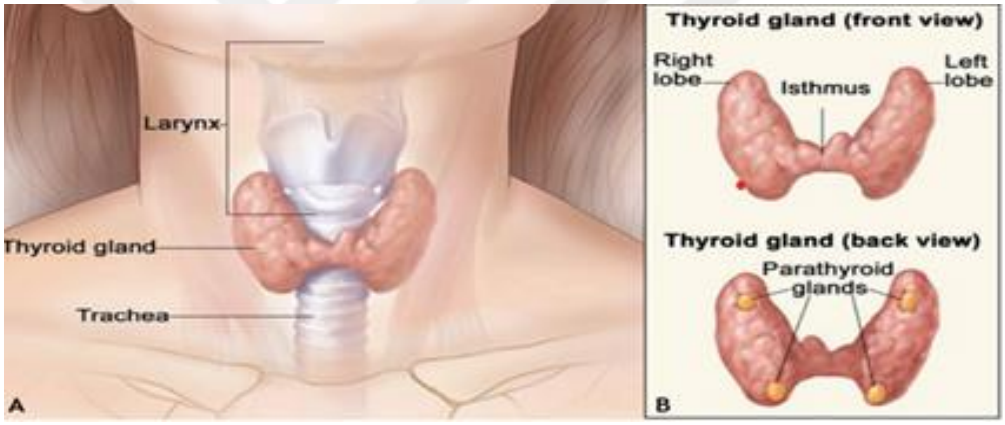

Figure 1.1. Thyroid Anatomy [11].

The gland arrangement consists of tightly packed follicles, spherical units formed by a colloid protein-containing lumen, surround by a single continuous layer of thyroid epithelial cells called thyrocytes or follicular cells (Figure 1-2). The diameter of the follicle, in even one gland, varies considerably but is approximately 200 nanometers on average. With the degree of glandular stimulation, the follicular cells differ in height, Active when columnar and inactive when cuboidal. Epithelium sits on a glycoprotein-rich membrane of a basement which divides the follicular cells from the capillaries surrounding them. By 2 connective tissue septa, between 20 and 40 follicles are demarcated to create a Lobule provided with a single blood vessel. A specific lobule may vary in its functional activity from its neighborhood. Thyroid hormone also includes follicle-producing cells called calcitonin or (C cells), which are usually found along the basement membrane of the thyroid gland epithelium [12].

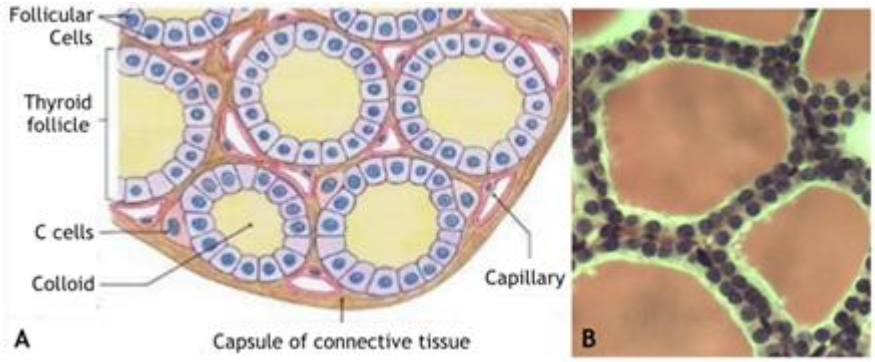

Figure 1.2. Thyroid histology[12].

1.2. Physiology and thyroid hormone synthesis

The assignment of the thyrocytes is to generate thyroid hormones triiodothyronine (T3), thyroxine (T4), iodine-derived homodimer peptides, and L-tyrosine amino acid binding (Figure 1-3).

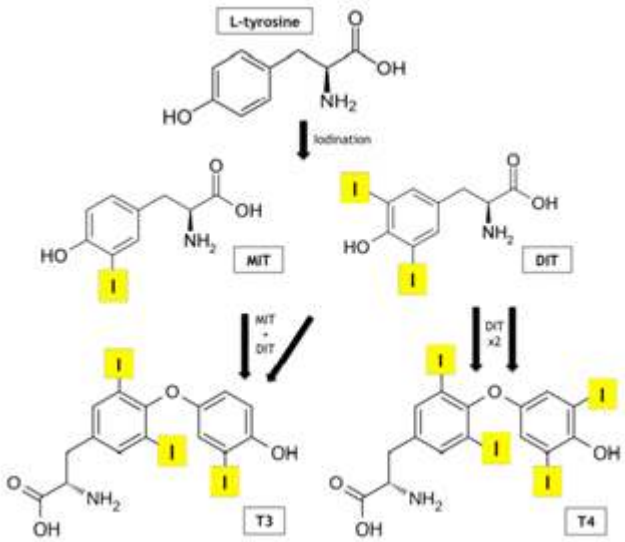

Figure 1.3. Structure and presence of thyroid hormones [13].

Thyroid hormone synthesis is a complex mechanism containing distinct and polarised thyrocyte activities (Figure 1-4) [14].

- Basal cell membrane into contact with the circulatory system through which the absorption of amino acids and ion iodide (I-) by symmetric sodium iodide (NIS), a glycoprotein across the membrane, for the synthesis of T3 and T4.
- Thyroglobulin (Tg) and thyroid peroxidase (TPO) two main thyroid proteins involved in the development of thyroid hormones. The secretion of Tg through the membrane into

the apical cavity follicular which is the main synthesis of the colloidal, and TPO is found on the apical membrane of thyrocytes and is a transmembrane protein.

- The primary site where T3 and T4 are synthesized in the apical border. I- It is carried by a carrier called pendrin, which is located on the apical membrane, in the lumen of the follicle. Tg homodimer residue treatment of tyrosine iodine in the follicular lumen to form 3-monoiodo-L-tyrosine (MIT) and 3,5-diiodo-L-tyrosine (DIT), a precursor to thyroid hormones. Catalyze the oxidation of TPO and its conversion to tyrosine, which requires the formation of hydrogen peroxide (H_2O_2), which is formed by the enzymes diploid oxidase 1 (DUOX1) and DUOX2. It is also stimulating the merger of two molecules DIT for the synthesis of T4 and one MIT and one DIT to generate T3 by TPO. MIT and DIT and still T3 and T4 connected to protein Tg and stored in colloid at this stage.

It is activated multiple process steps when the thyroid gland has to launch T3 and T4:

- Apical thyrocytes membrane: endocytosis of the follicular lumen colloid by both macropinocytosis (pseudopods) and micropinocytosis (small coated vesicles).
- Inside thyroid cells: integrate internal vesicles with lysosomes and determines the proteolysis launch iodotyrosines (T3, T4, MIT, DIT) of Tg. It is then removed iodine by MIT and DIT extractor iodotyrosine (IYD), encoded by gene IYD, to allow the return of iodine recycling with Tg.
- On the basolateral side of thyrocytes, T3 and T4 are secreted into the bloodstream through transmembrane protein transporters, including the monocarboxylate transporter 8 (MCT8), specific for the transport of thyroid hormones [15].

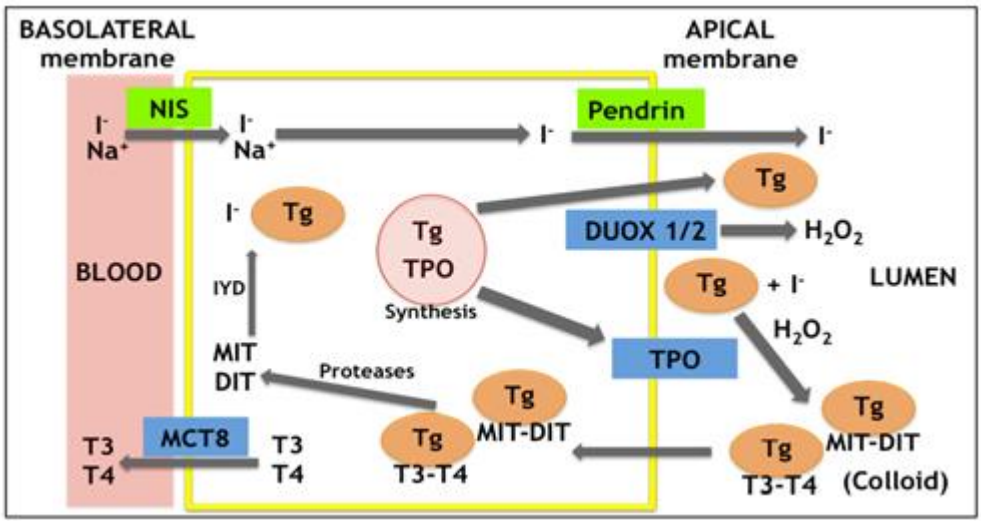

Figure 1.4. Thyroid cell structure and synthesis of thyroid hormones [15].

1.3. Regulate the Function of the thyroid gland Hypothalamus-Pituitary-Thyroid Axis

The thyroid stimulating hormone (TSH), a peptide formed in the anterior pituitary by endocrine cells called thyrotrophin, regulates the secretion of T3 and T4 in the thyroid [16]. Two interacting systems regulate the output of TSH (Figure 1.5.): 1) With open-loop by lowering factors nervous regulation of tissue hypothalamus 2) Thyrotropin-releasing hormone (TRH), a peptide secreted by nerve cells near the hypothalamus, is linked to a specific receptor on the thyroid plasma membrane and stimulates the development of the hormone TSH [17]. Other hormones also block the secretion of TSH, including estrogens, glucocorticoids, and possibly growth hormone, while cytokines are inhibited in the pituitary and hypothalamus [18]. On each of the pituitary gland and hypothalamus, exercise thyroid hormones, T4 and T3, control negative feedback, which determines a decrease in the release of TSH and TRH. T4 and T3 spin inside the brain, where they are converting T4 by kind II deiodinase to T3, T3, and then interacts with the hormone receptor thyroid [19].

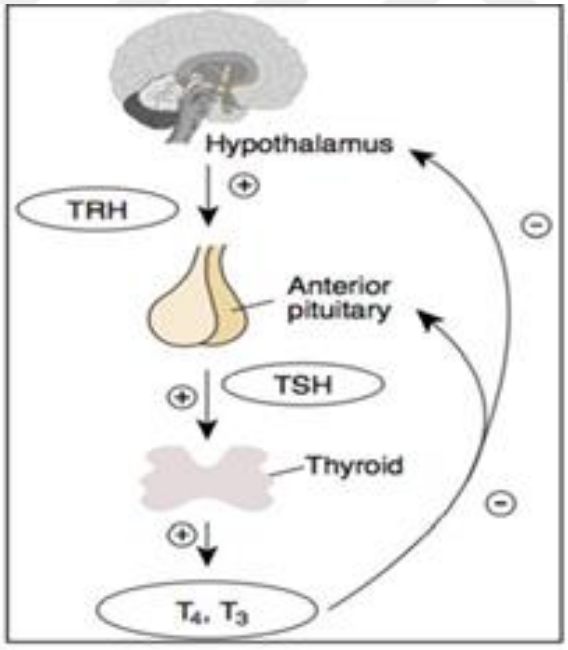

Figure 1.5. Axis of hypothalamic, pituitary-thyroid [20].

TSH is linked to its own future unique (TSHR) located on the basement membrane of the cells of the thyroid and causes many functions across different signals falls within cells. The key acts of TSH contain: 1) Stimulation of thyroid hormone composition 2) catalyze of the apical endocytic membrane, inducing colloid absorption and digestion with the consequent secretion of thyroid hormone from the lateral membrane into the blood 3) Maintain thyroid cells healthily and

equal [21]. In general, A) Induction of gene transcription TPO / NIS / Tg via adenylate cyclase (AC), an enzyme that stimulates the "transition from Tri-ATP (ATP) to pyrophosphate and 3', 5' cyclic adenosine monophosphate (CAMP) and pyrophosphate B) To generate H_2O_2 through Gq-phospholipase C, which leads to a series of increasing levels within cells of Diacylglycerol and calcium ion (Ca^2 +)[22]. TSH is a hormone glycoprotein composed of sub-units: alpha and beta. Beta sub-unit unique to the TSH is considered and therefore decided to privacy as a recipient. It was found in the unit area responder sub-alpha almost similar to other hormones, such as directed genital glands of human chorionic, follicle-stimulating hormone [23].

1.4. Transfer and metabolism of thyroid hormones

A thyroid gland with a ratio of 10:1 releases T4 and T3. Both are poorly water-soluble and therefore reversibly bind for plasma proteins, In specific, thyroxine binding globulin (TBG), and less so with albumin and transthyretin. To absorb the peripheral tissues, a small number of hormones is associated with these proteins is available, 0.02 percent for T4 and 0.3 percent for T3, respectively. This is referred to as the segment free-T4 (FT4) and free-T3 (FT3) and is determined by the level of concentration and saturation of these proteins, especially TBG, respectively. Thyroid gland case compatible with FT4 and FT3, not T4 and T3, in clinical practice for this reason. Standard serum concentrations normally range from 12 to 22 pmol/L for FT4 and from 3.1 to 6.8 pmol/L for FT3 [12]. There is a linear inverse relationship between the concentration of serum FT4 and TSH (log), making the concentration of TSH in serum measure very sensitive to the state of the thyroid gland in patients who have a healthy pituitary gland axis. Describes the word "euthyroidism" a condition in which FT4 and FT3 within the normal range for TSH during the description of the terms "hypothyroidism" and "overactive thyroid gland" case of low FT4 and FT3 / increase in TSH and increase FT4 and FT3 / low in TSH. T3 and T4 are transported across the cell membrane of the target peripheral member primarily by tanker proteins, including MCT88 [24]. The main respondent in the target cells is T3, while T4 is the most abundant hormone secreted by the thyroid gland 80 percent of it is derived from T4 by deiodinases in the peripheral tissues that stimulates the removal of one atom would like the size of 5 inches of T4 [25]. In particular, it was found three types of deiodinase: deiodinase 1 (D1) and deiodinase 2 (D2), and deiodinase 3 (D3) respectively. Each of the D1 and D2 develops T3 from T4, but they differ in terms of tissue site and organization. In particular, D1 is found primarily in the liver and kidney, while D2 is found primarily in the central nervous system and the pituitary gland and muscle structure and fatty tissue structure, placenta, and heart. D3 has a different function when it is deactivated in both T3 and T4 and is located mostly in the central nervous system, the placenta, liver, skeletal muscle. Decomposes T3 and T4 partially inside the cells

before going out through the peripheral deiodinase [10]. Another mechanism involved in the metabolism of T4 is glucuronidation hepatic group hydroxyl phenol by uridine diphosphate glucuronyl transferase (UDPGT) in the liver, but only small amounts of T3 are subject to this method [26].

1.5. Actions of hormones of the thyroid

Thyroid hormones work by linking them to a specific future thyroid hormone (TR). For TRs, T3 has a greater affinity correlation by 15 times of T4, which explains its function as the active thyroid hormone. When bound to T3, TR binds to DNA and is usually heterozygous for retinoid X receptors in specific sequences called thyroid hormone reaction elements (TREs). Alternative splicing is exposed to two TR genes, alpha, and beta, resulting in active and inactive gene products. The active proteins are TR alpha1, TRβ1, TRβ2, and TRβ3 [27]. To express different TRs, there is special preferences tissue, suggesting that it has different functions in different tissues [28]. In fact, in the hypothalamus and the pituitary gland, which is organized thyroid function, it is considered TR alpha, especially TR alpha2, task. [29]. T3 and T4 exercise multiple roles in the physiology of many different target devices and systems, including control of food representation rate, regulation of growth, effects of cardiac contraction affecting the temporal muscular contraction, and the development of the central nervous system [30]. The composition of members, during infancy and childhood after birth, important thyroid hormones are considered. At the age of puberty, it is still important thyroid hormones too, but clarify the main effects of thyroid hormones on target organs in the table (1.1) [12, 30, 31].

Table 1 Hata! Belgede belirtilen stilde metne rastlanmadı..**1.** Hormonal effect of the thyroid gland on the target organ [12].

CARDIOVASCULAR SYSTEM	▪ ↓ peripheral vascular resistance ▪ Positive chronotropic and inotropic cardiac effects
METABOLISM	▪ ↑ basal metabolic rate ▪ ↑ heat production ▪ ↑ both protein synthesis/degradation ▪ ↑ both lipogenesis and lipolysis
CENTRAL AND PERIPHERAL NERVOUS SYSTEM	▪ ↑ sensitivity to catecholamines ▪ Cortical and cerebellar development ▪ Myelination
SKELETAL SYSTEM	▪ Regulation of bone turnover
KIDNEY	▪ ↑ renal blood flow, glomerular filtration and tubular reabsorptive and secretory maxima
HEMATOPOIETIC SYSTEM	▪ ↑ erythropoiesis
REPRODUCTIVE SYSTEM	▪ Regulation of amplitude and frequency of LH/FSH pulses ▪ ↑ liver production of sex-hormone-binding globulin (SHBG) ▪ ↑ conversion of androgens to oestrogenic products
PITUITARY AND HYPOTHALAMUS	▪ ↓ TSH and TRH secretion

1.6. Thyroid Function Test:

a) Thyroid Stimulating Hormone (TSH):

To accurately diagnose thyroid abnormalities, serum TSH or thyrotropin assessment provides a sensitive index and the vast majority of thyroid dysfunctions are attributed to primary thyroid disease, in which the pituitary gland responds to predictable changes in the secretion of TSH. Patients with hyperthyroidism or thyrotoxicosis will often have subnormal TSH and also have an increased degree of hypothyroidism [32]. However as thyroid dysfunction results through pituitary or hypothalamic disorder and in patients with non-thyroidal disorders, TSH levels are confusing. Serum FT4, T4, and T3 estimations are helpful where the amount of TSH is beyond the reference range. Whenever the TSH level is high, calculations of serum FT4 can be carried out and estimations of FT4 and T3 values are also informative when the TSH is low. TSH is pulsed and has a circadian rhythm during the first hours of sleep, the maximum in the evening at 23 hours, and the normal value varies according to age [33, 34].

b) Total Thyroxine (T4) and Free Thyroxine (FT4):

T4/FT4 assays augment TSH which are used to validate a thyroid condition and its severity where an irregular TSH outcome indicates it. T4/FT4 is the most susceptible (T3/FT3) predictor of disease progression and is also favored for the confirmation of subclinical diseases already indicated by elevated TSH. The differential diagnosis is based on T4/FT4 where the findings of TSH are misleading in cases such as secondary (hypothalamic disorder) and tertiary (pituitary disorder) and inpatient care. The T4 assay tests both protein-bound and free hormone concentrations. The level of T4 depends on the transport protein concentration, specifically albumin, TBG, and transthyretin. Thus, any disorders that cause TBP levels will affect the levels of T4 (but not FT4) [35]. Due to estrogen (pregnancy, oral contraceptive hormone treatment, tamoxifen, selective estrogen receptor modulators, and inflammatory liver disease), thyroid-binding proteins are increased and decreased when TBG binding is diminished (androgens, nephrotic syndrome). The biologically active Thyroxine fraction is determined by the FT4 assay. The tissue availability of T4 is determined by Plasma FT4 and is therefore considered to be more reliable thyroid status parameters than T4, which can be affected by the variation in transport protein concentration [36, 37].

c) Total and Free Triiodothyronine (T3/FT3):

The main uses are to assess the level of hyperthyroidism which usually goes up before the T4 to receive the serum T3. The T3 assessment is helpful is used as a type of overactive thyroid gland with high abnormal levels of T3 and TSH levels of pent-up, with T4 levels in the normal or equivocal serum, for the diagnosis of thyroid poisoning supposed. In hypothyroidism, even though the T4 is strong, the T3 is also normal. Through acute sickness, hunger, drugs like Inderal, steroids, and amiodarone, T3 is decreased. T3 tests all hormones that are bound and free. Just

biologically active hormone-free. Since FT3 accounts for about 0.5 percent of total T3, the most accurate indicator of true thyroid status is the free hormone measurement. As a whole, the total T3 levels influence the TBP effect [38, 39].

d) The reverse of T3

Triiodothyronine in the opposite direction (3, 3 ', 5'-Triiodo-L- thyronine) is an inactive type of T3 produced when T4 to T3 peripheral deiodination is inhibited and reduces the level of T3.RT3 which, like T3, is unable to provide oxygen and energy to the cells. Hemorrhagic shock, anorexia nervosa, postoperative states, hepatic dysfunction, extreme infection, and many drugs raise the level of RT3 during times of malnutrition, severe physical discomfort, injuries seems that the RT3 level corresponds to the level of T4: low thyroid activity and overactive thyroid gland. In comparison, the observation in a chronically ill patient with an elevated RT3 level tends to exclude a hypothyroidism diagnosis [40].

e) Serum Thyroglobulin (TG):

TG is the thyroid gland's principal iodoprotein. Concentrations in women are much higher and in pregnant women, newborns, some folds are elevated, but the significant elevation is seen mostly in discrete thyroid tumors arising from follicular cells and destructive thyroiditis. The calculation of TG is fully used in thyroid cancer recurrence testing. Measurable levels suggest inadequate ablation or persistent cancer following total thyroidectomy and radio ablation. The TG assay interferes with Anti-TG, and the findings of TG in patients with positive antibodies are not accurate [40, 41].

f) Thyroid Binding Globulin (TBG):

TBG With thyroid hormone-binding activity is the main plasma protein. Therefore, an alteration in the circulating volume changes blood T4 and T3. In the comparative diagnosis of patients with elevated TH values, a TBG examination helps to show hypo or hyperthyroidism with no clinical signs or symptoms. Pregnancy, hormone utilization, and congenital TBG deficiency maybe because of elevated TBG levels [2, 42].

g) Anti-Thyroid Autoantibodies:

The diagnostic hallmark of the AITD; Graves' and HT The antibodies of the components of the thyroid gland are directed against one or more. Thyroid autoantibodies include thyroid-stimulating antibodies (TSAb), anti-TG, and anti-TPO in clinical treatment. Among these, anti-TPO has arisen as a beneficial marker for AITD diagnosis [35].

Thyroid Peroxidase Antibodies (Anti-TPO):

The most sensitive marker for the diagnosis of AITD is anti-TPO, historically identified as an anti-microsomal antibody. The principal microsomal portion recognized by these autoantibodies is the TPO enzyme, a 100 kD glycosylated protein responsible for iodinating tyrosine residues. TPO antibodies are of the G class of immunoglobulin formed by infiltrating

thyroid lymphocytes, with minimal contributions from the bone marrow and lymph nodes. Via complement activation and antibody-dependent cell cytotoxicity, they cause thyroid cell damage. Anti-TPO is present in about 100% and 80% of people with autoimmune hypothyroidism and Graves' disease, respectively. In patients with multiple non-thyroid autoimmune disorders and 15 to 20 percent of stable Euthyroid subjects, an irregular anti-TPO is identified [39, 43]. A significantly higher incidence of anti-TPO was observed in women of all age classes and the elderly in iodine-sufficient regions. Anti-TPO calculation is beneficial in addressing the L-thyroxine substitution problem in mild SCH and tends to be a risk component for the possible production of OH. Anti-TPO is not known to cause disease on its own although it may lead To its growth and chronicity, and serial measurement is not recommended for AITD care control [37].

Thyroglobulin Antibodies (Anti-TG)

Thyroglobulin-specific anti-TG is a 660kDa matrix protein produced in the thyroid gland and is the major component of TH synthesis involved in the lumen of the thyroid follicle. 70-80 percent of subjects with Hashimoto's disease, 30 percent with Graves' disease, and 10-20 percent with thyroid carcinoma have elevated serum levels. In 99 percent of cases with elevated anti-TG, anti-TPO is present. By adding an anti-TG, the diagnostic evidence supplied by anti-TPO assays is hardly enhanced. In monitoring discrete thyroid cancer, Anti-TG is mainly used as an adjunctive serum thyroglobulin assay to ensure that TG measurements do not interact and as an independent surrogate tumor marker [44].

Thyroid Receptor Antibodies (TRAbs)

The TSH receptor serves as the TSH receptor antibodies (TRAbs) antigen. There are two forms of TRAbs - Thyroid stimulating immunoglobulin (TSI) that mimic TSH activities and stimulate TH production and causing Graves' hyperthyroidism and Thyroid binding inhibitory immunoglobulin (TBII) to reduce TH production by blocking TSH binding to its receptor and triggering hypothyroidism. 70-100 percent of Graves' disease (85-100 percent to activate antibodies and 75-96 percent for blocking antibodies) and 1-2 percent of individuals infected with natural TRAns are [45]. In the differential analysis of the causes of an overactive thyroid gland, TSH receptor antibody (TSI) is a key pathogenic variable predicting the response to ant thyroid therapy and a valuable independent possible risk for Graves' ophthalmopathy. It would be useful to predict TRAbs in pregnant women with a history of Graves' hyperthyroidism in determining the possibility of its trans-placental transit. TBII is not regularly screened, can even become common, and may induce hypothyroidism during Graves' therapy [46].

1.7. Thyroid Disease:

Thyroid disease refers to a category of common illnesses that are among the most prevalent medical conditions. These diseases affect about 6.6% of the world's population, with the possibility of injury to women a form of deformation of the thyroid gland by 5-10 times more than men. Thyroid disease epidemiology differs greatly between various subpopulations; living in areas with iodine deficiency, Goiter and hypothyroidism are two of the main risk factors for development, as they account for a third of the world's population. The prevalence rate of hyperactivity of the thyroid in women 0.5-2% and 0.1-0.2% in men, while the lack of activity of the thyroid 0.06-1.2% in women and 0.1-0.4% in men [47]. Due to the influence of the thyroid gland on almost all main metabolic pathways, thyroid disease is associated with different metabolic disorders.

There are five forms of general diseases of the thyroid gland, each of the marks. It may be the person one type or more at the same time. What are the five categories, namely:

- Impaired function (hypothyroidism) due to a lack of sufficient free thyroid hormones.
- High function (hyperthyroidism) due to the abundance of free thyroid hormones.
- Structural irregularities, and often have a goiter (enlarged thyroid gland).
- Tumors that may be benign or precancerous or (non-cancerous).
- Non-natural assessments of the activity of the thyroid gland without any clinical signs (hypothyroidism or subclinical overactive thyroid gland under clinical) [48].

1.7.1. Subclinical Hypothyroidism:

Hypothyroidism is a thyroid disorder of some kind. That means that you have an underactive thyroid if you have hypothyroidism ("hypo-" means "under" or "below normal"). The thyroid does not produce enough thyroid hormones in people with hypothyroidism to keep the body functioning normally. It is characterized by high thyroid stimulating hormone (TSH) serum concentrations and normal FT4 and FT3. There are two types of subclinical hypothyroidism Based on the severity of the rise in serum TSH, With concentrations of 4.5-10 mU/L, TSH was considered mild and TSH >10 mU/L was considered extreme. However, disputes about the precise of the upper limit of the reference range of TSH in the blood are confused with the description and therapeutic importance of subclinical hypothyroidism [49]. For 4 to 20% of the adult population, subclinical hypothyroidism occurs, The continuity complex arises from differences in BMI, age, and sex, ethnicity, dietary iodine consumption, and serum TSH cut-off concentrations used to describe this disorder. Moreover in areas with abundant iodine intake, overt or subclinical hypothyroidism occurs more frequently than in areas with iodine deficiency, indicating that supplementation with iodine may increase the frequency of this disease. There

may be chronic or intermittent subclinical hypothyroidism. In some of these patients, the temporary expression of antibodies that block TSH receptors may indicate a restoration of thyroid function. Thus, it may be necessary to re-evaluate the antibodies for thyroid hormone, including antibodies that block receptors TSH, in patients who have been previously diagnosed with hypothyroidism, to determine whether the latter was intermittent or permanent. On average, according to age, gender, and the presence of thyroid antibodies, 2-28 percent of patients with subclinical hypothyroidism develop overt hypothyroidism [50]. However, it was found that the concentration of TSH basic is the most important factor associated with the transition to explicit thyroid deficiency. For example a recent prospective study, the prevalence of hypothyroidism frank about 10 percent in the total study population, but it was 2 percent and 20 percent and 73 percent in patients with primary from 5.0 TSH levels to 9.9 mmol / L [51].

Causes of Subclinical Hypothyroidism

- Having a close relative with an autoimmune disorder, such as a parent or grandparent.
- Hypothyroidism can occur at any age but increases the risk of infection with age. Hypothyroidism is more common in women than men, it is more common in young women than among young people. Increased risk of hypothyroidism during pregnancy and after childbirth and during menopause.
- The injury of another case of autoimmune diseases, such as type 1 diabetes mellitus or rheumatoid arthritis or multiple sclerosis or celiac disease or Addison's disease or pernicious anemia or blood vitiligo.
- Iodine injection of radioactive or exposure to the neck or upper chest.
- Undergo surgery of the thyroid gland.
- You have hereditary disorders Down syndrome or Turner.
- Getting bipolar (manic depression) disorder [50].

Symptoms of Subclinical Hypothyroidism:

A set of symptoms note hypothyroidism. Symptoms are all that can be observed, heard, and/or experienced. Many of these signs are also mistaken for other health problems. If your levels of thyroid hormone are too low, you can't get enough thyroid hormone from your body's cells. This causes the slowing down of your body functions. For example, the body produces less energy and less heat, causing organs like the brain and intestines to work slowly. You more find You feel cooler, you get tired more quickly, your skin becomes drier, you become forgetful and depressed, and you have begun to get constipated as your body slows. Not only does hypothyroidism cause problems, but it can also make other conditions worse [50]. For several months or years, the signs of hypothyroidism generally appear slowly. Some individuals,

however, experience hypothyroidism symptoms rapidly within a few months. Generally speaking, Whenever the hormone levels decreased thyroid gland, and the longer the period of decline, it increased the seriousness of the symptoms. It has hypothyroidism without clinical (mild) the ability to cause mild symptoms or no symptoms at all. Usually, severe hypothyroidism can cause severe symptoms. By the time they know their diagnosis, some people are very ill; Others whose blood tests indicate severe hypothyroidism have few if any symptoms. Because of very mysterious signs, blood tests are the only way to be positive that you have hypothyroidism [52].

Diagnostic of Subclinical Hypothyroidism:

Diagnosis is the art or act of recognizing a disorder by its signs and symptoms. When assessing or evaluating the type and cause of hypothyroidism, It is taking many factors into account by the health care provider, preferably a doctor, including:

- Your symptoms, medical history, risk factors, and family medical history (changes in how you feel).
- A physical test.
- Blood tests: TSH is the most sensitive measure. Other tests can be helpful in certain cases, such as FT4, FT4 index, and total T4.

You will be asked by your healthcare professional about your symptoms and what medications you are taking. He will inquire about your medical background and whether there is hypothyroidism in someone in your family. Your TSH level and FT4 will be shown by a blood test [53, 54].

Treatment Subclinical Hypothyroidism:

Cannot be cured of hypothyroidism but can be treated and regulated completely in most patients. It is treated with hypothyroidism by removing the amount of hormone that is no longer possible with the thyroid gland. This ensures the survival of T4 and TSH at normal levels. Pills contain synthetic thyroid hormone (also known as L- thyroxine or levothyroxine) on hormones such as T4, which are released naturally by the thyroid healthy. Lifetime, you must eat thyroid hormone artificial every day. Each dose lasts from thyroid hormone in the artificial blood work for about a week, just like T4 produced by the thyroid gland. This helps maintain the stability of T4 levels in the blood cells of the body so that you get a steady supply of T4. Treatment depends on the sum of the body's thyroid hormones. To get your thyroid hormone level back to normal, you may need thyroid hormone replacement medication [55].

1.8. Lipid profile:

The lipids in the blood (three fatty acids bound to the glycerol backbone) are cholesterol (both unesterified and esterified and triglycerides).Cholesterol many essential roles in the body: it is a structural component of cell membranes, and an introduction to the synthesis of steroids, and is used in the production of bile acid. The main functions of fat in the triple energy storage (fat) and energy consumption (liberated fatty acids consumed by the muscles during fasting and exercise and/or configure ketone). Through integration into the lipoprotein, very low density (VLDL) and low-density lipoprotein (LDL), and high-density lipoprotein (HDL), these become fat immiscible because fat does not dissolve in water (plasma) [56].

1.8.1. Lipoproteins

Fatty proteins that carry fat through the blood circulation to all vertebrates and even insects are compounds are soluble proteins (lipoproteins) and fat. In the liver is a fatty synthesis of proteins because of metabolic changes in the intestinal precursor. Cellular lipids and exogenous lipoproteins or lipoproteins are formed from lipoproteins or cell membranes. There is a complex cycle of proteins very fatty. Are subject to the enzyme reactions to fatty acids, fat transfer operations assistance and spontaneous, and transfers of proteins intrinsic soluble, and changes to the composition of fatty proteins in response to changes synthetic [57]. These proteins play a fatty function in the transfer of cholesterol and triglycerides, such as liver, muscle, and adipose tissue, are absorbed or absorbed from the place of origin to the place of use. Proteins Apo is the protein of fatty proteins part and helps in the transport and delivery process in three ways. First, they have structural functions (for example, the protein protease B100 (Apo-B100) is the structural protein basic for VLDL and proteins fatty medium density (IDL) and LDL; Apo-A-I is the main structural protein for HDL). Second, these cells operate in the form of links to the receptor (for example, targets Apo-B LDL for the future of LDL on the liver and peripheral tissues; Apo-E is also linked to the future of LDL which is important to identify the remains of the liver). Third, the fatty protein cofactors (Apo-C-II is a working assistant agent for fatty protein lipase, for example, and Apo-A-I is an assistant factor that is a lecithin cholesterol acyltransferase (LCAT)[58].

Structure and Classification of Lipoproteins

A lipoprotein particle is a mature ball consists of a surface layer phospholipid, and cholesterol is unstable and is surrounded by fatty proteins central nuclei (triple fat and cholesterol esters) (apoproteins) (Figure 1.6.). In practical terms, you can identify fatty proteins based on the size and characteristics of buoyancy [59].

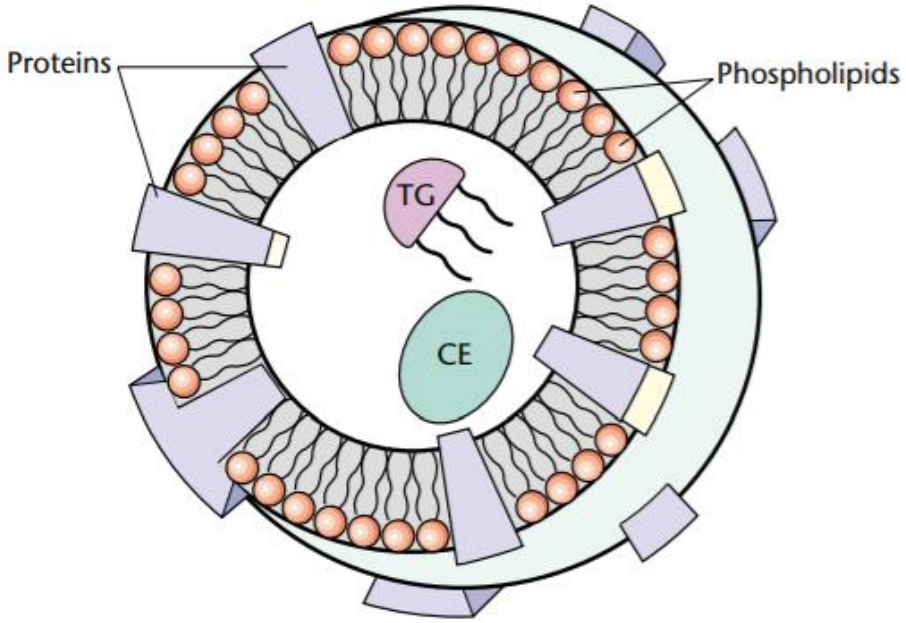

Figure 1.6. (A) Composition of central triglyceride (TG) particle, cholesterol ester (CE), phospholipid particle, and cholesterol and protein surface area [59].

1. Chylomicrons (CM)

The majority of the lipoprotein particles are CMs. The main structural protein is Apo-B-48. Triglycerides are the bulk (80%) of core lipids. These molecules are manufactured and secreted from the intestine and maintain external cholesterol, fatty acids, vitamins, and fat-soluble from digested foods [60].

2.VLDL

Is synthesized and secreted by the liver, and this particle-rich triple-fat (80% of central fat is triglycerides) is the introduction of fatty proteins medium density (IDL) and LDL. The main structural protein is Apo-B-100 [61].

3.IDL

The residue (VLDL) is formed from the lipoprotein lipase after the degradation of triglycerides in VLDL. Essential fats are made up of about 50 percent triglycerides and 50 percent of cholesterol ester. In clinical practice, the measurement of LDL and includes cholesterol fractions IDL and LDL [59].

4.HDL

The main structural protein is central Apo-A-I and lipids are also mainly cholesteryl ester. One of the functions in the transport of fatty proteins E and C-II between chylomicrons VLDL

and HDLIt also play an opposite role to atherosclerosis LDL by helping to remove cholesterol in the tissue transfer method of reverse cholesterol [62]. (not included in Figure 1.7.)

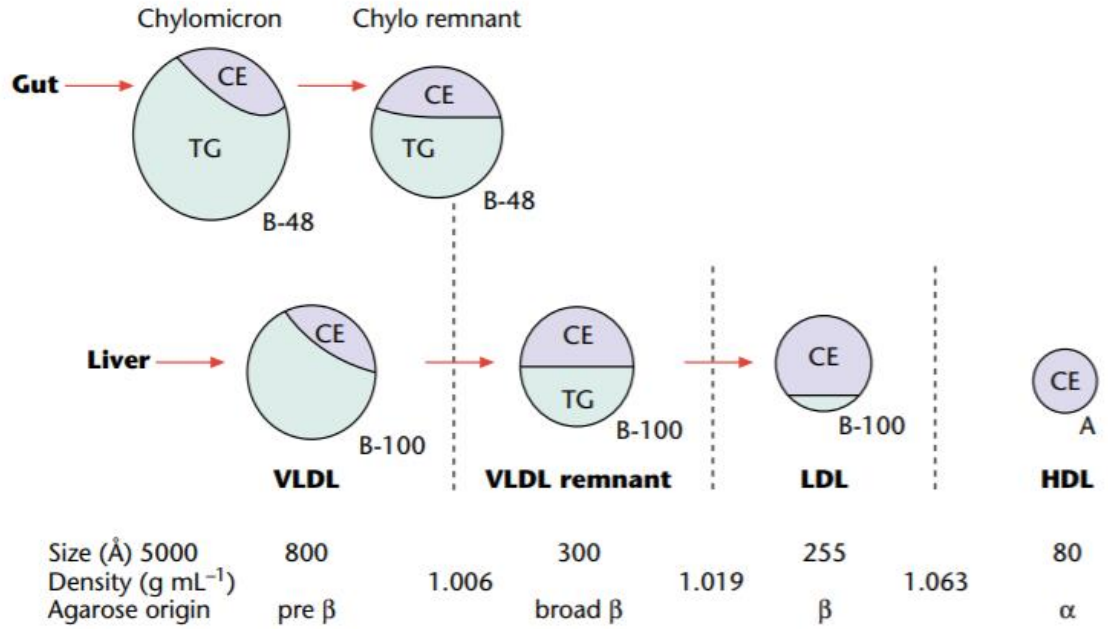

Figure Hata! Belgede belirtilen stilde metne rastlanmadı.**.7**. The size of fatty proteins and characteristics of the floats. CE, cholesteryl ester; LDL, low-density protein greasy; TG, triglycerides. VLDL, greasy protein is very low density. It can be characterized by fatty protein's material, size, or fat, and protein density[62].

5.LDL

This lipoprotein is the result of treating hepatic VLDL residues. Central fats, which represent a lot of cholesterol, circulate in the blood that is rich in cholesterol esters. It plays a major part in atherosclerosis promoting [63].

Lipoproteins Physiology:

Apo-B-100 is synthesized in the liver, in the endoplasmic reticulum. Most of this is locally degraded Apo-B. Apo-B is transported to the Golgi, where core lipids are inserted, destined to be secreted as VLDL. VLDL particle appearance (early) in plasma (Figure 1.8.). Where they are getting fatty proteins from high-density lipoproteins, such as Apo-C-II and Apo-E [59].

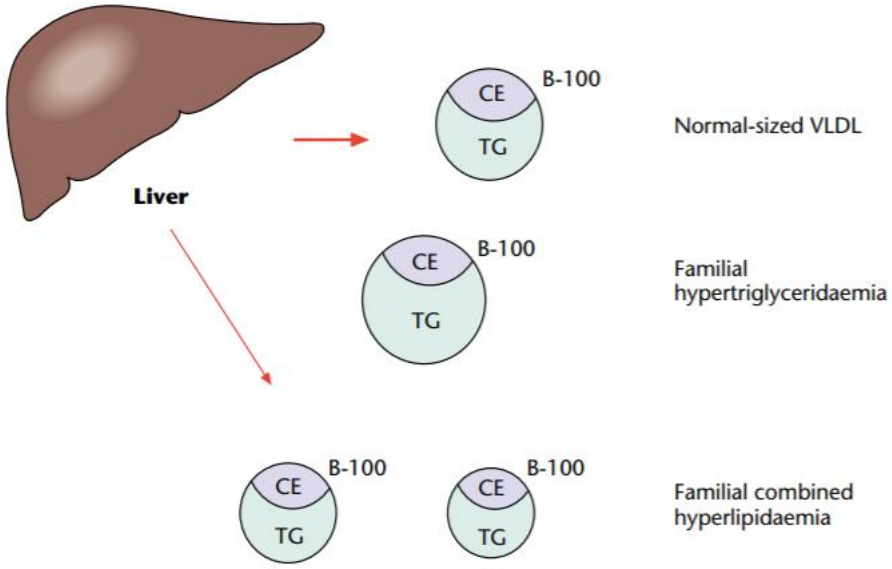

Figure 1.8. In familial hyperlipidemia, large VLDLs are secreted relative to normal VLDLs, and small VLDLs are secreted into co-familial hyperlipidemia [64].

Lipoproteins Pathophysiology

Two genetic variants of hyperlipidemia can cause defects in VLDL secretion: familial hypertriglyceridemia and familial combined hyperlipidemia. The overproduction of triglycerides is characterized by individuals with familial hypertriglyceridemia, The effect is a typical number of VLDL particles per particle with an excessive amount of triglycerides. On the other hand, people who suffer from excessive blood mixed hyperlipidemia family primarily excessive Apo-B100 in the VLDL and/or particles that LDL containing normal amounts of fat and cholesterol triple (Figure 1.8.). The molecular basis of fat triple and excessive production of Apo-B in these circumstances is unclear [64].

Lipoprotein (a):

Plasma lipoprotein human Lp (a) is a macromolecular complex first identified by the Norwegian physician Kåre Berg in 1963. Lipoprotein(a), a liver-synthesized particle consisting of a covalently bound apolipoprotein B100 (Apo B100) molecule to a very large glycoprotein known as apolipoprotein, is now considered to represent a distinct genetic form of low-density lipoprotein (LDL), Apo A is Lp (a) is connected by a second private links disulfide A poB100B100. Believes that Apo A reaction residues cysteine is identified at the end of carboxyl's happening in apoB100 K4366 through the remains of cystine is not associated. This explains why the correlation ApoB4a, a type of intestinal Apo B, which lacks the carboxyl end for ApoB100, Lp (a). It was found Lp (a) also in terms of size and density to be heterogeneous. Protein

differences may be responsible for this variation: fat, ApoB100: stoichiometric for Apo A, and scale Apo (a ratio) multi-forms, which ranges from 300 to 800 kDa. Although very small differences between individuals, the multiplicity of forms of scale Apo (a) may be one of the factors behind a wide range of differences between individuals in the plasma levels of Lp (a) that up to 1000 times [65]. Despite all these protein-level dilemmas and mysteries about the mechanisms of the adverse effects of Lp(a) on cardiovascular outcomes, good human genetic and epidemiological evidence supports the causal function of Lp(a) in cardiovascular arteriosclerotic disease and provide one of the best-supported genetic ties to aortic stenosis that have arisen from genome-wide association [66].

1.8.2. Association between subclinical hypothyroidism and lipoprotein (a) effect to cardiovascular disease:

One factor in the pathogenesis of a cardiovascular disease is subclinical hypothyroidism and abnormal lipid metabolism most studies believe that dyslipidemia is frequently correlated with patients with subclinical hypothyroidism [67]. Thyroid hormone and blood lipid levels were examined in several subclinical hypothyroidism patients and subclinical hypothyroidism was found to be linked to hypertriglyceridemia [67]. And Karthick studies have shown that (TC), (LDL-C), and triacylglycerol (TG) are significantly increased in patients with subclinical hypothyroidism relative to people with normal thyroid function. High thyroxine has a major effect on lipid metabolism. Thyroxine levels are usually present in most patients with subclinical hypothyroidism, and only TSH levels are elevated. However, even minor changes in TSH can influence the synthesis of lipids, which in turn influences the number of lipids in the blood. Lipoprotein (a), includes apolipoprotein Apo A and ApoB, etc [68]

Apolipoproteins are protein components of lipoproteins that bear circulating cholesterol and fat and are essential to cardiovascular disease and wellness. The risk of atherosclerosis because of indigestion blood lipids is commonly measured using cholesterol total, (HDL-C), (LDL-C). It has become clear in recent years that It is also expected that lipoprotein plays a significant role in atherosclerosis and thrombosis [69].

Lp (a) consists of a particle similar to that which LDL lipoprotein and B100 covalently bind to a protease through a second sulfide binding. Is determined plasma levels of Lp (a) genetically through the differences in the genetic coding of the protein protease Lp (a). Apolipoprotein (a) has a clear resemblance to structural with plasminogen. The meta-analysis found a correlation between a fixed and independent Lp (a) and the risk of coronary heart disease due to its composition [70]. Epidemiological data also suggest that high levels of Lp (a) in favor of atherosclerosis, although it is still unclear exact molecular mechanism that contributes to the Lp (a) in the atherosclerosis stage. It is estimated that the prevalence of Lp (a) greater than 50 mg

/ dL in the general population ranges from 7 to 26 percent. Lp (a) levels greater than 50 mg / dL were independently associated with a three-fold risk of cardiovascular adverse reactions in patients with coronary artery bypass grafting. Another study currently underway cases, such as blood clots and chronic heart failure due to low activity left ventricle, in large groups of patients [71]. Because the prevalence increases with age and is estimated to affect up to 2.7 percent of all individuals over the age of 65 years, the narrowing is the case of the aortic valve heart valvular increasingly important. Detection of risk factors for valvular calcification and AVS is of paramount importance as the burden of AVS is high and often leads to surgical or catheter treatment such as Transcatheter Aortic Valve Implantation (TAVI) [72]. It became clear in recent years that the development of AVS involved in the risk factors with atherosclerosis. There are also indications that high levels of Lp (a) contribute to an increased incidence of AVS. Moreover, very recent studies reported the existence of a link between the narrowing of the mitral valve and high levels of Lp (a) in patients with peripheral arterial disease friendly addition to heart symptoms, it can be high levels of Lp (a) are associated with atherosclerosis outside the heart, such as peripheral artery disease and narrowing of the arteries in the carotid artery [73]. Moreover, it was proposed Lp (a) to contribute to the initial healing of wounds, but to avoid the dissolution of external fibrin, which contributes to the subsequent problems at a subsequent phase of the healing operation. In extension, it has been reported to a greater risk of miscarriage in patients with high Lp (a)[65].

1.9. Aim of the research

This present study aims to show Lipoprotein (a) levels and risk of cardiovascular diseases among individuals with subclinical hypothyroidism.

1.9.1. Objectives of the study (should be in points)

The present study is designed to

1. Evaluate the serum lipoprotein (a) in patients with subclinical hypothyroidism

2. Ascertain the association between lipoprotein (a) and level of TSH in patients with subclinical hypothyroidism.

3. Compare the apolipoprotein B and apolipoprotein A ratio with that of LDL and HDL ratio in subjects with subclinical hypothyroidism.

2. MATERİALS AND SAMPLE RESEARCH STARTEGY

We explain the method of analysis in this chapter also the material I use in this study, also the instrument we used in this study, we explain below, also the software programmer we used to explain the statistical parameter.

2.1. Study and sample research startegy:

This study was carried out at the endocrinology unit in Azadi Teaching hospital, Emergency Teaching Hospital, VIN complex Hospital. The study spanned the period from February 2020 to November 2020 using convenient sampling techniques.

The research was performed with 50 newly diagnosed patient having subclinical hypo-thyroidism and 50 ages and sex-mached healthy controls. The subclinical hypothyroidism cases were considered as the group of people with elevated TSH (4.2 to 10 micro IU/ml) with normally standard levels of total free T3 /T4 values. The patients diagnosed, end-stage kidney disorder, liver disease, primarily known cardiac disorder, diabetic patients with both type I (T1DM) and type 2 (T2DM) mellitus, acute systemic disorder, elevated B.P, untreatmented with thyroxine/anti-thyroid drugs or antilipidemic medication, women with pregnancy and woman on oral contraceptive medications, the ones that didn´t agree and sign the study consent were excepted. And at the end, 50 newly diagnosed subjects of subclinical hypothyroidism were joined to the research, 50 normal healthy examples (ages & sexes match) were chosen randomly from subjects visiting outpatient departments of hospital for slight illness or regular checking.

The study group ages were between 18 and 55 years. Ethical clearance was obtained from the Directorate of Health, of Duhok city-Govern ate.

And informed consents were gathered from the participants joined the study before starting the analysis and data collection. All the subjects were evaluated basen on clinical invistigations.

The questionnaire form includes questions such as: name, age, date of birth, gender, family history of hypothyroidism, cardiovascular disease, and hyperlipidemia. Weight, Hight , WC, B.P, were obtained and BMI calculated.

2.2. Assessment of studied variables:

The samples were collected depend on inclusion criteria only 100 samples were selected from patients and asked to participate in this study project.

The study population was grouped into 7 different classes based on Age, WC (waist circumferences, BMI (body mass index), blood pressure, serum TC, Tg, LDL, HDL, Lp (a),. and thyroid function tests.

2.2.1. Class-based on age:

The subjects were grouped according to their age into four classes:

1.< 20 years.

2. Equal 20 to 29 years.

3. Equal 30 to 39 years.

4. Equal for more than 40 years.

2.2.2. Smoking habits

Based on smoking habits, the subjects were classified into two groups:

1. Nonsmoker.

2. Smoker (either light or heavy smoker).

2.2.3. Familial based history of hypothyroidism:

Subjects were grouped into two classes based on their familial causes:

1. Families with no history of hypothyroidism.

2. Families with a history of hypothyroidism.

2.2.4. Familial based history of CVD:

Subjects were grouped into two classes based on their familial causes:

1. Families with no history of CVD.

2. Families with a history of CVD.

2.2.5. Family history of Lipidema:

Subjects were grouped into two classes based on their familial causes:

1. Families with no history of lipidemia.

2. Families with a history of lipidemia.

2.2.6. Waist Circumference (WC)

Waist circumference has been taken on a high point of the iliac crest at minimal respiration to the nearest 0.1 cm. Central obesity is defined, following (Crook, 2007):

1. = > 102 cm for men.

2. = > 88 cm for women.

2.2.7. Body Mass Index (BMI):

BMI an approach used to determine if individuals based on their height and weight ratios are considered to be in the healthy weight range, underweighted. Overweighted or has obesity [41].

*BMI= weight (kg)/ (height (m))^2

1. Body mass index ranging in 18.5 and 24.9 kg/m2 were estimated normal weighted.
2. Body mass index ranging in 25.0 and 29.9 kg/m2 were estimated overweighted.
3. Body mass index equal to, or more than 30 kg/m2 were estimated obesed.

2.2.8. Blood pressure:

Based on B.P, the individuals were grouped into two classes:

1. Greater than, 130/80 Hypertension.
2. Equal and Less than, 130/80 Normal.

2.2.9. Thyroid function:

1. Thyroid - Stimulating Hormone (TSH):

Thyroid - Stimulating Hormone, the subject was classified into two groups:

1. Subclinical hypothyroidism, 4.2 to 10 μIU/mL.
2. Normal TSH, between 0.27and 4.2 μIU/mL.

2. Free Thyroxine (FT4):

Have only one group, normal 12 – 22 pmol/L.

3. Free Triiodothyronine (FT3):

Have only one group, normal 3.1- 6.8 pmol/L.

4. Thyroid Peroxidase Antibodies (Anti-TPO):

Anti-TPO, the subject was classified into two groups:

1. More than 34 IU/ml Hashimoto's disease.
2. Less than 34 IU/ml Normal.

2.2.10. Lipid profile status:

1.Total serum cholesterol:

1. S. total cholesterol < 200 mg/dl was regarded as normal.

2. S. total cholesterol between 200 and 239 mg/dl was regarded as borderline.
3. S. total Cholesterol = > 240 mg/dl was regarded as high.

2. Serum triglyceride (TG):

According to NCEP- normal range:

1. S. TG < 150 mg/dl was regarded as normal.
2. S. TG > 150 mg/dl was regarded as high.

3. Serum (HDL-C):

According to NCEP.

Males < 40 mg/dl (low HDL - Chol) major risk for CHD.

Female = < 60 mg/dl (low HDL- Chol) major risk for CDH.

	No risk of	Moderate risk	High risk
Females	> 65	45-65	< 45
Males	> 55	35-55	< 35

4.Serum (LDL-C)

Adult levels in terms of CHD risk.

Optimal < 100 mg/dl, Near optimal(100-129 mg/dl).

Border line (130-159 mg/dl), High (160 -189 mg/dl) ,Very High > =190 mg/dl.

5. Serum Apolipoprotein an (APO A):

Using serum from a healthy subject, the following values were obtained:

1. S.APO A less than 180 mg/dl was regarded as a low level for female
2. S.APO A between 108 and 225 mg/dl was regarded as normal for female
3. S.APO A more than 225 mg/dl was regarded high for female
4. S.APO A less than 104 mg/dl was regarded as a low level for male
5. S.APO A between 104 and 202 mg/dl was regarded as normal for male
6. S.APO A more than 202 mg/dl was regarded high for male

7. Serum Apolipoprotein b (APO B):

Using serum from a healthy subject, the following values were obtained:

1. S.APO B less than 60 mg/dl was considered low for women
2 .S.APO B between 60 and 141 mg /dl was considered normal for women
3. S.APO B more than 141 mg/dl was considered high for women
4. S.APO B less than 66 mg/dl was considered for mean
5. S.APO B between 66 and 144 was considered normal for mean
6. S.APO B more than 144 was considered high for mean

8. Lipoprotein A (LP (a)):

This suggests screening for elevated Lp(a) at intermediate or elevated risk of CVD/CHD and determines the desirable level of Lp(a):

1. LP (a) equal more than 50 mg/dl high.
2. Serum Lp (a) less than 50 mg/dl normal.

2.2.11. Serum Cardiac C - reactive protein (Latex) High Sensitive;

Consensus reference interval for adults.

IFCC/CRM

1. Serum level hs-CRP equal, more than 5 mg/dl high.
2. Serum level hs-CRP less than 5 mg/dl normal.

2.3. Methods:

We describe the sample collection and method of our study.

2.3.1. Collection and processing of blood sample:

One hundred subjects were collected in this study (50 patient's newly diagnosed subclinical hypothyroidism and 50 healthy individuals as a control group).

Participants who attended were fasting overnight and attended the biochemistry and endocrinology unit in the morning. The venous blood sample (5 mL) was collected between 8:30-11:30 am, by venipuncture using a vacuum from the anterior vein. Were collected immediately into gel separator tubes of the vacuum system. Using a centrifugation process, serum was isolated from whole blood after coagulation (Hitachi centrifuge, model O5P-21) at 5,000 rpm for 15 minutes, and serum was immediately treated to measure TSH, FT3, FT4, anti-TPO, TC, Triglycerides, HDL, LDL, APO A, APO B, LP (a) and CRP-HS. Standard technical guide for identifying Automated Cobas 6000 Immunoassay Analyzers based on Electrochemical Radiation Technology using ruthenium compound and measuring cell.

2.3.2. Thyroids analysis:

Thyroid analysis include TSH, T4, T3, and anti-TPO

Thyroid Stimulating Hormone (TSH):

Thyroid Stimulating Hormone level was tested using TSH 200 tests Roche/Hitachi, Cobas e 411, Cobas e 601/602 for more detailed information regarding the test principle and protocol refer to the leaflet with the reference number of 08429324 190.

Free Triiodothyronine (FT3):

Free Triiodothyronine hormone level was tested using FT3 200 tests Roche/Hitachi Cobas e 411, Cobas e 601/602 for more detailed information regarding the test principle and protocol refer to the leaflet with the reference number of 06437222 190.

Free Thyroxine FT4:

Free Thyroxine hormone level was tested using FT4 200 tests Roche/Hitachi Cobas e 411, Cobas e 601/602 for more detailed information regarding the test principle and protocol refer to the leaflet with the reference number of 07976836 190.

Thyroid peroxidase Antibodies Anti-TPO:

Thyroid peroxidase Antibodies hormone level was tested using Anti-TPO 100 tests Roche/Hitachi Cobas e 411, Cobas e 601/602 for more detailed information regarding the test principle and protocol refer to the leaflet with the reference number of 06368590 190.

2.3.3. Biochemical analysis:

Biochemical analysis that describes the analysis of TC, Tg, LDL, HDL, hsCRP

Total Cholesterol:

Total Cholesterol level was tested using CHOL2 Gen.2 400 tests Roche/Hitachi Cobas c 311, Cobas 501/502 for more detailed information regarding the test principle and protocol refer to the leaflet with the reference number of 03039773 190.

Triglycerides:

Triglycerides level were tested using TRIGL 250 tests Roche/Hitachi Cobas c 311, Cobas 501/502 for more detailed information regarding the test principle and protocol refer to the leaflet with the reference number of 20767107 322.

HDL-Cholesterol:

HDL-Cholesterol level was tested using HDLC4 Gen.4 350 tests Roche/Hitachi Cobas c 311, Cobas 501/502 for more detailed information regarding the test principle and protocol refer to the leaflet with the reference number of 075285566 190.

LDL-Cholesterol:

LDL -Cholesterol level was tested using LDLC3 Gen.3 200 tests Roche/Hitachi Cobas c 311, Cobas 501/502 for more detailed information regarding the test principle and protocol refer to the leaflet with the reference number of 07005717 190.

APOA-Apolipoprotein A:

APOA-Apolipoprotein A level was tested using APOAT ver.2 100tests Roche/Hitachi Cobas c 311, Cobas 501/502 for more detailed information regarding the test principle and protocol refer to the leaflet with the reference number of 03032566 122.

APOB-Apolipoprotein B:

APOB-Apolipoprotein B level was tested using APOBT ver.2 100tests Roche/Hitachi Cobas c 311, Cobas 501/502 for more detailed information regarding the test principle and protocol refer to the leaflet with the reference number of 03032574 122.

LPA2 – Lipoprotein (a):

Apolipoprotein (a) level was tested using LPA2 Gen.2 150 tests Roche/Hitachi Cobas c 311, Cobas 501/502 for more detailed information regarding the test principle and protocol refer to the leaflet with the reference number of 05852625 190.

C-Reactive protein –hs (CRP-HS)

C-reactive protein (Latex) High Sensitive level was tested using CRPHS 300 tests Roche/Hitachi Cobas c 311, Cobas 501/502 for more detailed information regarding the test principle and protocol refer to the leaflet with the reference number of 04628918 190.

2.4. Statistical analyses

The general characteristic of the study groups was presented in mean and Sta. Deviation or number and percentage. The homogeneity between patients with subclinical hypothyroidism and

healthy controls in terms of general characteristics were examined in an independent t-test or Pearson Chi-squared tests. The comparison of biochemical parameters between cases with subclinical hypothyroidism and healthy controls was examined in independent t-test, Pearson Chi-squared, or Fishers' exact tests. The correlation of Lpa and TSH concentration with biochemical parameters was examined in bivariate regression. In bivariate regression, the adjustment was made for age, gender, smoking, SBP, DBP, and BMI. The comparison of biochemical concentrations in patients with different age groups and BMI was examined in one-way ANOVA. The scatter plots were made by SAS JMP 14.3. The significant level of difference was determined in a p-value of less than 0.05. The statistical analyses were performed by statistical package for social sciences version 25 (IBM SPSS Statistics for Windows, Version 25.0. Armonk, NY: IBM Corp).

3. RESULT

In this section, we present the result we obtained in this study.

3.1. Demographic features

The results of the general feature of the groups It shows in Table 3.1 When clinical characteristics of 50 subjects of patient and the 50 normal control groups that age- and sex similar were compared as illustrated in Figure 3.1. and Figure 3.2. , the study found that most of the patients and subjects were females; (80%) in the control group and (78%) in the subclinical hypothyroid patients as showed in Figure **Hata! Belgede belirtilen stilde metne rastlanmadı.**.3, no significant differences were found depending on age or gender among the two groups. Although waist circumference was significantly higher in subclinical hypothyroidism 106.70 (18.92) than in control 92.62 (12.39) ($p < 0.001$) but in BMI and blood pressure no significant difference was observed between the groups. Furthermore, the study found that most of the participants were non-smokers in the two studied groups, 94%, and 82% in subclinical hypothyroidism and control, respectively ($p=0.065$);. Regarding the family history, it was observed that near half of the patients had a familial history of hypothyroidism (48.0% in the cases group and 26% in subject of the control group), but from both the cardiovascular disease and lipidemia (38.0%,44.0%) among the patient group and followed by 36.0% and 32.0% of both diseases among control, respectively.

Table 3.2. Comparison of general characteristics between cases with subclinical hypothyroidism and healthy controls

Characteristics	Study groups		P-Value (two-sided)
	Case (n=50)	Control (n=50)	
Age (yrs.) Mean (SD)	30.70 (9.31)	31.28 (9.32)	0.756[a]
Age distribution no. (%)			
<20 yrs.	5 (10.0)	5 (10.0)	0.995[b]
20-29	20 (40.0)	19 (38.0)	
30-39	14 (28.0)	14 (28.0)	
≥40	11 (22.0)	12 (24.0)	
Gender no. (%)			0.806[b]
Male	11 (22.0)	10 (20.0)	
Female	39 (78.0)	40 (80.0)	
Family history of hypothyroidism no. (%)			0.012[b]
Yes	24 (48.0)	12 (24.0)	
No	26 (52.0)	38 (76.0)	
Family history of CVD no. (%)			0.836[b]
Yes	19 (38.0)	18 (36.0)	
No	31 (62.0)	32 (64.0)	
Family history of Lipedemia no. (%)			0.216[b]
Yes	22 (44.0)	16 (32.0)	
No	28 (56.0)	34 (68.0)	
Smoking habit no. (%)			0.065[b]
Yes	3 (6.0)	9 (18.0)	
No	47 (94.0)	41 (82.0)	
Blood pressure no. (%)			0.161[b]
Normal blood pressure	40 (80.0)	45 (90.0)	
Hypertension	10 (20.0)	5 (10.0)	
SBP Mean (SD)	114.20 (7.85	115.80 (8.35	0.326[a]
DBP Mean (SD)	74.00 (10.69	69.80 (7.95	0.028[a]
BMI Mean (SD)	26.79 (5.83	24.84 (5.21	0.082[a]
BMI distribution no. (%)			0.599[b]
Normal weight	22 (44.0)	27 (54.0)	
Overweight	14 (28.0)	12 (24.0)	
Obese	14 (28.0)	11 (22.0)	
WC Mean (SD)	106.70 (18.92)	92.62 (12.39)	<0.001[a]
Male	102 (17.55)	93.94(10.48)	
Normal	7(63.6%)	8(80.0%)	
Abnormal	4(36.4%)	2(20%)	
Female	108.03(19.29)	92.29(12.92)	
Normal	4(10.3%)	16(40%)	
Abnormal	35(89.7%)	24(60%)	

[a] An independent t-test, and [b] Pearson Chi-squared test were performed for statistical analyses.

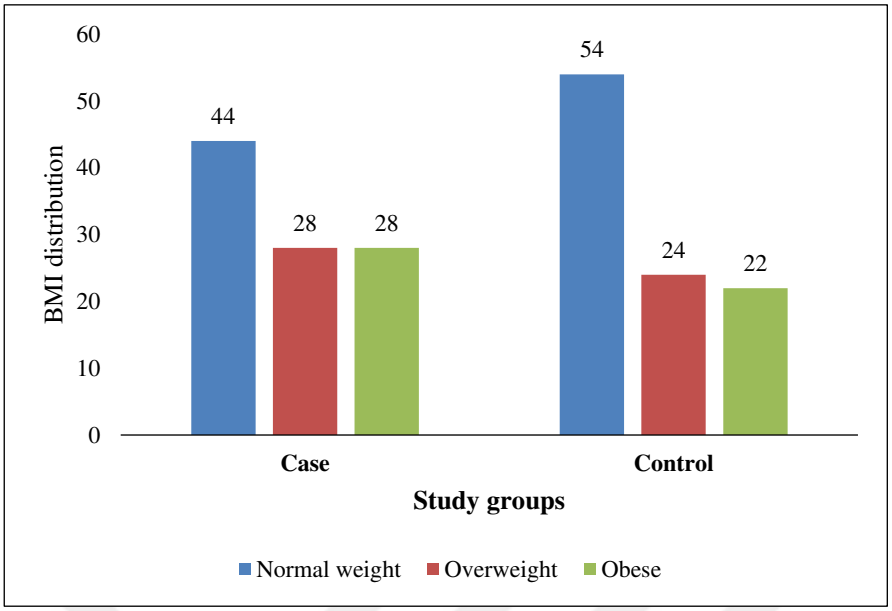

Figure 3.1. Comparison of BMI distribution between patients with subclinical hypothyroidism and healthy controls.

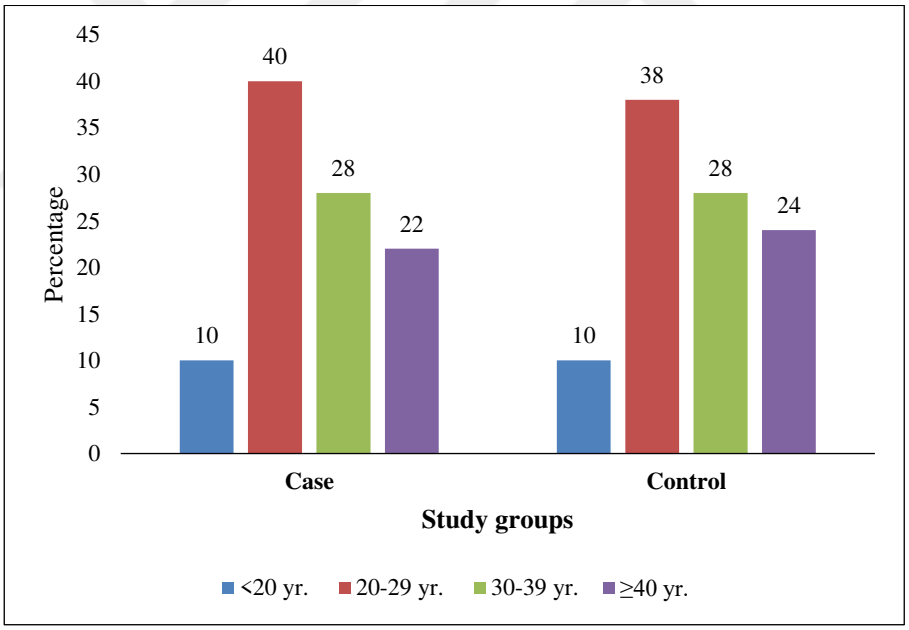

Figure 3.2. Comparison of age distribution between patients with subclinical hypothyroidism and healthy controls

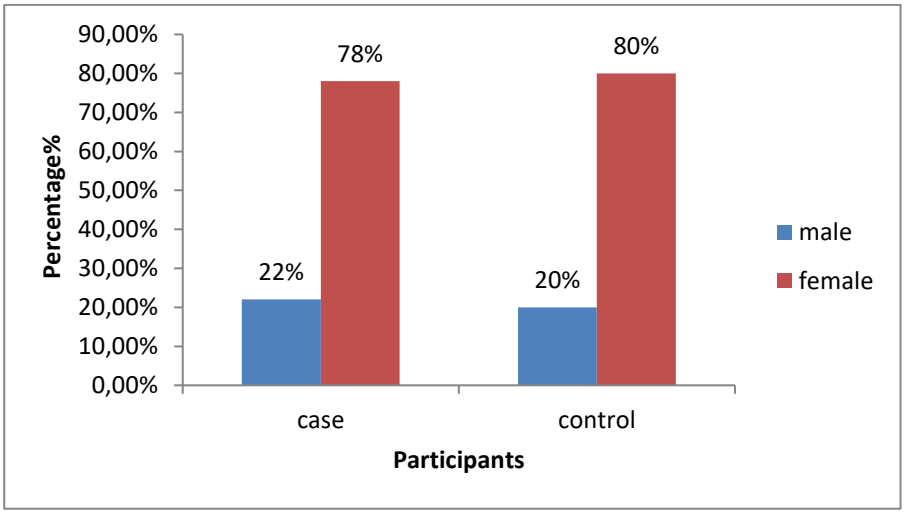

Figure 3.3. Comparison of gender distribution between patients with subclinical hypothyroidism and healthy controls.

3.2. Biochemical Parameters

Thyroid function tests, lipid profile, and cholesterol panel of the patients included in the research were examined. The data for these parameters are illustrated in Table 3.2. The comparison of the thyroid status of the subjects among the groups found a significant difference in the thyroid markers. Thyroid hormones level FT3 5.03 (0.63) were non-significantly greater in the case compared to in the control groups ($p>0.05$), and FT4 levels 14.27 (2.10) though still within the normal range as expected by diagnosis were lower in the subclinical state whereas TSH 7.24 (1.65), anti-TPO 133.23 (166.08) , low-density lipoprotein (LDL-C) 96.54 (24.72)and serum total cholesterol (TC) 152.98 (26.25),) was significantly ($p<0.05$) increase in the subclinical hypothyroidism group compared to control group. However, There was Triglyceride (TG) 112.34 (54.39) in the subclinical hypothyroidism group no significant higher than the normal thyroid function group ($P>0.05$); (HDL) 41.94 (9.32) and LDL to HDL ratio 2.33 (0.86) apromixtly same with control groups 2.21 (0.85).

Table 3.3 Comparison of biochemical parameters between cases with subclinical hypothyroidism and healthy controls

Characteristics	Study groups Mean (SD)		P-Value (two-sided)
	Case (n=50)	Control (n=50)	
TSH Mean (SD)	7.24 (1.65)	2.37 (1.14	<0.001[a]
FT3 Mean (SD)	5.03 (0.63)	4.98 (0.59	0.662[a]
FT4 Mean (SD)	14.27 (2.10	15.25 (1.82	0.014[a]
ATPO Mean (SD)	133.23 (166.08	16.71 (4.87	<0.001[a]
ATPO Normal Abnormal	 28 (56.0% 22 (44.0%	 43 (86.0% 7 (14.0%	0.001[b]
Total Cholesterol Mean (SD) Cholesterol distribution Normal Borderline Abnormal	152.98 (26.25 48 (96.0% 2 (4.0% 0 (0.0%	138.72 (29.48 48 (96.0% 1 (2.0% 1 (2.0%	0.012[a] 0.495c
Triglyceride Mean (SD) Triglyceride distribution Normal Abnormal	112.34 (54.39 39 (78.0% 11 (22.0%	105.16 (39.75 44 (88.0% 6 (12.0%	0.453[a] 0.183[b]
HDL Mean (SD) HDL distribution No risk High risk Moderate risk	41.94 (9.32 3 (6.0% 30 (60.0% 17 (34.0%	44.40 (9.45 5 (10.0% 21 (42.0% 24 (48.0%	0.193[a] 0.193[c]
LDL Mean (SD) LDL distribution Optimal Nera optimal Borderline high	96.54 (24.72 30 (60.0% 15 (30.0% 5 (10.0%	86.38 (22.68 32 (64.0.0% 18 (36.0% 5 (5.0%	0.035[a] 0.082[c]
LDL/HDL Ratio	2.33 (0.86)	2.21 (0.85)	0.445[a]
[a] An indecent t-test, [P]earson Chi-squared , and 'Fishers' exact tests were performed for statistical analyses.			

3.3. CVD risk marker parameters

I have compared the results of CVD Risk markers of the participants in subclinical hypothyroidism patients and healthy control groups. These include Lp(a), Apo A-I, Apo B, APOB/APOA ratio, and hsCRP.

The CVD markers and have been depicted in Table 3.3. with a comparison of these parameters in subclinical hypothyroidism and control groups. It is clear from the table that the mean values Lp(a) were significantly ($p<0.05$) increase in the subclinical hypothyroid population 37.23 (41.08) than in controls 21.19 (13.45) as shown in Table 3.3 and Figure 3.4. and other parameters (Apo B, APOB/APOA ratio, and hsCRP) concentration in subclinical hypothyroidism and controls was (85.84 (23.96), 81.32 (18.43)),(0.65(0.2),0.61 (0.17))and (1.93 (1.42),1.91 (1.42)) are nonsignificantly ($p>0.05$) higher in subclinical hypothyroidism. Except for Apo A-I.

The mean value of Apo A-I (135.78 (20.05)) was non-significantly (p>0.05) lower in subclinical hypothyroidism compared to controls.

Table Hata! Belgede belirtilen stilde metne rastlanmadı..**4.** Comparison of biochemical parameters between cases with subclinical hypothyroidism and healthy controls.

Characteristics	Study groups Mean (SD)		P-Value (two-sided)
	Case (n=50)	Control (n=50)	
APOA Mean (SD)	135.78 (20.05	138.32 (23.86	0.566
APOA distribution			
Low	4 (8.0%	6 (12.0%	0.505
Normal	46 (92.0%	44 (88.0%	
APOB Mean (SD)	85.84 (23.96	81.32 (18.43	0.293
APOB distribution			
Low	5 (10.0%	4 (8.0%	
Normal	44 (88.0%	46 (92.0%	0.741 Fishers'
High	1 (2.0%	0 (0.0%	
APOB/APOA ratio Mean (SD)	0.65 (0.20)	0.61 (0.17)	0.276
Lpa Mean (SD)	37.23 (41.08	21.19 (13.45	0.011
Lpa distribution			
Normal	34 (68.0%	38 (76.0%	0.373
Abnormal	16 (32.0%	12 (24.0%	
Hs.CRP Mean (SD)	1.93 (1.42	1.91 (1.42	0.933
Hs.CRP distribution			
Normal	44 (88.0%	46 (92.0%	0.505
Abnormal	6 (12.0%	4 (8.0%	

[a] An indecent t-test, [P]earson Chi-squared , and [C]Fishers' exact tests were performed for statistical analyses.

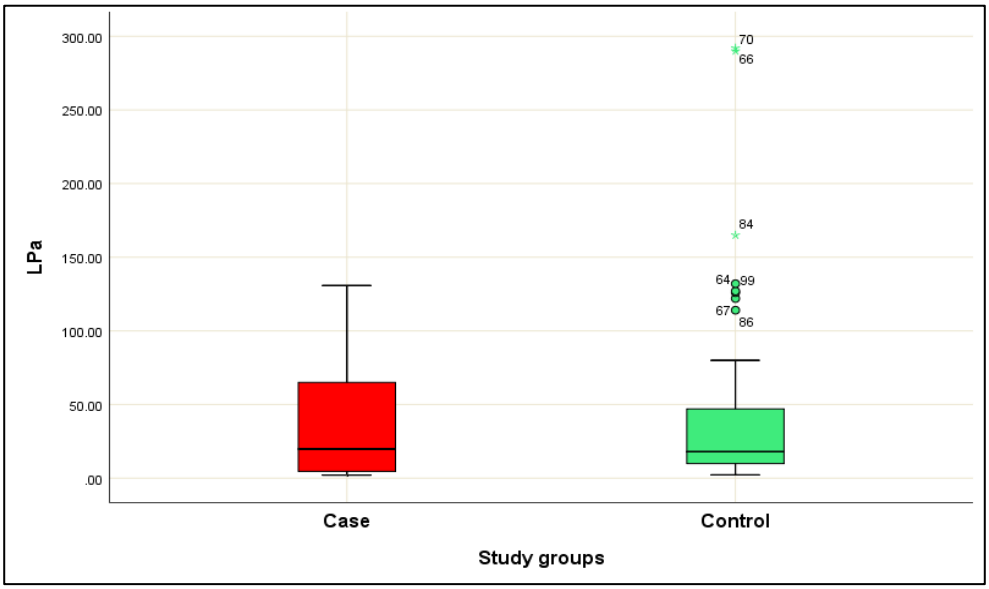

Figure 3.4. Comparison of Lpa between cases with subclinical hypothyroidism and healthy controls.

3.3.1. Correlation between Lp(a) with subclinical hypothyroidism :

Table 3.4 is the Pearson's correlation analysis of Lp (a) with biochemical parameters components revealed the following aspects.

Lp (a) had no correlations with age, BMI, TSH, FT3, FT4, ATPO. APOA and hs CRP, however CHO, LDL, APOB, whenever a ratio of APOB to APOA had a significant positive correlation with Lp a ($p<0.05$), while a ratio of LDL/HDL had a nonsignificant positive correlation with Lpa ($p<0.05$), that have been show in Figure 3.5, 3.6, 3.7, 3.8.

Table 3.5. Correlation of Lpa with biochemical parameters concentration in persons with subclinical hypothyroidism.

Control Variables	Lpa	
	Correlation	Significance (2-tailed)
Age (Yrs.)	0.026	0.861
BMI	0.119	0.416
TSH	0.073	0.618
FT3	-0.155	0.289
FT4	-0.19	0.192
ATPO	-0.04	0.786
CHO	**0.314**	**0.028**
TRI	-0.06	0.682
HDL	-0.138	0.343
LDL/HDL	0.272	0.059
LDL	**0.339**	**0.017**
APOA	-0.056	0.700
APOB	**0.407**	**0.004**
APOB/APOA	**0.348**	**0.014**
Hs.CRP	0.068	0.644

The adjustment was made for gender.
The bold numbers show the significant correlating.

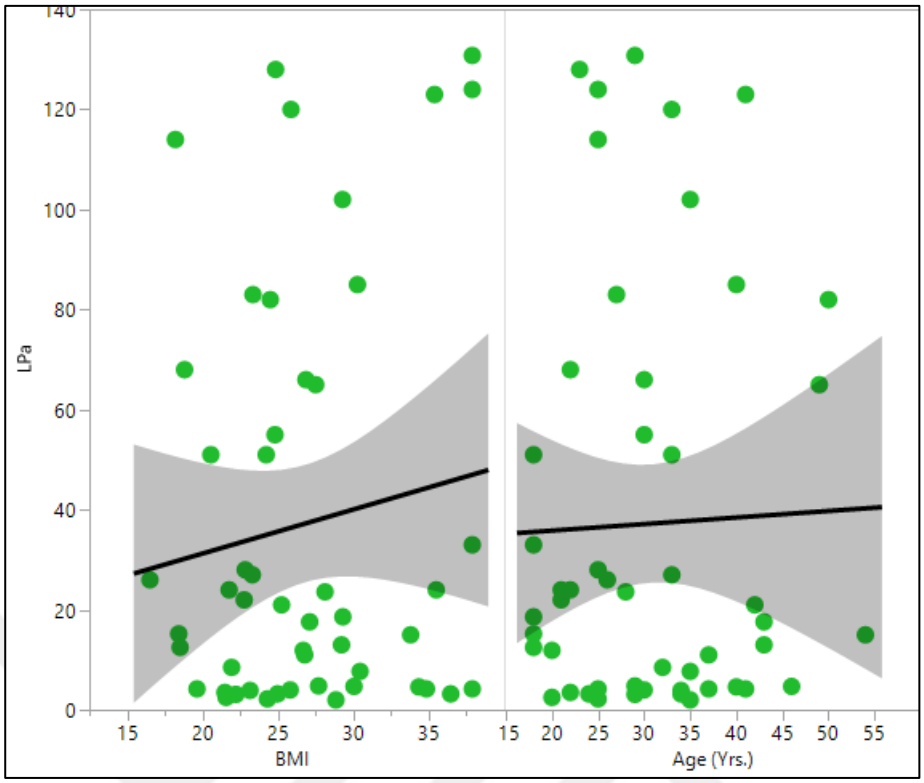

Figure 3.5. Scatter plot of Lpa with age and BMI concentration in persons with subclinical hypothyroidism

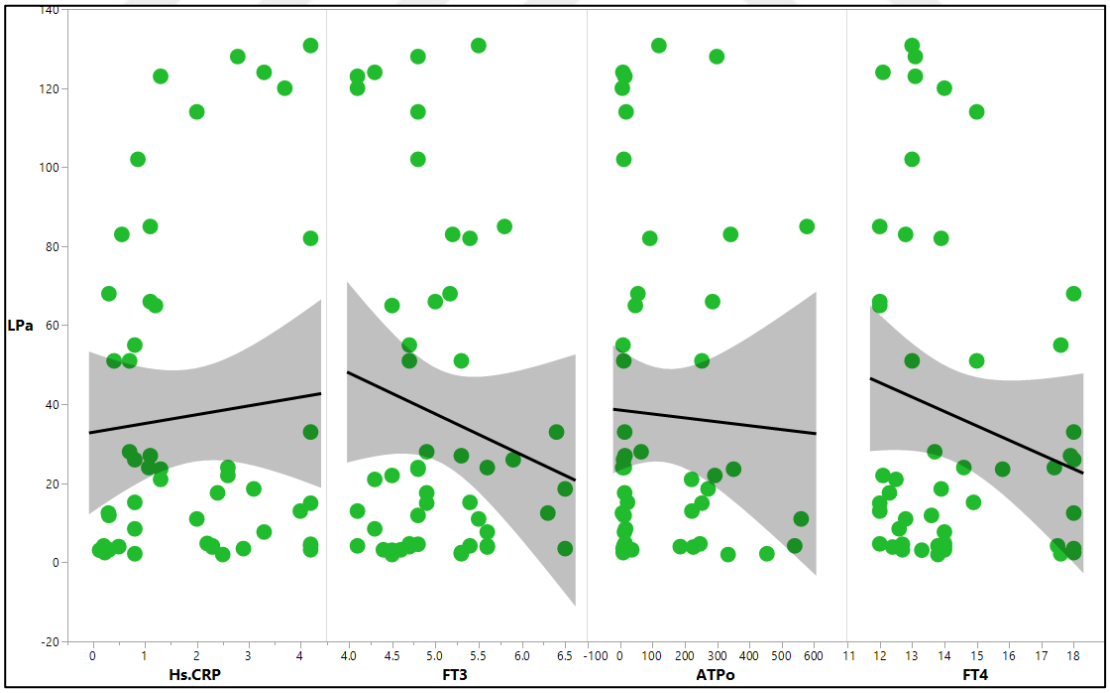

Figure 3.6 Scatter plot of Lpa with HsCRP, FT3, ATPO, and FT4 concentration in persons with subclinical hypothyroidism.

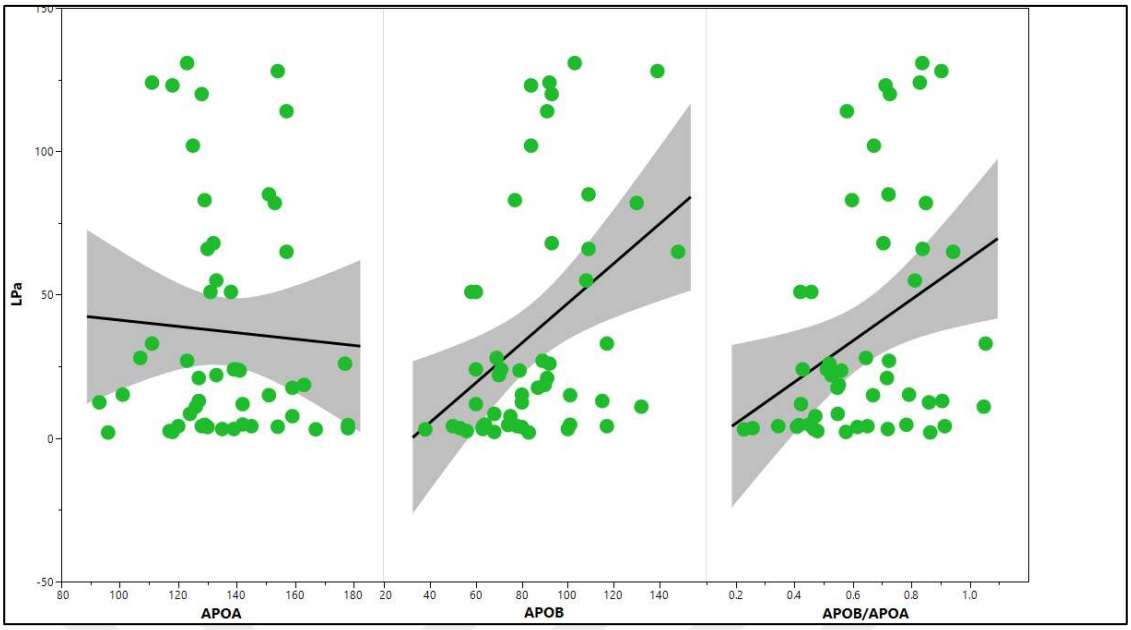

Figure 3.7. Correlation of Lp (a) with Apo A, APO B, APO B/Apo A with adjustment for gender in with subclinical hypothyroidism

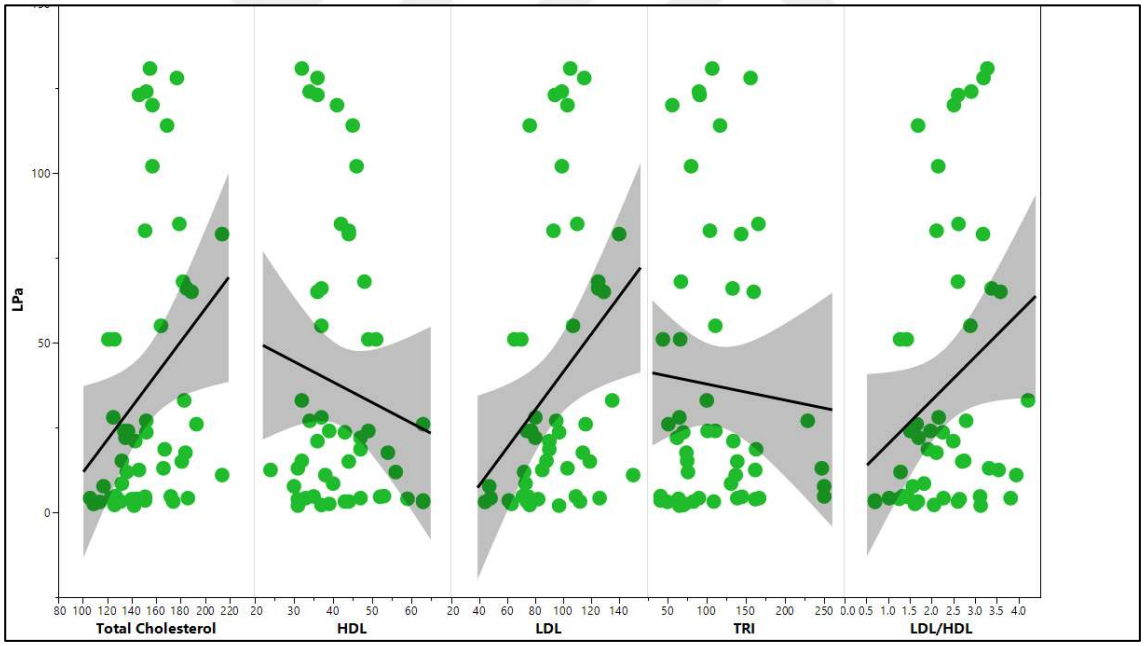

Figure 3.8. Correlation of Lp (a) with lipid profile parameters with adjustment for gender in patients with subclinical hypothyroidism.

3.4. Correlation of Thyroid stimulated hormone (TSH) in subclinical hypothyroidism patients with biochemical parameters

Table 3.5 our results in the whole study population, according to Pearson correlation, showed that serum TSH level had a positive significant correlation with age, ATPO, and CHO ($p< 0.05$), conversely we found that TSH had a negative significant correlation with FT4

(P<0.05). Eventually, there is no correlation between TSH with the other parameters, as show in Figure 3.9, 3.10, 3.11, 3.12.

Table 3.6. Correlation of TSH with biochemical parameters with adjustment for gender in patients with subclinical hypothyroidism

Control Variables	TSH	
	Correlation	Significance (2-tailed)
LPa	0.073	0.618
Age	0.386	0.006
BMI	0.034	0.819
FT3	0.133	0.361
FT4	-0.44	0.002
ATPO	0.489	<0.001
CHO	0.311	0.029
TRI	0.28	0.051
HDL	-0.028	0.849
LDL/HDL	0.179	0.219
LDL	0.278	0.053
APOA	0.149	0.307
APOB	0.283	0.049
APOB/POA	0.157	0.280
Hs.CRP	0.167	0.250
The adjustment was made for gender.		

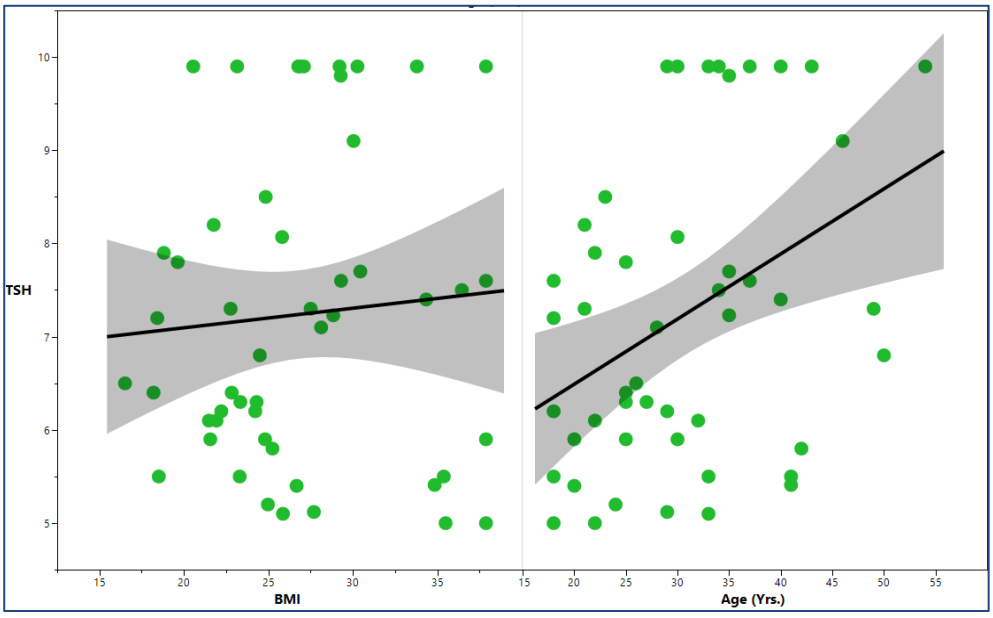

Figure 3.9. Scatter plot of TSH with age and BMI concentration in persons by subclinical hypothyroidism

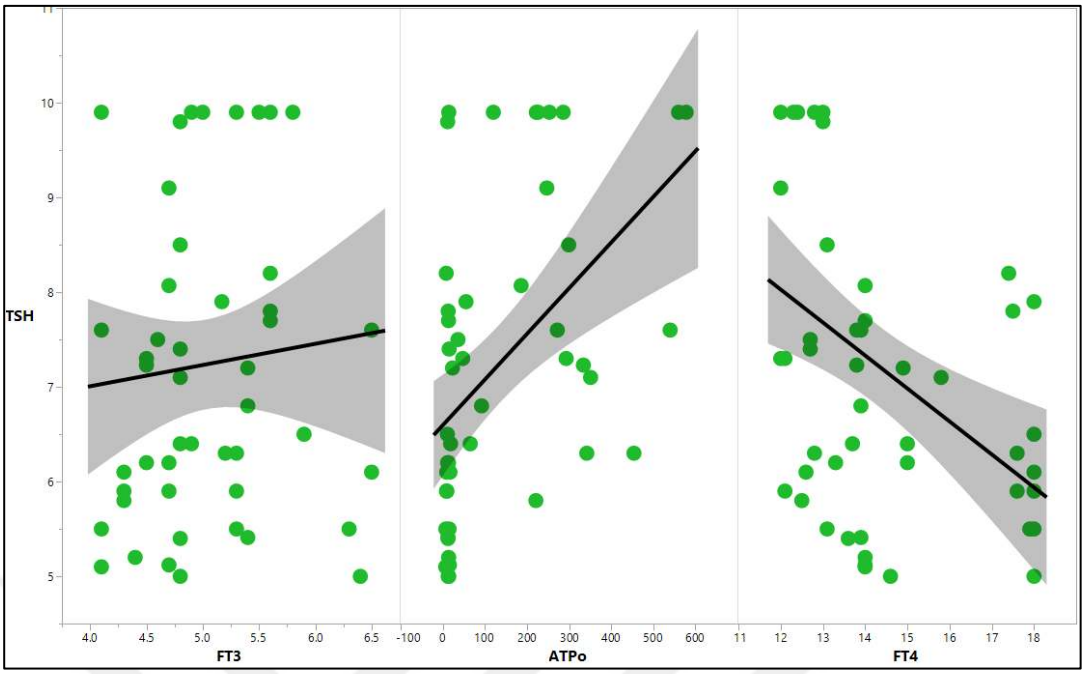

Figure 3.10. Scatter plot of TSH with thyroid function test concentration in persons by subclinical hypothyroidism

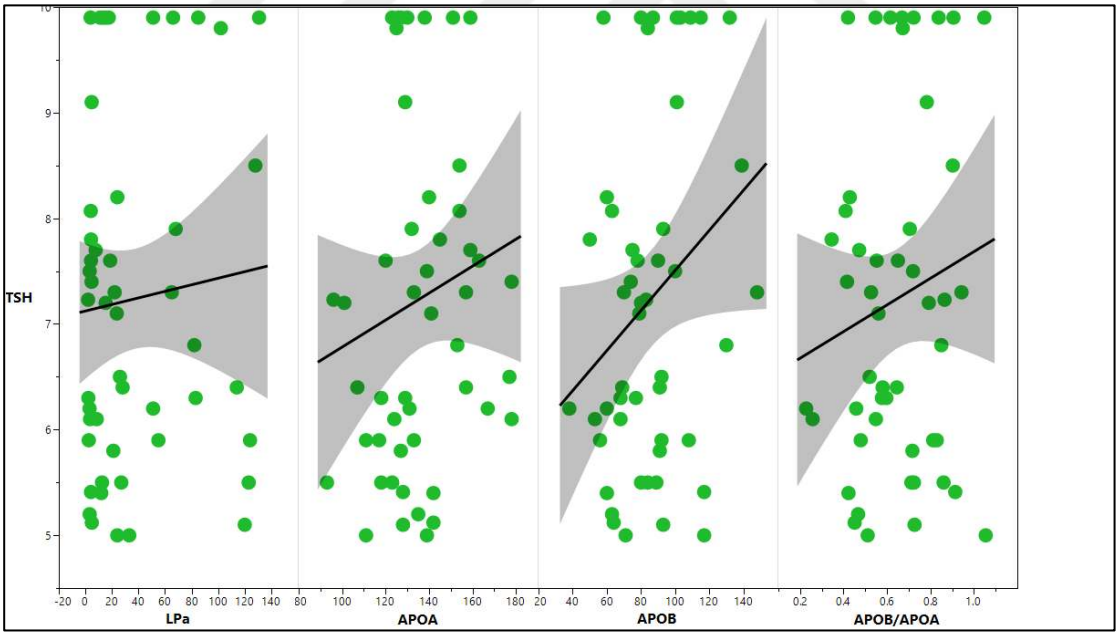

Figure 3.11. Correlation of TSH with lipoprotein profile with adjustment for gender subclinical hypothyroidism patient.

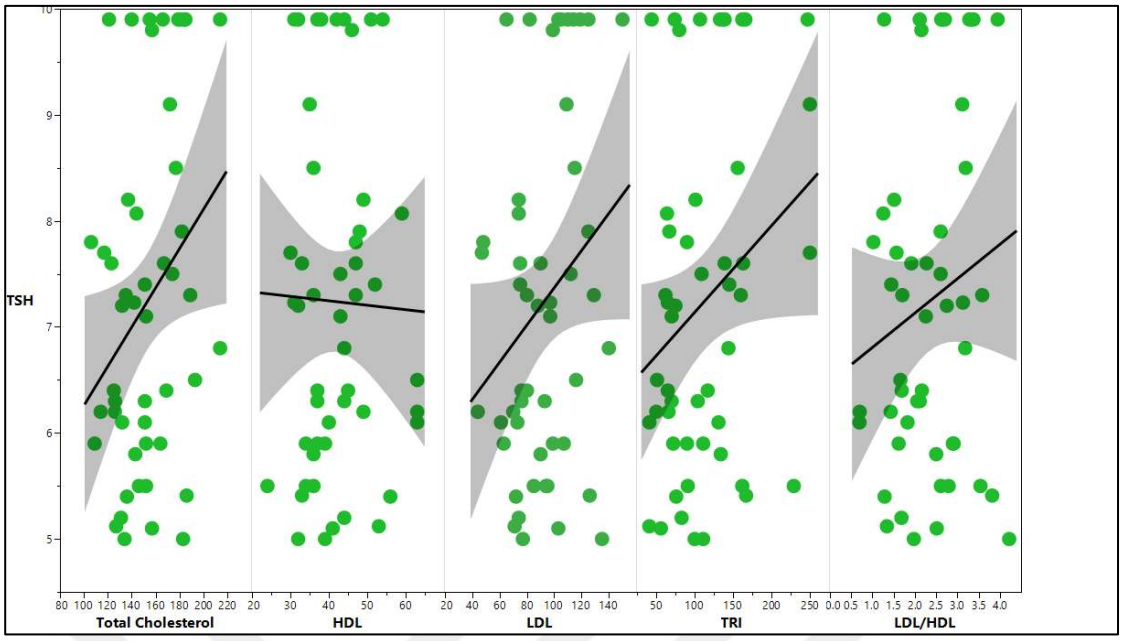

Figure 3.12. Correlation of TSH with lipid profile parameters with adjustment for gender in patients with subclinical hypothyroidism.

3.4. Correlation of APO A and APO B with LDL and HDL:

Table.3.6. represents the correlation coefficient among either of APOA with HDL and LDL, and APOB with HDL and LDL.

The correlation result analysis exposed a positive significant (P<0.001) correlation among APO A with HDL, although LDL has a negative correlation but was not statistically significant. Also Analysis of the correlation, outcome showed a strong positive correlation between apolipoprotein B and LDL and a negative significant (p>0.05) correlation among APO B and HDL as depicted in Figure 3.13.

Table 3.7. Correlation of APO A and APO B with LDL and HDL concentrations in persons with subclinical hypothyroidism

Control Variables		HDL	LDL
APOA	Correlation Significance (2-tailed)	0.740 <0.001	-0.169 0.274
APOB	Correlation Significance (2-tailed)	-0.401 0.007	0.856 <0.001
The adjustment was made for age, gender, smoking, SBP, DBP, and BMI.			

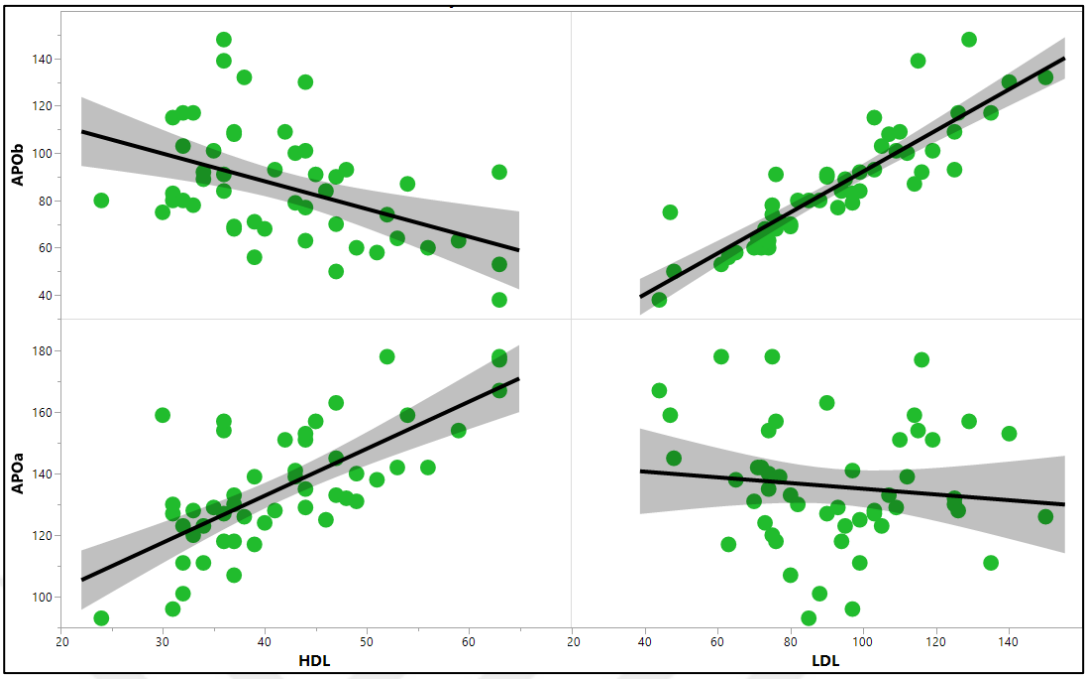

Figure 3.13. Scatter plots of APO A and APO B with LDL and HDL concentrations in persons with subclinical hypothyroidism.

4. DİSCUSSİON

One of the prevalent endocrine disorders in the Middle East is hypothyroidism, affecting one in ten adults with a higher prevalence in females. Hypothyroidism is a classified condition that entails the full-blown failure of the thyroid however an early, mild type of hypothyroidism, a disorder in which the body does not produce enough thyroid hormones, is subclinical hypothyroidism. Several studies have reported a 9% to 11% prevalence of SCH [74, 75]. The prevalence of SCH was identified by two recent studies as 9.5 and 9.4 percent. The prevalence of SCH in both studies increased with age and was primarily present towards women. It is currently difficult to accurately determine the true prevalence of SCH, as the current concept of SCH was not applied consistently in previous researches [76, 77]. In the current study, 78 % of SCH subjects were women (Table 3.1). for this consistent gender difference observed, a logical explanation is not yet available. It is thought that it may be the most common cause of subclinical hypothyroidism and hypothyroidism in women due to the high prevalence of Hashimoto thyroiditis [78].

In the present study, regarding age, the maximum number of age subjects (40%) of SCH was present in the 20-29 year age group and (28%) in the 30-39 year age group, while the lowest (10%) were in the <20 year age group (Table 3.1). These results are in line with the research group that recorded the highest prevalence in the adolescents and adult population age group [79].

The family history of SCH and healthy control subjects is reflected in (Table 3.1) in the current study. A significant number of subjects with SCH founded a family history of SCH (48%). Lipidema alone (44%), and CVD (38%), which may lead to the incidence of metabolic syndrome. A lower number of healthy control subjects reported a family history of these conditions, and as a person chi-square (Table 3.1) there were extremely significant differences ($p<0.012$) between SCH subjects and family history of SCH so Positive family history has shown a tendency to subclinical hypothyroidism than negative family history in the present study, which is following a study group which had a positive family history between SCH and family history [51].

Hypothyroidism is well accepted to be related to weight gain however. There is an absence of clarification in the subclinical state [80]. It was found that subjects in all two groups ranged from normal weight, overweight to obese regardless of their thyroid status when applying Asian cut-off values of BMI <23kg/m^2 as normal weight, about 23kg/m^2 as overweight, and about 25kg/m^2 as obese [81]. The association of elevated TSH with BMI is still controversial, proposing that TSH may be significant in assessing excess body weight. On the other hand, research further supports the theory that obesity itself contributes to alteration in the parameters of circulating thyroid function. Researchers found that an increased propagation of higher TSH, predictive of

SCH in morbidly obese subjects, may not be a symptom of thyroid hypofunction [82]. A positive correlation of weight and TSH was observed in a community-based longitudinal study; however the study focused that TSH levels are not a determinant of potential weight change; correspondingly, BMI is not a factor of changes in TSH levels [83].

Observed waist circumference among SCH patients (males, females) and controls is given in Table 3.1. This shows that the average waist circumference of SCH patients was 106.70 (18.92) and that for controls was 92.62 (12.39) differences between them were statistically significant P<0.001. Comparisons of males versus female SCH reveal that the average waist circumference of females (108.03) was more than that of the males (102.00).

Both groups were compared in terms of blood pressure (Table 3.1). As a result, hypertension was identified in 10 (20%) of patients with SCH, and 5 (10%) of the control group. However, we distribute our participants based on blood pressure (systolic and diastolic) and its outcome in terms of aspects.

This study showed no significant difference in systolic blood pressure, 114.20 (7.85) in SCH compared to 115.80 (8.35) in stable control groups. Both the subclinical and control groups of hypothyroid subjects were associated with elevated systolic blood pressure (Table 1). These findings include the study conducted by Shende et al [84]. However, The present study shows a statistically significant difference in the diastolic blood pressure of SCH compared to control groups. The SCH subjects have higher diastolic blood pressure relative to the healthy control groups (Table 3.1) .the result was present is 74.00 (10.69) in SCH, in contrast in healthy group was present 69.80 (7.95) There was an increase in diastolic blood pressure in this study is consistent with the study conducted by this research [84]. This is in line with the research carried out by this study [85].

In study participants, altered thyroid status was observed, which is common for a subclinical status. In all subjects with subclinical hypothyroidism, TSH levels were greater than 5 mIU/L, but in the control group were in the normal range. Also, the level of T3 and T4 between the SCH and the healthy control group was statistically similar, which is an acceptable finding because T4 to T3 peripheral deiodination in subclinical hypothyroidism was not affected. SCH also demonstrated a connection with inflammatory markers as shown in (Table 3.2). AntiTPO and TSH are independent inflammation mediators and promote the production of different inflammatory cytokines. In this study, significant elevated anti-TPO antibody titer was observed in SCH subjects as compared to control groups p<0.001 as shown in (Table3.2) that is Similar findings were obtained in earlier studies [86].

In general, the metabolic effects of subclinical hypothyroidism be similar to those of the hypothyroidism state to a lesser degree and are thought to affect lipids by the same mechanism. Previous studies have shown contradictory findings on the impact of SCH on serum lipids [87].

The variation in previous SCH studies was due to the following differences: the design of the research, age, sex, TSH cut-off description, and degree and duration. But in this study, we reduce the effect of this factor by taken match participants according to these factors.

In the current research, newly diagnosed SCH participants had significantly elevated TC and LDL-C levels relative to control groups, but within the normal reference range. Although not statistically significant a marginal increase in TGs and decline in HDL has been reported (112.34 (54.39)) and (41.94 (9.32)) with $p>0.05$ respectively. In earlier studies, lipid abnormalities were observed in adult subjects with SCH, particularly until the TSH is greater, the effect of TSH is more lipid abnormally [88]. A recent study indicates that SCH is commonly associated with higher serum TC and LDL concentrations than with TG and HDL abnormalities [3].

We further studied the relationships between thyroid function and risk factors for CVD in patients with SCH in this research. Complying with an earlier study [89].

A significant positive association with TSH was shown by cardiometabolic variables. This suggests that elevated TSH leads to metabolic abnormalities in SCH by the degree of hypofunction demonstrated [85].

Although there is an ongoing controversy about the degree of lipid change caused by SCH In this study, the correlation between TSH and lipid profile parameters in SCH patients showed that thyroid-stimulating hormone had a positive relationship with TC, LDL, and TG and APOB ($p <0.05$). There was no relation in SCH between thyroid-stimulating hormone (TSH) and APOA and HDL as illustrated in table 3.5 and figure 3.11 and figure 3.12 respectively. The findings of this study showed consistency with the earlier study results [90, 91].

The studies with Apo A and Apo B stand out among the new lipid fractions that were studied. The coefficient of correlation between APOA with HDL and LDL and APOB with HDL and LDL is represented in table 3.6 figure 3.13.

A positive significant correlation ($P<0.01$) between APO A and HDL was reported in the correlation result study, while LDL had a negative correlation that was not statistically significant. Regarding the correlation study, the findings showed a strong positive correlation between apolipoprotein B and LDL and a negative significant correlation ($p>0.05$) between APO b and HDL as shown.

The founding supported our hypothesis that, as seen in hypothyroidism, subclinical hypothyroidism patients would have alteration in lipid profiles, also, to identify the effect of an SCH on CVD; we analyzed and compared Lp (a) in the case with the control group. However, correlated the LP (a) with other parameters in the case groups. The clinical significance of Lp (a) was greatly noticeable because Earlier studies have also shown evidence that Lp (a) plays a significant role in atherosclerosis and is one of the top cardiovascular disease risk elements [92]. So Lipoprotein (a) was observed as a significant and independent risk factor in the current

research. Lp (a) is a particle similar to LDL that has been connected to a glycoprotein called apolipoprotein (a). There is essential evidence to indicate that increase Lp(a) levels contribute significantly to CVD production. However, Lp(a)'s physiological function and metabolism are not fully clear. How serum Lp (a) levels are affected by thyroid hormone of thyroid remains unclear in cases of subclinical hypothyroidism and conflicting reports on the impact of thyroid hormone replacement on Lp (a) levels have been recorded [93]. While a changed lipid profile is often found in SCH subjects, the association among Lp(a) and thyroid status is opposite in published evidence [94]. Trapping LDL particles after Lp (a) is bound by its Apo B portion to arterial wall proteoglycans, upon absorption by macrophages of Lp(a) particles after interaction of these cells with low oxidized LDL [26]. These could play a part in consisting of the increase in the levels of Lp (a). The present result showed elevated levels of Lp (a) In SCH subjects 37.23 (41.08) when compared with control groups 21.19 (13.45) and the difference was significant ($p<0.01$) as depicted in figure 3.3 Our research agrees with previous studies [95, 96]. Distribution of participants by Lp(a), categories, and diagnosis of SCH has been given in table 3.2. According to the distribution of participants with elevated Lp(a) levels, 68.0% (34) presented a diagnosis of subclinical hypothyroidism, in contrast to the proportion of individuals with normal Lp(a) levels: 32.0% (16) as shown in table 3.2 and figure 3.2 Our research agrees with previous studies [95, 96].

Pearson's correlation analysis of Lp(a) thyroid status and a risk parameter of CVD among case groups revealed the following aspects. As illustrated in table 3.4 and figure 3.4. ApoB was found to have a highly positive significant correlation ($p<0.01$) with elevated Lp(a) levels, whereas HDL had a non-significant negative correlation and apoA-1,hs-CRP did not correlate with varying Lp(a) levels but TC, LDL have positive statistically significant with LP (a), the present study showed similarity with the findings of the previous study, (Kung, A., Pang, R., and Janus, E). Therefore, our result, in combination with previously study evidence indicates that an impairment of the catabolism of apo B-containing particles, such as Lp(a), predominates in a subclinical hypothyroid state; and that increased secretion of apo B and Lp(a), possibly in combination with lowered catabolism, could be postulated. The lower lipolytic activity of lipase enzymes may have caused this effect [97].

The possible association in the general population among Lp(a) and thyroid function status may be considered as an essential feature of cardiovascular risk prediction and prevention. In subclinical hypothyroid subjects, we and some other studies have pointed out the correlation among TSH and Lp(a) levels, indicating the impact of thyroid hormones on the metabolism of Lp(a) [98].

When Lp a compared to thyroid status is a non-significant positive correlation with TSH however, Lp a have a significant negative correlation with FT4 p-value < 0.05 as depicted in table

3.5 and figure 3.5. Thus, they concluded that TSH and Lp(a) had a weak relationship. On the other hand, we concluded that Lp(a) increased in subjects with SCH and show a weak correlation in our results with TSH, the weak association observed by some researchers is usually correlated with the presence of milder types of thyroid dysfunction as described by normal basal TSH.

Subclinical hypothyroidism is correlated not only with more levels of LDL and less levels of HDL but also with elevated levels of lipoprotein (a). Therefore further increases the risk of developing cardiovascular disease.

5. CONCLUSİONS

Our studies show that subclinical hypothyroidism is a transitional state between euthyroidism and hypothyroidism, with smaller-scale Cardiometabolic evidence resembling hypothyroidism. When TSH is over 5 IU/mL and with elevated anti-TPO, these modifications are noticeable in this study we funded lipid profile is more obvious in the subclinical state when TSH is elevated. Serum lipids and lipoprotein (a) had a linear association across the entire range of TSH. Anti-TPO positivity in SCH patients is coloration with alterations in thyroid function, lipid profile, and lipoprotein (a) thus contributing to an increased cardiovascular risk.

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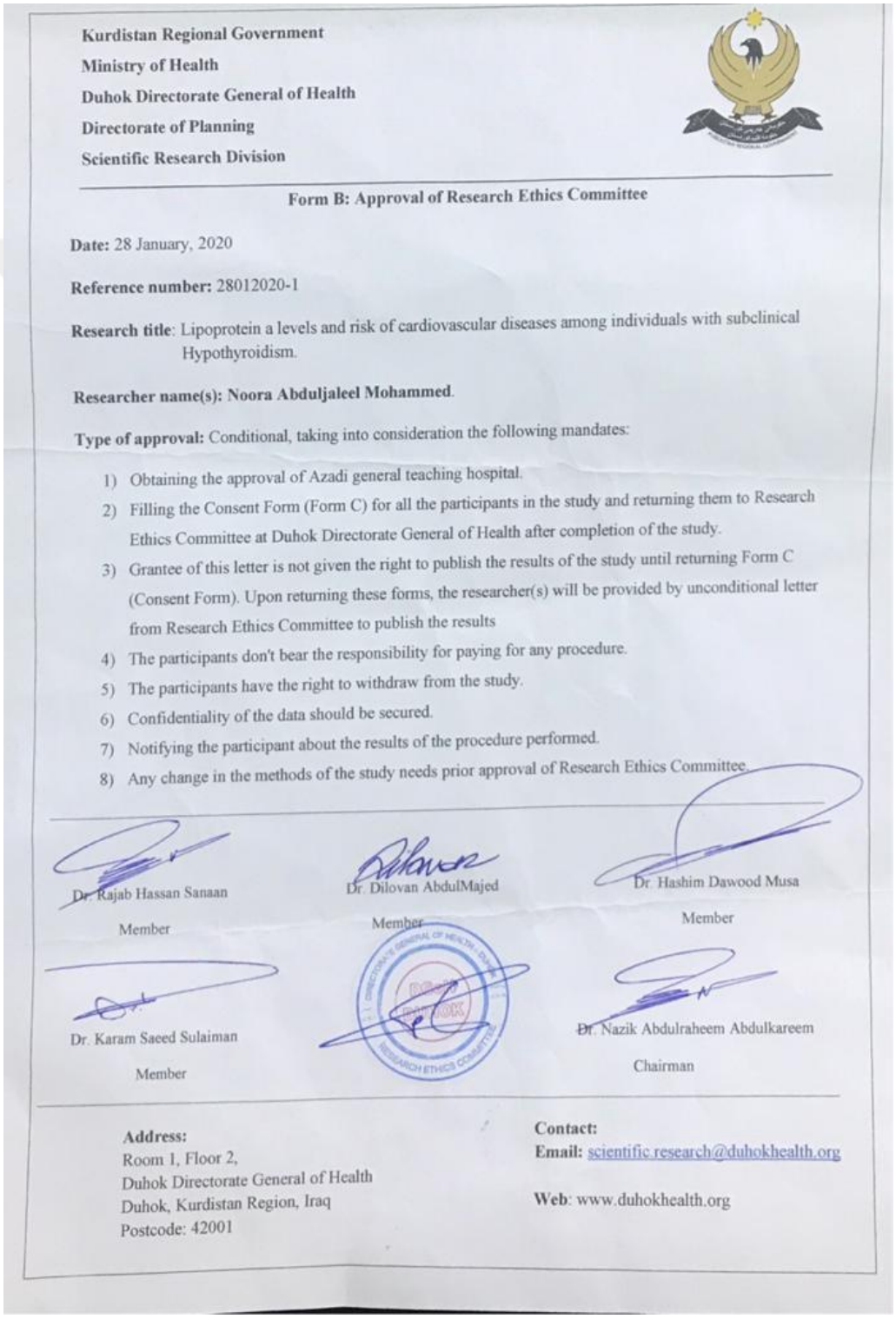
APENDİCES

APPENDIX 1:APPROVAL OF RESEARCH ETHICS COMMITTEE



Kurdistan Regional Government
Ministry of Health
Duhok Directorate General of Health
Directorate of Planning
Scientific Research Division

Form B: Approval of Research Ethics Committee

Date: 28 January, 2020

Reference number: 28012020-1

Research title: Lipoprotein a levels and risk of cardiovascular diseases among individuals with subclinical Hypothyroidism.

Researcher name(s): Noora Abduljaleel Mohammed.

Type of approval: Conditional, taking into consideration the following mandates:

1) Obtaining the approval of Azadi general teaching hospital.
2) Filling the Consent Form (Form C) for all the participants in the study and returning them to Research Ethics Committee at Duhok Directorate General of Health after completion of the study.
3) Grantee of this letter is not given the right to publish the results of the study until returning Form C (Consent Form). Upon returning these forms, the researcher(s) will be provided by unconditional letter from Research Ethics Committee to publish the results
4) The participants don't bear the responsibility for paying for any procedure.
5) The participants have the right to withdraw from the study.
6) Confidentiality of the data should be secured.
7) Notifying the participant about the results of the procedure performed.
8) Any change in the methods of the study needs prior approval of Research Ethics Committee.

Dr. Rajab Hassan Sanaan
Member

Dr. Dilovan AbdulMajed
Member

Dr. Hashim Dawood Musa
Member

Dr. Karam Saeed Sulaiman
Member

Dr. Nazik Abdulraheem Abdulkareem
Chairman

Address:
Room 1, Floor 2,
Duhok Directorate General of Health
Duhok, Kurdistan Region, Iraq
Postcode: 42001

Contact:
Email: scientific.research@duhokhealth.org

Web: www.duhokhealth.org

APPENDIX 2: RESEARCH QUESTIONNAIRE

Research Questionnaire

1. Names of Patient : ……
2. Patient's number ……
3. Age of Patient : …….
4. Gender: ……..
5. Address: ……..
6. Tel.: …….

Diseases History

- Family history thyroid disorders: Yes No
  -Hypothyroidism: Yes No
  -Hyperthyroidism: Yes No
  -Duration : ……
- Family history cardiovascular diseases: Yes No
  (Mother) (Father) (Brother) (Sister) (Other)(Alive) (Died)
- Age of the CVD …….
- Family history dyslipidemia: Yes No
  (Cholesterol) (Triglyceride)

Physical examinations

1.Weight of Patient: …….. (Kg)
2.Height of Patient: …….. (cm)
3. BMI of Patient……... (Kg/m2)
4. Blood pressure: …systolic / diastolic……
5.Width circumferences (WC)…… ( )

Exclude criteria

- Smoking : (smoker) (nonsmoker)
- Pregnancy
- Liver diseases
- Renal diseases
- Other

Chemical investigations

1. S. TSH ……
2. S. FT4 ……
3. S.FT3 ……
4. S. Cholesterol ……
5. S. Triglyceride ……
6. S. HDL ……
7. S. LDL ……...
8. S. APO (A) ……
9. S. APO (B) ……
10. S.Lp (a) ……
11. S. Anti TPO ……..
12. S. CRP HS ……...

APPENDIX 3: COBAS 6000 ANALAYAZER SERIES

CURRICULUM VITAE

[redacted]

PERSONAL INFORMATION

[redacted]

[redacted]

[redacted]

EDUCATION

Bachelor **:** University of Duhok /College of Science /Chemistry Department

RESEARCH EXPERIENCES

- ✓ Laboratory Instruments you use, such as test systems etc. you know
- ✓ Computer Programming languages available (C-C++, MATLAP, LABVIEW, vb.)
- ✓ Computer programs you can use (CAT/CAM, PROTEUS, CALCULUS, vb.)

WORK EXPERIENCE

2011 - 2012 : Teaching From September 2011 to June 2012, at Safeen preparatory school for girls, chemistry unit for grade 10 and 11.

2012-2019 :I worked in the clinical chemistry department in the Dohuk Emergency Teaching Hospital

2013-2019: Work in Clinical chemistry and immunology department at Vin Medical Laboratories in VIN Hospital and Medical Complex.

2019- 2021: I studied master degree.