TURKISH REPUBLIC OF NORTHERN CYPRUS

NEAR EAST UNIVERSITY

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RELATIONSHIP BETWEEN THYROID HORMONE LEVELS AND LIVER FUNCTION TESTS IN LIBYAN POPULATION

MOHAMED ALHOSEN ALI DEGM

MASTER OF SCIENCE THESIS

MEDICAL BIOCHEMISTRY PROGRAM

NICOSIA

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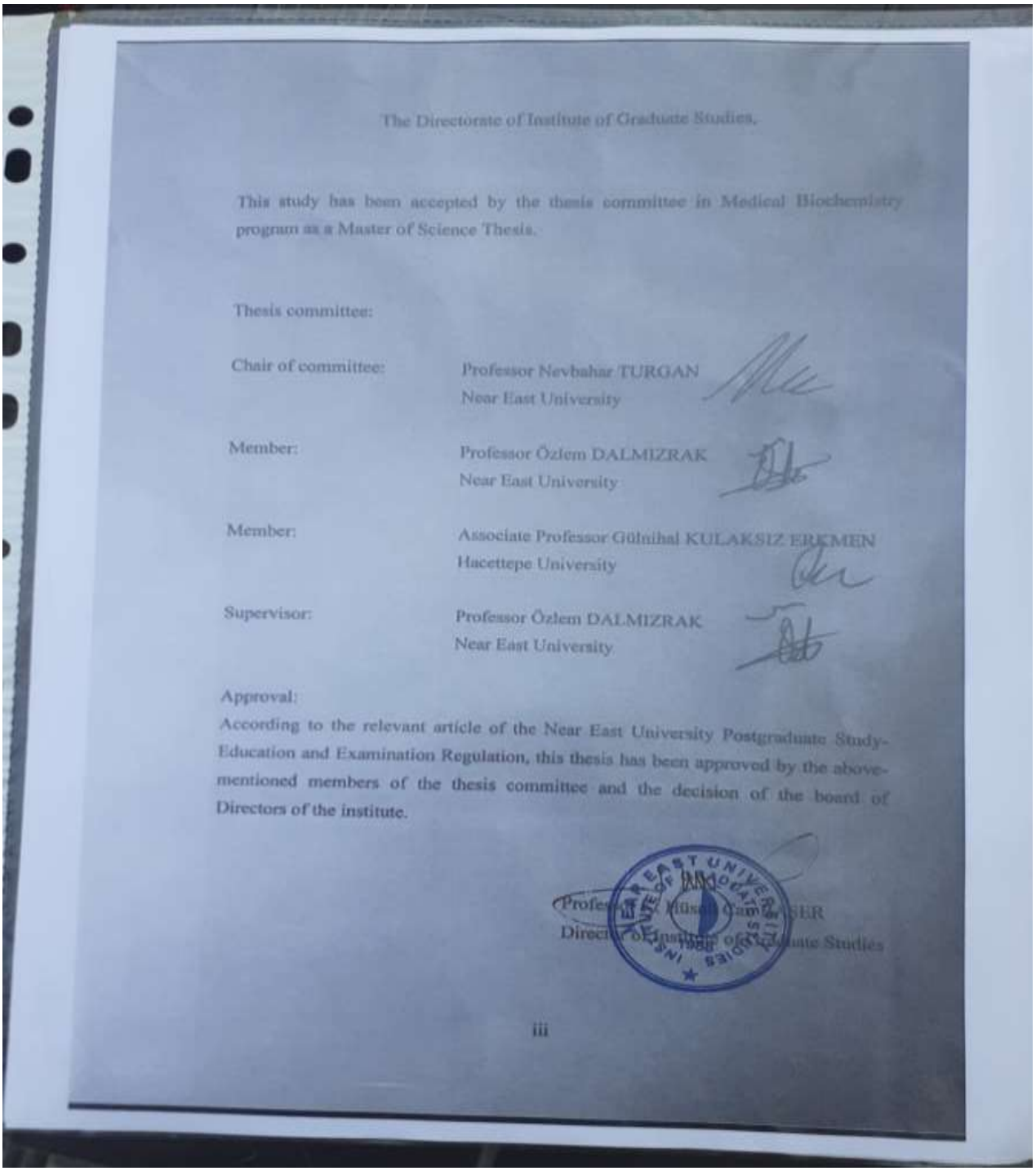

The Directorate of Institute of Graduate Studies,

This study has been accepted by the thesis committee in Medical Biochemistry program as a Master of Science Thesis.

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Approval:

According to the relevant article of the Near East University Postgraduate Study-Education and Examination Regulation, this thesis has been approved by the above-mentioned members of the thesis committee and the decision of the board of Directors of the institute.

Prof[illegible] Hüsnü Can BA[illegible]SER
Director of Institute of Graduate Studies

iii

DECLARATION

Hereby I declare that this thesis titled “Relationship between thyroid hormone levels and liver function tests in Libyan population” study is my own study. I had no unethical behavior in all stages from planning of the thesis until writing thereof, I obtained all the information in this thesis in academic and ethical rules. I provided reference to all of the information and comments which could not be obtained by this thesis study and took these references into the reference list and had no behavior of breeching patent rights and copyright infringement during the study and writing of this thesis.

MOHAMED DEGM

Date: 17/02/2021

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TABLE OF CONTENTS

Page No

ABBREVIATIONS

AITD	Autoimmune thyroid disease
ALP	Alkaline phosphatase
ALT	Alanine aminotransferase
AST	Aspartate aminotransferase
ATP	Adenosine triphosphate
B-BIL	Direct Bilirubin
DUOX	Dual oxidase
FT3	Free T3
FT4	Free T4
GGT	Gamma glutamyltransferase
GPX	Glutathione peroxidase
LDL	The low-density lipoprotein
NIS	Sodium iodide symporter
SREBPs	Sterol regulatory element binding proteins
T-BIL	Total Bilirubin
Tg	Thyroglobulin
TPO	Thyroid peroxidase
TRE	Trans regulatory element
TRH	Thyrotropin-releasing hormone
TSB	Total serum bilirubin
TSH	Thyroid stimulating hormone
T3	Triiodothyronine
T4	Thyroxine

LIST OF FIGURES

LIST OF TABLES

Thesis Title: Relationship Between Thyroid Hormone Levels and Liver Function Tests in Libyan Population

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Department: Department of Medical Biochemistry

ABSTRACT

Aim: The liver plays a pivotal role in thyroid hormone metabolism. The human body is very complex and must operate within specified range in order to work properly, a functional change in one biological parameter may alter the functions of one or more other parameters that are interrelated. Therefore, this study investigated the association between thyroid hormone levels, liver function tests, vitamin D, B6, B9 and B12 levels in Libyan population.

Materials and Methods: Samples were collected from Endocrinology Department of National Centre for Diabetes and Endocrinology, Libya. The study groups were divided into three groups namely control group, hyperthyroidism, hypothyroidism with each group having 150 participants. COBAS INTEGRA 400 plus analyzer was used for biochemical analysis while Elecsys COBAS E411 was used for assessment of the levels of thyroid hormones and vitamins. Results were evaluated by statistical analysis.

Results: Both patient groups had more females. Groups showed no difference with regards to age and gender. Albumin, vitamin D, vitamin B6, vitamin B12, calcium and magnesium levels showed no statistical difference among groups. However, ALT, AST, ALP, GGT, D-BIL, T-BIL, Vitamin B9 and Ferritin levels were found to be statistically different ($p<0,05$) compared to control subjects.

Conclusion: There is a correlation between thyroid hormones and liver function tests. This correlation could be used for patients that have thyroid dysfunction to prevent liver damage in advance.

Key Words: Thyroid hormones, Hyperthyroidism, Hypothyroidism, Liver Function Tests, Vitamins, Minerals

Tez Başlığı: Libya Popülasyonunda Tiroid Hormon Düzeyleri ile Karaciğer Fonksiyon Testleri Arasındaki İlişki

Öğrencinin Adı/Soyadı: Mohamed Alhosen Ali Degm

Danışman: Prof. Dr. Özlem Dalmızrak

Bölüm: Tıbbi Biyokimya Anabilim Dalı

ÖZET

Amaç: Karaciğer, tiroid hormonu metabolizmasında çok önemli rol oynamaktadır. İnsan vücudu çok karmaşık olduğundan ve düzgün çalışması için belirli aralıkta çalışması gerektiğinden, bir biyolojik parametrede meydana gelen bir işlevsel değişiklik, birbiriyle ilişkili bir veya daha fazla başka parametrenin işlevlerini değiştirebilir. Bu nedenle, bu çalışma Libya'da tiroid hormon seviyeleri, karaciğer fonksiyon testleri, D vitamini, B6, B9 ve B12 düzeyleri arasındaki ilişkiyi araştırmak amaçlanmıştır.

Gereç ve Yöntem: Örnekler Libya, Ulusal Diyabet ve Endokrinoloji Merkezi'ndeki Endokrinoloji Bölümü'ne başvuran hastalardan alınmıştır. Çalışma grupları her bir grupta 150 katılımcı olmak üzere kontrol grubu, hipertiroidizm ve hipotiroidizm olarak belirlenmiştir. Biyokimyasal analizler için COBAS INTEGRA 400 plus analizörü kullanılırken, Elecsys COBAS E411 ile tiroid hormonları ve vitamin seviyeleri elde edilmiştir. Sonuçlar istatistiksel olarak değerlendirilmiştir.

Bulgular: Hipotiroidizm ve hipertiroidizm daha fazla kadında gözlenmiştir. Gruplar arasında yaş ve cinsiyet bakımından farklılık bulunmamaktadır. Albümin, D vitamini, B6 vitamini, B12 vitamini, kalsiyum ve magnezyum gruplar arasında istatistiksel olarak fark göstermemektedir. Bununla birlikte, ALT, AST, ALP, GGT, D-BIL, T-BIL, Vitamin B9 ve Ferritin'de farklılıklar istatistiksel olarak anlamlı bulunmuştur.

Sonuçlar: Tiroid hormonları ile karaciğer fonksiyon testleri arasında korelasyon saptanmıştır. Bu korelasyon tiroid hormone bozukluğu olan hastalar için erken önlem alınarak karaciğer hasarının önlenmesinde kullanılabilecek bir belirteç olabilir.

Anahtar Kelimeler: Tiroid hormonları, Hipertiroidizm, Hipotiroidizm, Karaciğer Fonksiyon Testleri, Vitaminler, Mineraller

1. INTRODUCTION

The human body is a complex interconnection of various structures that work individually and collectively to maintain normal metabolic process. For example, the heart and the lungs work individually to pump blood (oxygenated and deoxygenated) and inhalation-exhalation of oxygen and carbon dioxide, respectively. However, they collectively work to make sure oxygenated blood is carried to the various parts of the body, and excreted carbon dioxide is taken out of the body. The thyroid gland and the liver have been shown to have some kind of relationship between them. Also, some vitamins are thought to have a relationship with both the thyroid gland and the liver. Previous studies have shown the role of metal ions like calcium and magnesium in controlling the thyroid hormone production as well as metabolism and hence an association may exist between thyroid hormones and several other minerals (Susanna et al., 2016).

Since the human body is complex and must operate within a specified range in order to work properly, a functional change in one biological parameter may alter the functions of one or more other parameters that are interrelated. In the thyroid gland, two important hormones affect the natural process of growth, development, and proper functions of organs. These hormones are the thyroxine and tri-iodothyronine hormones. They are responsible for the regulation of the rate of cellular basal metabolism. Thyroid hormones have a functional role in stabilizing metabolic basal rates of hepatocytes. Hepatocytes are also involved in the modulation of vital liver functions. Therefore, there is a clear relationship between the thyroid gland and the liver through hepatocytes. Furthermore, the liver functions are responsible for mobilizing all thyroid hormones for response as well as the regulation of the systemic endocrine effects from the thyroid hormones, further accentuating the interrelationship between the two organs.

It is clear that the thyroid gland and the liver have very important functions that benefit them mutually. An alteration in the functions of the thyroid gland (thyroid dysfunction) will result in an alteration in the functions of the liver (liver diseases) and vice versa. An abnormal functioning liver is capable of modulating metabolic activities

of thyroid hormones as well as a diverse range of disorders to both the liver and the thyroid gland (Malik and Hodgson, 2002).

The prevalence of thyroid dysfunction is high among Libyan population. Therefore, the present study aims to investigate the association between thyroid hormone levels, liver function tests, vitamin D, B_6, B_9 and B_{12} levels among the Libyan population.

2. GENERAL INFORMATION

Thyroid hormones are beneficial in regulating several metabolic activities in adults, and they exert feedback on multiple organs that help to maintain body homeostasis (Shahid et al., 2020). Imbalance in the function of thyroid hormones to maintain cellular homeostasis leads to different metabolic disorders that include cardiovascular diseases, chronic liver disease and diabetes (Chi et al., 2013).

2.1. The Thyroid Gland

In anatomical description, the thyroid gland is shaped like a butterfly because of its two lobes which makes it a bi-lobed gland. It is located within the lower part of the neck, specifically laying in the anterior part of the trachea. Regarding the weight, it is estimated that the average weight of a matured thyroid is just about 20 grams. In the trachea, their specific level starts from C5 to T1 level. The central isthmus is the main connection point between the two lobes of the thyroid gland. There is a adequate supply of blood from the heart to the thyroid. Blood is supplied mainly from the superior and inferior arteries to the thyroid gland. In a microscopic level, the thyroid consists of follicles shaped spherically. Each of the follicles consists of only one layer of columnar cells which is encompassed colloids that have adequate protein content in the lumen. In the colloids, the content mainly constitutes of glycoprotein which is usually referred to as thyroglobulin. It is in the colloids that synthesis of thyroid hormones occurs inside the protein of the thyroglobulin (Summers and Macnab, 2017).

The thyroid gland is specifically regarded as an endocrine gland that is responsible for secreting hormones that are vital for a diverse range of the processes of body's metabolism. The hormones that the thyroid gland is responsible for secreting include the thyroxine (T4) and triiodothyronine (T3) which are all regulated by the thyroid stimulating hormone (TSH) (Figure 2.1) (Summers and Macnab, 2017). The synthesis as well as the processing of all these vital hormones are controlled by negative feedback mechanism. In the circulation, T4 and T3 are bound reversibly to carrier proteins with a percentage of 99.97 and 99.7, respectively. From these view, it is clear that the majority of these hormones are bound to carrier proteins and only a very negligible amount is available for free in the circulation. The fractions that are

available freely are referred to as free T3 (fT3) and free T4 (fT4) which are the biologically active forms of thyroid hormones (Burtis et al., 2008).

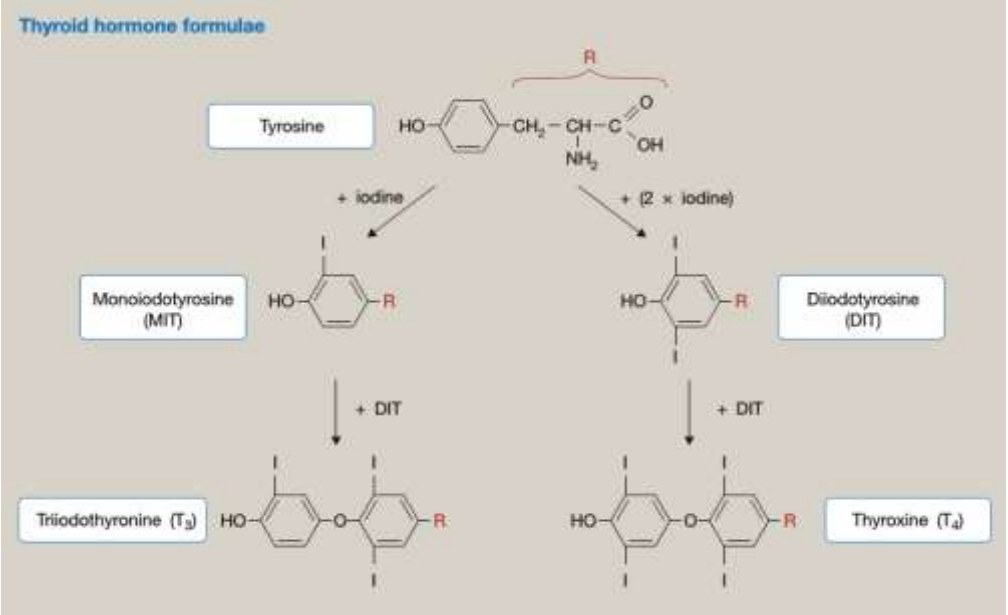

Figure 2.1. Chemical formulae of thyroid hormones (Summers and Macnab, 2017)

2.2. Synthesis of Thyroid Hormones

Hypothalamic thyrotropin-releasing hormone (TRH) is responsible for the secretion of thyroid stimulating hormone (TSH) from anterior pituitary gland. When stimulated by TSH, the thyroid gland secretes thyroid hormones; thyroxine (T4) (80%) and T3 (20%). TSH functions to regulate the level of hormones produced by the thyroid gland such as the T3 and T4 which are present in the bloodstream. In case of a depletion of both T3 and T4, the resultant event is an increase in the production of TSH hormone. On the other hand, when there is high level of both T3 and T4, there is a decrease in TSH hormone production. This shows an opposite relationship between T3/T4 hormones and TSH hormone which are very good examples of a negative feedback mechanism (Figure 2.2) (Estrada et al., 2014).

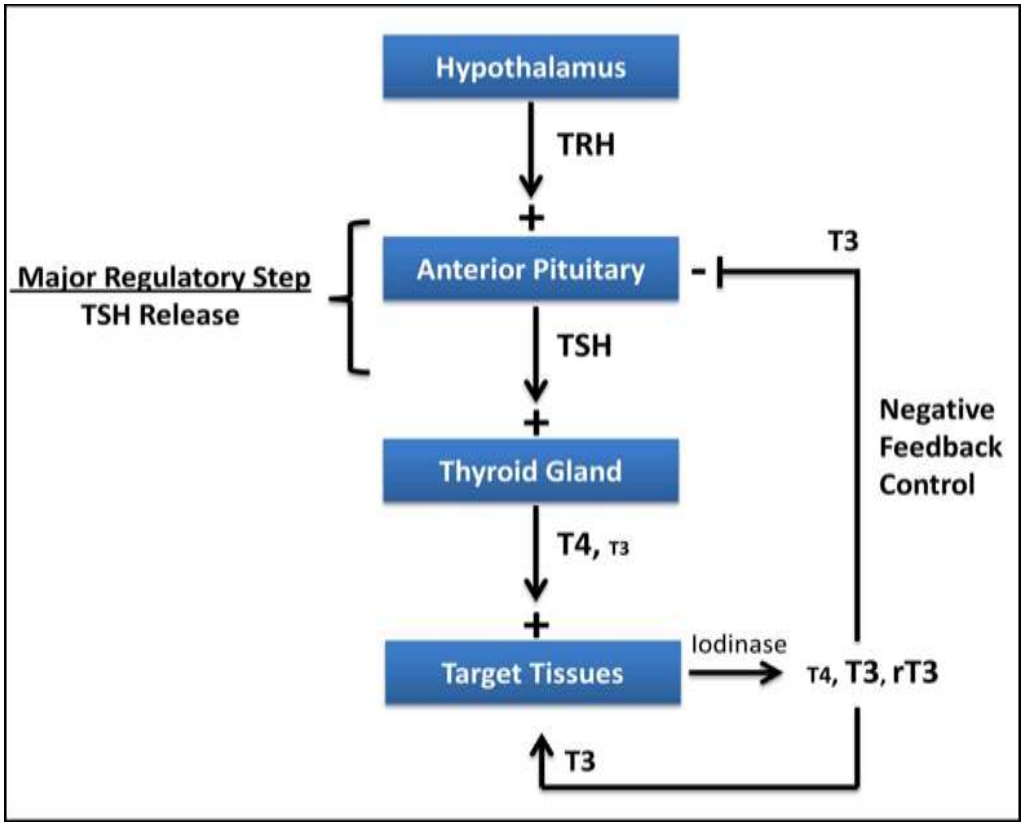

Figure 2.2. Synthesis and regulation of thyroid hormone formation (Negative feedback control) (http://www.pathwaymedicine.org/thyroid-hormone-regulation)

2.3. Functions of Thyroid Hormones

As mentioned earlier, the two hormones triiodothyronine (T3) and thyroxine (T4) produced by the thyroid gland have significant effects on several metabolic processes such as maintaining homeostasis and regulation of the basal metabolic rate in almost all tissues and cells. Furthermore, in humans, appetite regulation, absorption of nutrients (including minerals and vitamins) through the walls of the intestine (gastrointestinal tract) and their transport are influenced by the level and activity of triiodothyronine (T3) and thyroxine (T4). Additionally, T3 and T4 hormones have considerable influence on the stimulation of glycolysis and breakdown of fats. The influence of thyroid hormones also extends to improve the amount of cholesterol excretion through the bile, thereby significantly reducing and maintaining physiological levels of cholesterol in the body (Guyton and Hall, 2011).

The thyroid hormones also affect cardiovascular activities and the maintenance of normal body temperature. This is achieved through increasing the strength and rate of

each heart beat responsible for pumping and distribution of blood and oxygen. Body temperature, respiration rate, oxygen consumption, as well as the activities of mitochondria are also under the control of these hormones (Harvard Health Publishing, 2019). Lastly, thyroid hormones are also involved in the process of growth and development in children, adolescents and young adults. These hormones have vital roles in the process of brain maturation especially during the development of the fetus and in the newborn (Guyton and Hall, 2011). In conclusion, thyroid hormones are very important in human growth and development. Figure 2.3 depicts the major effects of these hormones including their role in growth and development

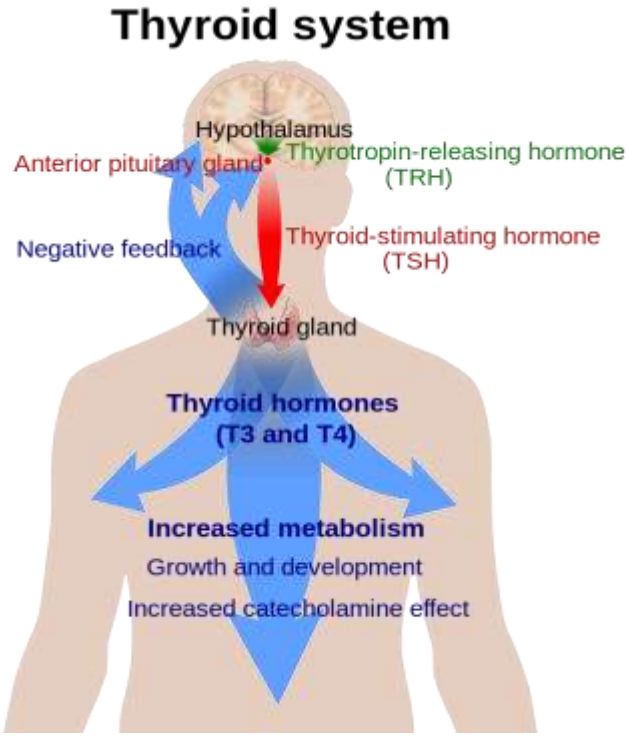

Figure 2.3. Functions of T3, T4, TRH, and TSH thyroid hormones (https://www.thyroidchange.org/about-thyroid-disease.html)

By now, it is even more clear that the thyroid hormones have direct or indirect effects on the function of virtually every organ system which makes the thyroid gland a very critical and important body part in the normal functioning of the human body. Stimulation of adenosine triphosphate (ATP) synthesis through catabolic pathways as well as maintenance of ionic gradients are the other functions of thyroid hormones.

Thyroid hormones also play important roles in the modulation of calorigenesis as a result of the enhancement of mitochondrial metabolic processes (Summers and Macnab, 2017).

2.4. Action Mechanism of Thyroid Hormones

Thyroid gland secretes two iodine containing tyrosine-derivative hormones; L-thyroxine (T4) and 3,5,3'-L-tri-iodothyronine (T3). Through the plasma membrane, free T3 and T4 find their way into the cells to bind to nuclear T3 receptor which is a member of the nuclear receptor superfamily. Retinoic acid, retinoid X, vitamin D and peroxisome proliferator receptors are the other members of this receptor superfamily. All these receptors share the common characteristic of having six domains in each of them. Among these six domains, two are ligand-binding regions with a central region that binds DNA constitutively (Malik and Hodgson, 2002).

Thyroid hormone receptors inside the cells are directly or indirectly responsible for providing ligand-activated transcription factors that are vital for the regulation of direct expression of target genes through DNA response elements such as thyroid response elements and trans-regulatory elements (TREs). Constitutively, the receptors have a very significant property of binding to thyroid response elements. The consensus sequence recognized by nuclear receptors often contains a hexamer AGGTCA known as the half site; two half-site sequences with a specific orientation are required for efficient binding and function (Malik and Hodgson, 2002).

2.5. The Liver and its Metabolism

The liver is a unique organ in the human body that has a wide variety of vital functions including detoxification. It is responsible for regulating the levels of a diverse range of chemicals in blood. From this regulation, bile is produced and excreted into the duodenum. In fact, it is compulsory for all the blood coming from the stomach and the intestines to pass through the liver before reaching their final destination and the process is repeated again. The liver as an organ only weighs on average about 3 pounds which is approximately 1.36 kg. It is reddish brown color with a rubbery feel to the touch and is also shaped as cone as shown in Figure 2.4 (Johns Hopkins Medicine, 2021).

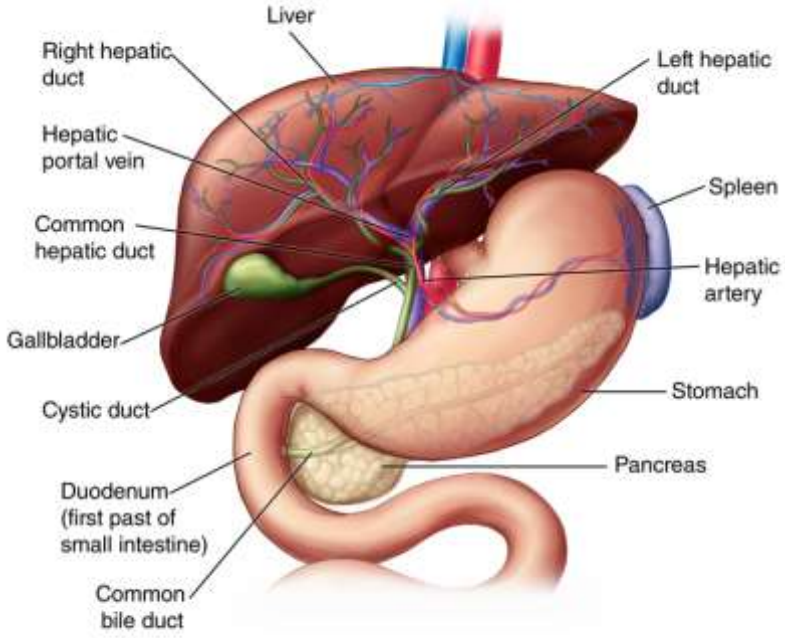

Figure 2.4. The anatomy of the liver (Johns Hopkins Medicine, 2021)

The liver is an important site for the metabolism of cholesterol and triglycerides. This is one site that the liver and the thyroid gland may have a relation because the thyroid gland has a role in the homeostasis of hepatic lipids (Brent, 2012). Furthermore, hormones produced in the thyroid gland also have the function of increasing the rate of low density lipoprotein (LDL) receptor expression in hepatocytes as well as increasing lipid-lowering enzyme activities in liver which in turn reduces the levels of LDL (Sinha et al., 2018). The process of expressing apolipoprotein A1 protein is also influenced by thyroid hormones. Apolipoprotein A1 is a significant component of high-density lipoproteins in the body (Malik and Hodgson, 2002).

The liver's function is not only to detoxify and secrete bile, but also it functions for a wide range of body's metabolism including production of plasma proteins like albumin. The liver is responsible for regulating the composition of blood plasma. Other functions of the liver include controlling the flow of nutrients and energy in the tissues (Moriles and Azer, 2020). The liver is also actively involved in storing vitamins and minerals including iron, catalyzing digestion process and removing toxins from the body (Blocka, 2018).

The relationship between the liver and the thyroid gland cannot be over emphasized because the liver is the primary organ that aids the metabolic processes of the hormones produced in the thyroid gland. Moreover, the relation between the liver and thyroid gland is the reason why a dysfunction in one of the organs, result in dysfunction in the other (Evlice and Aksoz, 2017). Because of the relationship between the two organs, there are a diverse range of pathological illnesses that can simultaneously alter the function of both the liver and the thyroid gland. One of such examples is autoimmune diseases whose occurrence may happen time to time in multisystem autoimmune disorder setting (Malik and Hodgson, 2002). A diseased or dysfunctional liver may significantly affect the metabolic processes of thyroid hormones. On the other hand, a diseased or dysfunctional thyroid gland may significantly affect the metabolic processes of the liver (Khanam, 2017).

A condition in which an individual constantly craves for food intake, weight loss and dissipating high levels of energy is referred to as hypermetabolism. Hypermetabolism occurs as a result of an increase in the levels of thyroid hormones in the blood (Kim, 2008). Importantly, all of these conditions are the results of a direct relationship between hormones produced in the thyroid gland and target organs such as the heart, liver, adipose tissue as well as muscles of the skeletal system (Silva 2006; Mullur et al., 2014).

2.6. Liver Function Tests

Liver has long been known as the center of metabolism and detoxification of drugs. Dysfunction in the liver results in the presence of certain markers (usually enzymes) in the blood serum that can be utilized in the diagnosis of different diseases.

2.6.1. Alkaline phosphatase

Alkaline phosphatase (ALP) is an enzyme found on most cell membranes and is responsible for hydrolysis of many monophosphate esters. Elevated ALP level is usually associated with many physiological processes such as bone marrow development (Lowe et al., 2020). Recently reported that ALP is positively associated with metabolic syndrome which renders it an additional biomarker in the measurement of metabolic syndrome (Kim et al., 2020). Decrease in ALP level in hypothyroid

patients has been reported by Al-Hindawi et al. for its use as a biomarker for hypothyroidism (Al-Hindawi et al., 2018). Other studies restrict the decrease in ALP level to be associated with hypothyroidism only (Mane and Baghwat, 2011).

2.6.2. Alanine aminotransferase

Alanine aminotransferase (ALT) is the most widely used marker to assess hepatic injury. Alanine aminotransferase is one of the enzymes belonging to the group aminotransferases (Vroon and Israili, 1990). Alanine aminotransferase (ALT) is virtually present in all areas of the body including kidney, myocardium, skeletal muscle, brain, pancreas, spleen and the lung. However, its presence of significant proportion is found in the cytosol of hepatocytes. The functions of ALT include the catalysis of the amino groups from L-alanine to alpha-ketoglutarate which produces L-glutamate and pyruvate. The transfer of amino groups is very significant in tricarboxylic acid cycle in liver (Moriles and Azer, 2020).

Alanine aminotransferase is the most widely used marker to assess liver diseases as its levels were found to be elevated (McGill, 2016). Elevation in ALT has previously been reported to be associated with a disturbance in the mitochondria as a result of hyperthyroidism (Upadhyay et al., 2004) while in hypothyroidism it is found to be decreased (Dullaart et al., 2014). Excess thyroid hormone disrupts the liver, causing the elevation of liver marker enzymes such as ALT which may be found in minute quantity (Silva et al., 2016).

2.6.3. Aspartate aminotransferase

Aspartate aminotransferase (AST) is one of the enzymes belonging to the group aminotransferases. Just like its sister enzyme (ALT), it is responsible for the process of transferring amino groups between aspartate and glutamate. It is mostly concentrated in cytoplasm of hepatocytes but can be found in small quantities in other tissues such as the muscles, heart, and kidney etc. (Vroon and Israili, 1990).

AST can leak from the cytoplasm (intracellular) to the extracellular areas when there is a significant injury to the hepatocytes which results in increased activities of AST in serum (Aulbach and Amuzie, 2017). The activities of both AST and ALT are usually measured in serum (Tavakoli et al., 2011). Both enzymes are mostly

concentrated in the liver compared to any part of the body (Vroon and Israili, 1990). AST was found to be the third predominant liver marker in autoimmune thyroid disease after ALT and GGT (Hsieh et al., 2019).

2.6.4. Gamma-glutamyl transferase

Gamma-glutamyl transferase (GGT) is an enzyme that is widely used as a biomarker to investigate liver damage in alcoholics. It is also popular for checking disorders of the liver. The activity of GGT like other enzymes is usually measured in serum. Elevated GGT is commonly due to high alcohol intake, liver obstruction diseases, as well as the side effects of enzyme-inducing medications. Any of these conditions result in an increase in the production of free radicals which can cause depletion of glutathione. Moreover, the production of free radicals can also be as a result of the GGT itself (especially when iron is present), because GGT reaction produces products that influence the production of free radicals (Mayo Clinic, 2021). Elevated serum GGT activity is associated with increased mortality rate (Whitfield, 2001). GGT is one of the most sensitive independent bio-indicator enzymes used to investigate liver diseases. The activity of GGT is increased in any type of liver disorder making it a very important indicator for diagnosing liver diseases (Mayo Clinic, 2021). GGT can be found primarily in the liver (hepatobiliary system) in high concentrations, however, it can also be found in other places in the body such as kidney, pancreatic cells and other tissues etc (Whitfield, 2001).

2.7. Vitamins

2.7.1. Vitamin D

Vitamin D is a fat-soluble vitamin that is very important throughout the lifespan of the human body (Harvard School of Public Health, 2021). It is mostly synthesized in the skin when the skin is exposed to sun light (especially early morning sunlight) (DeLuca, 2004). Because of this, vitamin D is sometimes called the sun shine vitamin. Synthesis of vitamin D in the skin involves a vigorous process of photolytic reaction of 7-dehydrocholesterol with light to produce a vitamin called previtamin D, then a slow process of isomerizing previtamin D to vitamin D_3 which is the natural existing form of vitamin D synthesized in the skin (Velluz and Amiard, 1949).

One of the major functions of vitamin D is the process of bone formation, however, it is also important for other body functions such as reducing the risk of degenerative diseases and cancer (Harvard School of Public Health, 2021). In the bone formation, vitamin D regulates the concentration of calcium and phosphorous in the blood, as well catalyze the absorption of calcium which not only form, but also maintain the strength of the bone (Mayo Clinic, 2021).

There are studies that have found an association between long term low vitamin D levels and the possibility to develop autoimmune thyroid disorder (Bozkurt et al., 2013). On the contrary, Zhou et al. found no association between the level of thyroid hormones and the level of vitamin D in the body (Zhou et al., 2016). This creates a controversy in the sense that the role of vitamin D in relation to the thyroid gland may not be significant enough to cause any alteration in the functions of one another. But recently, studies reported a strong link between thyroid hormones/thyroid antibodies and vitamin D (Chahardoli et al., 2019). Wang et al. also reported an association between vitamin D and autoimmune thyroid disease (AITD) (Wang et al., 2015). Supplementation with vitamin D has also been reported to ameliorate thyroid defect (Krysiak et al., 2019).

2.7.2. Vitamin B6

Vitamin B6 (pyridoxine) serves as a coenzyme in many enzymatic processes in the human body (Parra, 2018). Functions of vitamin B6 in metabolic processes include metabolism of homocysteine, production of hemoglobin, myoglobin, myelin sheath and most importantly production of neurotransmitters (Parra, 2018). Deficiency of pyridoxine has been shown to cause hypothyroidism due to a decrease in thyrotropin-releasing hormone (TRH) synthesis (Dakshinamurti et al., 1990). Deficiency of vitamin B6 have been reported in hypothyroidism, and its supplementation normalized the abnormality (Kostiukow et al., 2018).

2.7.3. Vitamin B9

Vitamin B9 (folate) cannot be synthesized by humans and is therefore taken to the body via food, especially by green leaves. It plays a crucial role in homocysteine metabolism. One of the functions of folate is that it aids in forming DNA and RNA. It

is also involved in the metabolic processes of proteins. Folate also has an active role in the breakdown of homocysteine in the body (Harvard School of Public Health, 2021). Other functions of folate include production of healthy erythrocytes and aid rapid growth/development.

Folate depletion has been reported in hyperthyroidism usually due to the high demand in hypermetabolic circumstances (Kostiukow et al., 2018). Vitamin B9 supplementation (excess) was found to lower the levels of T3 and T4 in adolescence which could be due to methylation of the TSH (Sittig et al., 2012). This motive could be the rationale behind the memory impairment in the aforementioned study. In a recent study on prediabetic and diabetic patients, serum folate level tends to decrease with an increase in the level of TSH (Hammouda and Mumena, 2019).

2.7.4. Vitamin B12

Vitamin B12 also popularly referred to as cobalamin is type of vitamin that can dissolve in water. It has vital functions in several body's metabolism. One of the functions of cobalamin is aiding the process of forming erythrocytes, formation of DNA, and the essential nerve function (Mayo Clinic, 2017). Vitamin B12 deficiency is a very rare condition because the body has capacity to store large amount that can sustain the individual for several years. However, studies have shown that vegans and vegetarians have high risk of vitamin B12 deficiency (Javid and Christensen, 2016).

Vitamin B12 deficiency has a significant role in trapping the availability of folate thereby causing a decrease in its level, and a significant correlation exists between folate and TSH in hypothyroidism (Tripathi et al., 2018). Vitamin B12 was recently found to be associated with autoimmune hypothyroidism which could be as a result of malabsorption of vitamin B12 (Aktas, 2020).

2.8. Minerals

Minerals are very important for normal functioning of the human body. Particularly iodine, iron, selenium as well as zinc are crucial for the function of the thyroid gland as shown in Figure 2.5 (Moncayo and Moncayo, 2017). Among all of these, iodine has been proven to have a very vital role in the process of synthesizing thyroid hormones (Chung, 2014). The process of thyroid hormone synthesis requires

a number of mechanisms. These mechanisms are usually required by the thyroid follicular cells and include sodium iodide symporter (NIS) and thyroperoxidase (TPO). NIS is responsible for transporting iodide into cells, however, TPO is responsible for oxidizing iodide to iodinium (I+). In turn, the iodinium (I+) iodinates thyroglobulin (Tg) (Ke and Liu, 2014).

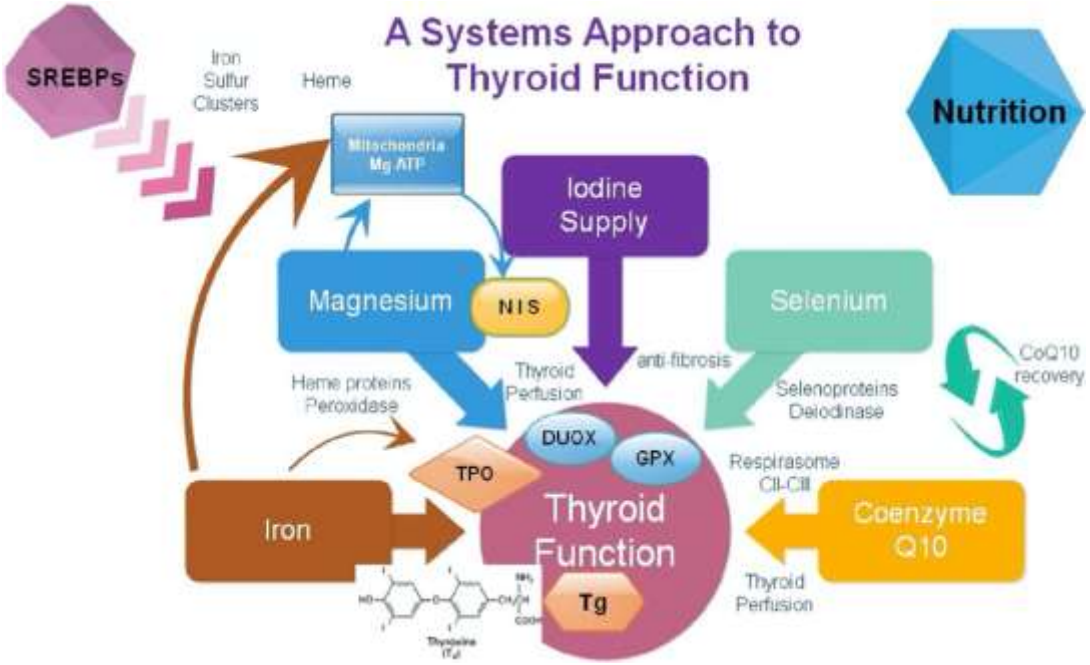

Figure 2.5. Interaction of Mg, Fe, and other elements in maintaining the proper function of the thyroid (Moncayo and Moncayo,2017). Tg: thyroglobulin; TPO: thyroid peroxidase; GPX: glutathione peroxidase; DUOX: dual oxidase; NIS: natrium iodide symporter; SREBPs: sterol regulatory element binding proteins

2.8.1. Calcium

This element is a divalent cation available in almost every part of the body. More than 99% of the calcium is stored in the skeleton, while only about 1% is present in the blood and it is partly bound to a protein and partially ionized. Calcium has an important task of transmitting nerve impulses which includes the regulation of heart beats. It is also involved in blood clotting and hormone secretion (Bongard et al., 2008). Several studies have attempted to find the relationship between calcium and the thyroid hormones; however, the status of the relation is still unclear. Most of the

studies such as the study of Abdullah et al., found a weak correlation between calcium and thyroid hormones (Abdullah et al., 2015).

2.8.2. Magnesium

Magnesium plays an important role in energy production in glycolysis, and it is also involved in many biological reactions including regulation of many enzymatic reactions (Pilchova et al., 2017). It works indirectly in the production of thyroid hormones, iodine absorption and iodine removal (Nussey and Whitehead, 2001). There is a link between low levels of thyroid hormones and low magnesium concentration. Low magnesium level is also correlated with increased TSH level (Kolanu et al., 2019).

There are several evidences showing the link between the level of magnesium and the function of thyroid gland. The first evidence was indicated by Kleiber et al. who observed thyroid gland enlargement in magnesium deficient rats (Kleiber et al., 1941). Magnesium is related to the regulation of the functions of thyroid gland. The thyroid gland may influence both magnesium and calcium metabolism (Mosikidle and Christensen, 1977; Wiang et al., 1992). A preliminary study has predicted the relationship between thyroid hormones and the principal macro metals calcium and magnesium (Hasey et al., 1993).

Increased thyroid activity causes more magnesium to be consumed by the tissues of the gland, thus favoring hypomagnesaemia. In a serum containing thyroxine and magnesium, thyroxine reduces the amount of magnesium and vice versa (Tortora and Grabowski, 1996). A significant increase in thyroid size was reported in rats fed with a magnesium deficient diet (Corradins and Parker, 1986). Evidence suggests that magnesium metabolism in thyroid dysfunction is affected not only by thyroid hormone levels but also by the duration of disorder. Plasma magnesium level was significantly lower in hyperthyroid patients than in euthyroid or hypothyroid individuals. Furthermore, magnesium levels were negatively correlated with the duration of hyperthyroidism (Shibutani et al., 1989).

2.9. Ferritin

Ferritin is important for several functions in the human body. It is an intracellular protein that has a universal function as an iron storage protein. It stores adequate amounts of iron and it is present in almost all cells and tissues. The relationship between the level of iron and storage protein ferritin is linear, as the levels of ferritin in the blood correlate well with the storage of iron in the body. Iron is required for the production of T4 and T3 in the thyroid gland, the conversion of T4 into the more active T3, and iron is required for the utilization of T3 inside the cells. (Wang et al., 2019). Reports indicate alterations in serum ferritin levels in patients with thyroid diseases. These reports claim that changes in the levels of serum ferritin are related to a dysfunctional thyroid gland that may be caused by some pathogens (Sachdeva et al., 2015).

In hyperthyroidism, the level of iron and ferritin are increased, while their decrease was observed in hypothyroidism (Hernik et al., 2019). There are links between T3 and the regulation of ferritin expression. Also, there is a positive relationship between T4 / T3 and serum ferritin levels (Akhter et al., 2012). Thus, it is preferable to measure the level of ferritin in the blood, which helps in evaluating the action of thyroid hormones in the tissues.

Iron deficiency is a serious global problem as it is reported to have affected more than 800 million people either as anemia or iron deficiency (Shaban et al., 2020). Most of the people who are affected are women and mostly pregnant women because of the nutritional requirement of the developing fetus (Stevens et al., 2013; Breymann, 2015). Ferritin plays a role in buffering against both acute and chronic depletion of iron (He et al., 2018). Therefore, ferritin is an important indicator for diagnosis and treatment of iron deficiency and anemia (Saito, 2014).

2.10. Albumin

Albumin is a protein that is secreted by the liver. It is a type of globular protein and present in the blood (Chaudhary and Muddeshwar, 2016). Proteins belonging to the albumin family are hydrophilic which means water-soluble. Albumin is the general transporter protein in blood and it transports hydrophobic compounds such as fatty

acids, hormones, bilirubin and thyroid hormone (T4). It also has a vital part in the regulation of blood osmotic pressure (Chaudhary and Muddeshwar, 2016).

2.11. Thyroid Dysfunction

Thyroid disorders are among the most prevalent endocrine disorders worldwide. Globally, 300 million people are suffering from thyroid disorders (Sumathi et al., 2019). Thyroid dysfunction is more prevalent among women compared to men. One in every eight women during their lifetime is at risk of developing thyroid disorder. Though the exact mechanism is not known, it is believed to be associated with estrogen and progesterone. Iodine intake, sex, age, ethnicity and geographical factors influence the incidence of thyroid disorders (Sumathi et al., 2019).

A healthy thyroid gland and the normal functioning of thyroid hormones are dependent on adequate amount of some trace elements and minerals. Iodine, iron, selenium and zinc are some of the examples of minerals and trace elements vital for thyroid hormone metabolism. Trace elements associated with the normal function of the thyroid gland are useful for the processes of both metabolism and synthesis of thyroid hormones (Betsy et al., 2013). Selenium is an important constituent of deiodinase enzyme which is responsible for converting triiodothyronine (T3) to thyroxine (T4). Too much exposure of thyroid gland to iodide could alter the normal function of the thyroid gland and the thyroid hormones. According to Takamatsu et al., low iron, or more specifically, low ferritin, is one of the most overlooked causes of thyroid dysfunction (Takamatsu et al., 1985).

The two well-known risk factors for thyroid dysfunction are iodine deficiency and autoimmune dysfunction. Exposure to environmental chemicals is also among the suspected risk factors. Other risk factors include depression; which has been reported to cause different abnormalities and a decrease in the levels of total T3.

The liver as a key organ in the body is known to regulate the activity of the thyroid gland, therefore, any disturbance in the activity of the liver may affect thyroid hormones (Khan et al., 2010). Untreated thyrotoxicosis has been associated with the high prevalence of certain abnormalities in liver biochemical tests. The abnormalities include high serum alkaline phosphatase (ALP), alanine aminotransferase (ALT), gamma glutamyltransferase (GGT), aspartate aminotransferase (AST) and elevated

bilirubin. This renders thyroid hormone dysfunction as a risk factor for liver diseases (Lin et al., 2017).

The production of thyroid hormones should be tightly regulated at specific points for preventing abnormally high or low hormone levels since both conditions will result in abnormal functioning of the liver and the gland itself. Decreased production of thyroid hormones by the thyroid gland is called hypothyroidism; it can be either primary or secondary (Shahid et al., 2020). In primary hypothyroidism, the thyroid is incapable of producing adequate levels of thyroid hormones needed for vital functions. Primary hypothyroidism is the most prevalent form. On the other hand, secondary or central hypothyroidism is less prevalent and occurs when the activities of the pituitary gland or hypothalamus interfere or causes pathology of the thyroid gland (Patil and Rehman, 2020). An increase in serum TSH (above 10 mIU/L) along with a decreased concentration of serum T4 and T3 is called overt hypothyroidism. In sub-clinical hypothyroidism, serum TSH is between 4-10 mIU/L and is associated with a normal concentration of serum T4 and T3 (Pillai and Bennet, 2018). Hypothyroidism can occur as a result of infection or dysfunction of hypothalamus, pituitary, as well as chronic low iodine levels (Gupta and Lee, 2011).

A condition in which there is an increase in the level of thyroid hormones is referred to as hyperthyroidism (Malik and Hodgson, 2002). Hyperthyroidism can be caused by several factors including thyroid infections, autoimmune destruction of the gland and elevated pituitary production of TSH (Franco et al., 2013). The symptoms of hyperthyroidism consist of a diverse range of clinical features that can manifest in almost all systems of the human body. One of the common forms of hyperthyroidism is thyrotoxicosis, the clinical condition which presents with high levels of thyroid hormones (Blick et al., 2020). Thyrotoxicosis causes liver injury of both hepatic and cholestatic types, manifested by elevated activities of aspartate aminotransferase (AST) and alanine aminotransferase (ALT) in the hepatic type and elevated serum alkaline phosphatase (ALP) in the cholestatic type (Malik and Hodgson, 2002).

Hyperthyroidism and hypothyroidism exert oxidative stress that results in disturbances in the level of vitamins (Sworczak and Wiśniewski, 2011). Variation in vitamin levels in thyroid patients also leads to a disturbance in mineral levels such as

serum calcium and magnesium; however, magnesium shows seasonal variation unlike calcium (Aldrees et al., 2020).

Determining the level of serum TSH is usually the first biochemical test that is used to check for hypothyroidism. It is widely used because it is cost effective, safe, and provides accurate results (Brenta et al., 2013). However, some recent studies have found higher levels of serum TSH in people without any thyroid problems (Duntas and Yen, 2019). This means checking for TSH serum alone cannot always be reliable for diagnosing hypothyroidism.

Furthermore, hypothyroidism can be caused by the negative feedback exerted production of the hormones TRH and TSH. This negative feedback is caused by the alteration in the levels of both T3 and T4. This effect is mostly exerted by T3 hormones. When T4 hormone is produced less than normal, it triggers the production of the TSH hormone in the pituitary gland. When pituitary gland produces more TSH hormone, it results in hypertrophy and hyperplasia of the thyroid parenchyma which in turn triggers T3 hormones production (Patil and Rehman, 2020).

During early detection of T3 and T4, their level might be low but certain antibodies such as thyroid peroxidase antibody, anti-microsomal antibody, and thyroglobulin antibody are shown to be increased (Kollerová et al., 2015). However, the diagnosis of hypothyroidism is dependent on several factors including gender, genetic predisposition, thyroid insufficiency, age and even the environment (Brix et al., 2000; Laurberg et al., 2005; Thvilum et al., 2013; Duntas and Yen, 2019). For example, the serum TSH level in the age range of 20–29 years old is 3.56 mIU/L, however, a report shows a significant difference in the levels of serum TSH for the age group more than 80 years (7.9 mIU/L) (Hollowell et al., 2002). Furthermore, hypothyroidism occurs more within the female gender especially in pregnant women with characteristic features such as a small stature and body mass during childhood. It has been reported that about 10% of women may experience postpartum thyroiditis (Patil and Rehman, 2020).

In a clinical setting, diagnostic testing for hypothyroidism can be performed by simple blood tests to check for levels of TSH and/or thyroid hormones. The hypothyroidism is diagnosed if the levels of TSH hormone is higher than normal or if thyroxine is lower than normal (Mayo Clinic, 2021). Symptoms and signs of

hypothyroidism are often mild or subtle and, when there is clinical suspicion, thyroid function tests are needed; if serum TSH level is raised, free T4 and thyroid peroxidase antibody should be measured. Usually, symptoms of hypothyroidism can be helpful in determining the presence of hypothyroidism. It is more effective and efficient to combine the result of blood test and the clinical symptoms of hypothyroidism (Patil and Rehman, 2020). Ultrasensitive or third generation TSH assays are able to measure low levels of TSH than conventional TSH assays and thus are important for differentiating normal from hyperthyroid (Brenta et al., 2013).

2.12. Relationship Between Liver and Thyroid Hormone Dysfunction

Thyroid function affects liver function both in health and disease. Thyroid hormones modulate liver function by regulating the basal metabolic rate of hepatocytes, and the liver regulates the effects of thyroid hormones since it is the major organ that metabolizes T3 and T4. Some reports suggest that hypothyroidism may have a direct effect not only the on the function but also the structure of the liver. A few studies have found a link between cholestatic jaundice (decreased excretion of bilirubin and bile) and hypothyroidism (Malik and Hodgson, 2002).

Liver dysfunction associated with hyperthyroidism has been reported for decades, but the pathophysiology is yet to be determined. Drugs used to treat hyperthyroidism or liver dysfunction due to thyrotoxic heart failure, as well as medications including oral contraceptives may be implicated in the hepatic dysfunction in hyperthyroidism (Evlice and Aksoz, 2017). The mechanism of thyrotoxicosis does not only cause injury to the liver, it is capable of causing other nonspecific abnormalities in liver biochemistry, mostly in the form of modest elevation in transaminases or rarely severe hyperbilirubinemia and hypoalbuminemia (Evlice and Aksoz, 2017).

The female gender is one of the strongest independent predictors of hypothyroidism (Aminorroaya et al., 2016). A recent study by Hu et al. revealed that the male gender is also a strong predictor for early hypothyroidism (Hu et al., 2020). This finding was similar to a previous study from Malaysia by Wan Mohamed et al. which also reported that men have the highest incidence of hypothyroidism (Wan Mohamed et al, 2018).

Patients with type A hepatic encephalopathy were reported to have abnormal thyroid hormone concentrations (Wang et al., 2017). A study by Hasan et al. revealed a high correlation between hypo and hyperthyroidism with liver biomarker enzymes such as ALT, AST, ALP and TSB. It is clear from the aforementioned sources that there is a high correlation between thyroid hormone abnormality and liver dysfunction (Hasan et al., 2016).

3. MATERIALS AND METHODS

3.1. Study Design

This study was conducted at the National Centre for Diabetes and Endocrinology, Tripoli, Libya. Samples were collected from participants (control and patients) that comply with the target of the research (patients that have thyroid related complications) and the age range of the study was set between 20 to 60 years. Details of the research and aim were explained to the participants prior to sample collection. Informed consent form was completed and signed by both parts. The detailed information about the participant such as name, age, gender, symptoms-disease history, family history and other related problems was collected Control subjects were chosen from the completely healthy individuals. This study was approved by the Near East University, Health Sciences Ethics Committee (Registration Number: YDU/2019/75-943).

Exclusion criteria for control and patient groups was set prior to sample collection.

1. Participants in the study who have tested positive for hepatitis B surface antigen as well as hepatitis C virus antibodies were selected.
2. Participants in the study who have been previously administered with drugs for treating thyroid or preventing any disorder of the thyroid (antithyroid drugs).
3. Participants in the study who have been clinical tested and proven to have some problems in their cardiovascular systems or any positive diagnosis of any autoimmune liver diseases.
4. Participating patients in the study who have been administered drug-induced liver disorder and nonalcoholic fatty liver disorder.
5. Participating patients in the study who have been diagnosed with secondary hyperthyroidism or secondary hypothyroidism.
6. Participating patients in the study who have been diagnosed with hepatobiliary disease.

3.2. Biochemical Measurements

Two red capped tubes (Figure 3.1) were used to collect 3 mL blood each by a specialist phlebotomist. Samples were then let to settle and centrifuged at 2000*g* for 10 minutes in a Hettich EBA 200 centrifuge (Figure 3.2). Serum was aspirated from the tube for analysis. COBAS INTEGRA 400 plus analyzer (Figure 3.3) was used for biochemical analysis. It is a random-access analytical system that is operated automatically and controlled by a software. These features make it ideal for efficient lab operations. It is also capable of performing large quantity of tests per day. Other features of the system include modular design, utilization of serum/plasma/whole blood, capable of analyzing diverse range of analytes using quantitative test *in vitro*, as well as photometric assays and ion-selective electrode measurements (Gundersen Health Systems, 2019).

Elecsys COBAS E411 (Roche Diagnostics International Ltd.), (Figure 3.4) was used for assessing the level of thyroid hormones and vitamins. This diagnostic tool is a well designed automatic (random access) immunoassay that is controlled by software. The immunoassay analysis system has three test principles: the competitive test principle which enables the detection of extremely small analytes, the one or two steps sandwich principle for larger analytes and a bridging principle for the detection of the antibodies in the sample. The system uses the electrochemiluminescence (ECL) technology in which stable precursors on the electrode are transformed into very reactive species interacting with one another to produce light (Gundersen Health Systems, 2019).

Thyroid hormone and TSH levels were evaluated in order to classify the patients as hyper- or hypothyroid. Individuals with normal TSH and thyroid hormone levels (T4, T3, FT3 and FT4) were considered as the control group. Each group was planned to have 150 individuals.

3.3. Statistical Analysis

For each group (control + hyperthyroidism + hypothyroidism) liver function tests (ALT, AST, GGT, ALP) and other biochemical parameters (T-Bilirubin, D-Bilirubin, albumin, calcium, magnesium, ferritin, vitamin D, vitamin B6, Vitamin B9 and vitamin B12) were statistically evaluated using IBM SPSS Statistics (version 20)

(SPSS Inc. Chicago, IL, USA). First, the groups were compared by using Kruskal-Wallis test and $p < 0,05$ was accepted as statistically significant. Then Mann-Whitney U test was used to compare the two groups. Finally, Pearson's correlation analysis was performed to see the correlation between to parameters.

Figure 3.1. Plain blood collection tubes

Figure 3.2. Hettich EBA 200 centrifuge

Figure 3.3. COBAS INTEGRA 400 plus

Figure 3.4. Elecsys COBAS E411

4. RESULTS

Our study included 148 patients with hypothyroidism, 147 patients with hyperthyroidism and 150 control subjects. The majority of the patients were females with a percentage of 90.5 among the hypothyroid and 84.4 among the hyperthyroid groups. However, there was no statistically significant difference in the ages of the subjects in each group ($p = 0,285$).

Since our measurements for each parameter in each group did not show normal distribution, nonparametric statistical tests were used for the evaluation of our findings. First of all, groups were compared by using Kruskal-Wallis test. Albumin, vitamin D, vitamin B6, vitamin B12, calcium and magnesium levels did not show any statistically significant difference among groups (Table 4.1), but the levels of TSH, FT4, FT3, T4, T3, D-BIL, T-BIL, vitamin B9, ferritin and activities of ALT, AST, ALP and GGT differed significantly ($p < 0,05$) (Table 4.1).

In patients with hypothyroidism TSH, FT4, FT3, T4, T3, ALT, AST, GGT, D-BIL, T-BIL, albumin and ferritin levels showed significant difference compared to control group ($p < 0,05$). There was an increase in the levels of liver function enzymes (ALT, AST, ALT, GGT), total and direct bilirubin and albumin, whereas ferritin level decreased in patients with hypothyroidism (Table 4.2).

Table 4.1. Baseline and biochemical characteristics of study groups

	Control (n = 150)		**Hypothyroidism (n = 148)**		**Hyperthyroidism (n = 147)**		**p**
	Mean ± SD	Median (min-max)	Mean ± SD	Median (min-max)	Mean ± SD	Median (min-max)	
Age (yrs)	39,65 ± 10,36	38 (20-60)	41,39 ± 10,70	42 (20-60)	40,00 ± 11,96	41 (19-75)	0,285
Gender (F/M)	85 / 65		134 / 14		124 / 23		
TSH (0,27-4,2 mIU/L)	1,99 ± 0,99	1,9 (0,3-4,1)	23,60 ± 25,52	12,89 (4,30-100)	0,027 ± 0,036	0,010 (0,0-0,2)	**0**
FT4 (11,5-22,7 pmol/L)	15,31 ± 2,93	15,04 (10,3-31,1)	7,51 ± 5,43	6,74 (0,20-46,1)	36,39 ± 16,88	31,10 (7,3-100)	**0**
FT3 (2,8-7,1 pmol/L)	4,10 ± 1,17	3,94 (1,56-7,02)	1,57 ± 1,51	1,30 (0,10-12,90)	13,87 ± 11,09	10,03 (5,12-100)	**0**
T4 (66-181 nmol/L)	114,58 ± 32,11	113,05 (1,61-179)	39,53 ± 22,26	38,21 (0,42-128)	233,25 ± 49,59	225 (50-328,60)	**0**
T3 (1,3-3,1 nmol/L)	1,95 ± 0,50	1,91	1,01 ± 0,53	1,02	9,58 ± 6,49	7,70	**0**

		(0,8-3,4)		(0,10-3,40)		(0,0-36,90)	
ALT (up to 40 IU/L)	28,35 ± 9,06	28 (11-69)	32,93 ± 12,09	33 (8-62)	37,09 ± 8,55	38 (13-62)	**0**
AST (up to 45 IU/L)	28,35 ± 7,56	27 (13-51)	34,83 ± 11,12	36 (7-63)	37,52 ± 8,77	39 (14-56)	**0**
ALP (up to 270 IU/L)	135,71 ± 54,14	123,50 (19-270)	153,81 ± 65,92	137 (53-288)	173,59 ±64,61	182 (36-295)	**0**
GGT (24,8-50 IU/L)	25,95 ± 7,22	25 (12-51)	32,59 ± 12,43	31,75 (13-58)	38,00 ± 11,75	40 (12-60)	**0**
D-BIL (up to 0,25 mg/dL)	0,17 ± 0,10	0,15 (0,10-0,80)	0,20 ± 0,10	0,20 (0,10-0,70)	0,19 ±0,09	0,20 (0,10-0,60)	**0,001**
T-BIL (0,5-1,2 mg/dL)	0,58 ± 0,28	0,53 (0,10-1,20)	0,71 ± 0,56	0,60 (0,10-6,40)	0,74 ± 0,29	0,70 (0,10-1,60)	**0**
Albumin (3,5-5 g/dL)	3,70 ± 0,69	3,62 (2,10-6,1)	3,88 ± 0,68	3,89 (2,20-6,50)	3,75 ± 0,61	3,66 (2,10-6,10)	**0,025**
Vit D (30-100 ng/mL)	24,66 ± 17,81	19 (5,70-90)	26,80 ± 18,31	20,65 (4,60-76)	30,16 ± 20,39	22,50 (3,10-91)	0,058

Vit B6 (3,6-18 ng/mL)	11,53 ± 4,86	11,16 (2,20-26)	10,70 ± 4,58	10,79 (2-24,20)	10,59 ± 4,45	10,33 (1,30-24,20)	0,298
Vit B9 (1,1-20 ng/mL)	11,45 ± 6,68	10,90 (2-65)	11,99 ± 5,08	12 (2-25)	14,25 ± 7,59	13,40 (2-46)	**0,001**
Vit B12 (190-630 pg/mL)	317,92 ± 124,37	301 (80-673)	308,08±125,32	290 (17-618)	327,44 ± 130,64	315 (99-613)	0,487
Ferritin (20-250 ng/mL)	48,11 ± 34,26	38 (8-171)	34,62 ± 27,67	28,70 (5-193)	53,47 ± 34,65	45 (11-186)	**0**
Calcium (8,6-10,2 mg/dL)	8,90 ± 0,98	8,77 (6,20-11,7)	8,77 ± 0,90	8,70 (5,70-11,0)	8,83±0,89	8,60 (6,50-11,10)	0,55
Magnesium (1,7-2,25 mg/dL)	1,85 ± 0,30	1,81 (1,10-3,80)	1,82 ± 0,30	1,81 (1,00-2,60)	1,83 ±0,37	1,80 (1,00-3,80)	0,821

p value testing the difference between groups (through Kruskal-Wallis test) <0,05 is significant. Reference values are given in parenthesis.

Table 4.2. Comparison of biochemical parameters between hypothyroidism and control group

	Control (n = 150)		**Hypothyroidism (n = 148)**		
	Mean ± SD	Median (min-max)	Mean ± SD	Median (min-max)	p
Age (yrs)	39,65 ± 10,36	38 (20-60)	41,39 ± 10,70	42 (20-60)	0,108
Gender (F/M)	85 / 65		134 / 14		
TSH	1,99 ± 0,99	1,9 (0,3-4,1)	23,60±25,52	12,89 (4,30-100)	**0**
FT4	15,31 ± 2,93	15,04 (10,3-31,1)	7,51 ± 5,43	6,74 (0,20-46,1)	**0**
FT3	4,10 ± 1,17	3,94 (1,56-7,02)	1,57 ± 1,51	1,30 (0,10-12,90)	**0**
T4	114,58±32,11	113,05 (1,61-179)	39,53 ± 22,26	38,21 (0,42-128)	**0**
T3	1,95 ± 0,50	1,91 (0,8-3,4)	1,01 ± 0,53	1,02 (0,10-3,40)	**0**
ALT	28,35 ± 9,06	28 (11-69)	32,93±12,09	33 (8-62)	**0**
AST	28,35 ± 7,56	27 (13-51)	34,83±11,12	36 (7-63)	**0**
ALP	135,71±54,14	123,50 (19-270)	153,81±65,92	137 (53-288)	0,48
GGT	25,95±7,22	25 (12-51)	32,59±12,43	31,75 (13-58)	**0**
D-BIL	0,17 ± 0,10	0,15 (0,10-0,80)	0,20 ± 0,10	0,20 (0,10-0,70)	**0**

T-BIL	0,58 ± 0,28	0,53 (0,10-1,20)	0,71 ± 0,56	0,60 (0,10-6,40)	**0,038**
Albumin	3,70 ± 0,69	3,62 (2,10-6,1)	3,88 ± 0,68	3,89 (2,20-6,50)	**0,009**
Vit D	24,66±17,81	19 (5,70-90)	26,80±18,31	20,65 (4,60-76)	0,370
Vit B6	11,53 ± 4,86	11,16 (2,20-26)	10,70 ± 4,58	10,79 (2-24,20)	0,214
Vit B9	11,45 ± 6,68	10,90 (2-65)	11,99 ± 5,08	12 (2-25)	0,091
Vit B12	317,92±124,37	301 (80-673)	308,08±125,32	290 (17-618)	0,644
Ferritin	48,11±34,26	38 (8-171)	34,62±27,67	28,70 (5-193)	**0**
Calcium	8,90 ± 0,98	8,77 (6,20-11,70)	8,77 ± 0,90	8,70 (5,70-11,60)	0,351
Magnesium	1,85 ± 0,30	1,81 (1,10-3,80)	1,82 ± 0,30	1,81 (1,00-2,60)	0,858

*Control and hypothyroidism groups were compared by Mann-Whitney U test. $p < 0,05$ was accepted as statistically significant.

In patients with hyperthyroidism, TSH, FT4, FT3, T4, T3, ALT, AST, ALP, GGT, D-BIL, T-BIL, vitamin D and vitamin B9 levels were statistically different than controls ($p < 0,05$). Liver function enzymes (ALT, AST, ALP, GGT) direct and total bilirubin, vitamin D and vitamin B9 levels were elevated (Table 4.3).

Table 4.3. Comparison of biochemical parameters between hyperthyroidism and control group

	Control (n = 150)		**Hyperthyroidism (n = 147)**		
	Mean ± SD	Median (min-max)	Mean ± SD	Median (min-max)	p
Age (yrs)	39,65±10,36	38 (20-60)	40,00 ± 11,96	41 (19-75)	0,706
Gender (F/M)	85 / 65		124 / 23		
TSH	1,99 ± 0,99	1,9 (0,3-4,1)	0,027 ± 0,036	0,010 (0,0-0,2)	**0**
FT4	15,31 ± 2,93	15,04 (10,3-31,1)	36,39 ± 16,88	31,10 (7,3-100)	**0**
FT3	4,10 ± 1,17	3,94 (1,56-7,02)	13,87 ± 11,09	10,03 (5,12-100)	**0**
T4	114,58±32,11	113,05 (1,61-179)	233,25±49,59	225 (50-328,60)	**0**
T3	1,95 ± 0,50	1,91 (0,8-3,4)	9,58 ± 6,49	7,70 (0,0-36,90)	**0**
ALT	28,35 ± 9,06	28 (11-69)	37,09 ± 8,55	38 (13-62)	**0**
AST	28,35 ± 7,56	27 (13-51)	37,52 ± 8,77	39 (14-56)	**0**
ALP	135,71±54,14	123,50 (19-270)	173,59±64,61	182 (36-295)	**0**
GGT	25,95 ± 7,22	25 (12-51)	38,00 ± 11,75	40 (12-60)	**0**
D-BIL	0,17 ± 0,10	0,15 (0,10-0,80)	0,19 ±0,09	0,20 (0,10-0,60)	**0,020**

T-BIL	0,58 ± 0,28	0,53 (0,10-1,20)	0,74 ± 0,29	0,70 (0,10-1,60)	**0**
Albumin	3,70 ± 0,69	3,62 (2,10-6,1)	3,75 ± 0,61	3,66 (2,10-6,10)	0,367
Vit D	24,66±17,81	19 (5,70-90)	30,16 ± 20,39	22,50 (3,10-91)	**0,017**
Vit B6	11,53 ± 4,86	11,16 (2,20-26)	10,59 ± 4,45	10,33 (1,30-24,20)	0,154
Vit B9	11,45 ± 6,68	10,90 (2-65)	14,25 ± 7,59	13,40 (2-46)	**0**
Vit B12	317,92 ± 124,37	301 (80-673)	327,44±130,64	315 (99-613)	0,501
Ferritin	48,11±34,26	38 (8-171)	53,47 ± 34,65	45 (11-186)	0,093
Calcium	8,90 ± 0,98	8,77 (6,20-11,70)	8,83 ± 0,89	8,60 (6,50-11,10)	0,344
Magnesium	1,85 ± 0,30	1,81 (1,10-3,80)	1,83 ±0,37	1,80 (1,00-3,80)	0,521

*Control and hyperthyroidism groups were compared by Mann-Whitney U test. $p < 0,05$ was accepted as statistically significant.

Table 4.4 and Figure 4.1 shows the correlation between thyroid hormones and the studied biochemical parameters in patients with hypothyroidism. TSH levels showed positive correlation with AST, ALP, GGT and magnesium levels. T3 level was positively correlated with ALT, AST, ALP and GGT levels. No correlation was detected in terms of other studied parameters (Table 4.4).

In patients with hyperthyroidism, TSH showed a negative correlation only with ALP, while positive correlations were detected between FT4 and ALT; FT3 and ALT, ALP; T4 and ALT, AST, GGT; T3 and ALP, total bilirubin (Figure 4.2) (Table 4.5).

Table 4.4. Correlation between thyroid hormones and studied parameters in hypothyroidism

	TSH	FT4	FT3	T4	T3
ALT	r=0,114 p=0,168	r=-0,071 p=0,392	r=-0,096 p=0,247	r=0,067 p=0,416	**r=-0,180** **p=0,029**
AST	**r=0,224** **p=0,006**	r=-0,132 p=0,110	r=-0,023 p=0,777	r=0,007 p=0,934	**r=-0,175** **p=0,034**
ALP	**r=0,226** **p=0,006**	r=-0,063 p=0,450	r=-0,111 p=0,178	r=-0,084 p=0,311	**r=-0,247** **p=0,003**
GGT	**r=0,249** **p=0,002**	r=-0,076 p=0,358	r=-0,148 p=0,072	r=-0,122 p=0,139	**r=-0,163** **p=0,047**
D-BIL	r=-0,043 p=0,6	r=0,073 p=0,375	r=0,159 p=0,054	r=0,059 p=0,479	r=-0,061 p=0,462
T-BIL	r=0,021 p=0,796	r=0,048 p=0,560	r=-0,006 p=0,944	r=-0,001 p=0,987	r=0,006 p=0,946
ALB	r=0,005 p=0,954	r=-0,040 p=0,631	r=0,054 p=0,513	r=0,043 p=0,602	r=0,020 p=0,807
Vit D	r=0,033 p=0,694	r=-0,058 p=0,480	r=-0,100 p=0,228	r=-0,081 p=0,329	r=-0,019 p=0,818
Vit B6	r=-0,037 p=0,653	**r=0,225** **p=0,006**	r=0,093 p=0,259	r=0,003 p=0,967	r=-0,069 p=0,404
Vit B9	r=-0,089 p=0,280	r=0,001 p=0,993	r=-0,072 p=0,385	r=-0,098 p=0,235	r=-0,065 p=0,434
Vit B12	r=-0,028 p=0,734	r=-0,005 p=0,952	r=0,020 p=0,808	r=-0,030 p=0,715	r=-0,009 p=0,910

Ferritin	r=-0,106 p=0,200	r=-0,047 p=0,568	r=-0,045 p=0,584	r=-0,018 p=0,825	r=0,028 p=0,732
Calcium	r=-0,048 p=0,564	r=0,079 p=0,339	r=0,070 p=0,398	r=0,027 p=0,742	r=0,009 p=0,913
Magnesium	**r=-0,171** **p=0,038**	r=-0,018 p=0,826	r=-0,043 p=0,608	r=0,059 p=0,479	r=0,100 p=0,225

*Correlation was analyzed by Pearson's correlation coefficient

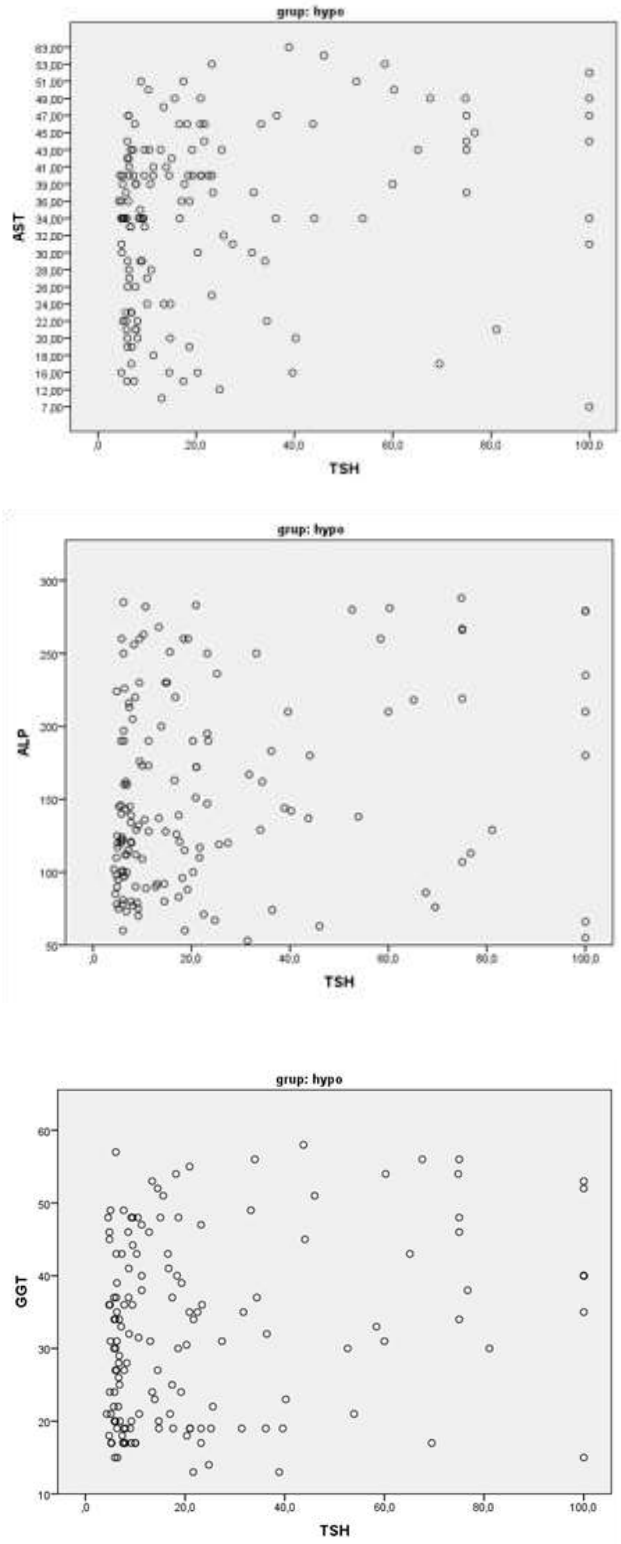

Figure 4.1. Correlation plots between TSH and AST, ALP and GGT in patients with hypothyroidism

Table 4.5. Correlation between thyroid hormones and studied parameters in hyperthyroidism

	TSH	FT4	FT3	T4	T3
ALT	r=0,055 p=0,508	**r=0,202** **p=0,014**	**r=0,191** **p=0,021**	**r=0,298** **p=0**	r=0,124 p=0,134
AST	r=-0,035 p=0,678	r=0,084 p=0,310	r=0,126 p=0,127	**r=0,203** **p=0,014**	r=0,087 p=0,296
ALP	**r=-0,164** **p=0,047**	r=0,036 p=0,661	**r=0,213** **p=0,009**	r=0,134 p=0,104	**r=0,178** **p=0,031**
GGT	r=0,015 p=0,857	r=0,077 p=0,351	r=0,124 p=0,135	**r=0,230** **p=0,005**	r=0,090 p=0,279
D-BIL	r=-0,091 p=0,275	r=-0,025 p=0,760	r=0,065 p=0,437	r=-0,023 p=0,780	r=0,039 p=0,641
T-BIL	r=-0,034 p=0,679	r=-0,022 p=0,788	r=0,152 p=0,066	r=0,069 p=0,406	**r=0,171** **p=0,039**
ALB	r=-0,035 p=0,676	r=-0,013 p=0,875	r=0,042 p=0,617	r=0,028 p=0,732	r=-0,061 p=0,466
Vit D	r=-0,022 p=0,787	r=-0,078 p=0,348	r=-0,082 p=0,322	r=0,046 p=0,581	r=-0,027 p=0,748
Vit B6	r=-0,030 p=0,721	r=0,135 p=0,104	r=-0,062 p=0,454	r=0,029 p=0,724	r=-0,088 p=0,291
Vit B9	r=-0,060 p=0,469	r=0,102 p=0,219	r=-0,103 p=0,213	r=0,022 p=0,793	r=-0,127 p=0,127
Vit B12	r=0,013 p=0,874	r=0,101 p=0,226	r=0,079 p=0,342	r=0,081 p=0,328	r=0,016 p=0,846

Ferritin	r=-0,122 p=0,142	r=0,030 p=0,722	r=0,003 p=0,976	r=0,089 p=0,281	r=0,113 p=0,175
Calcium	r=0,026 p=0,758	r=-0,060 p=0,470	r=-0,028 p=0,735	r=-0,028 p=0,736	r=-0,103 p=0,214
Magnesium	r=0,080 p=0,334	r=-0,103 p=0,215	r=-0,046 p=0,610	r=-0,003 p=0,969	r=-0,073 p=0,379

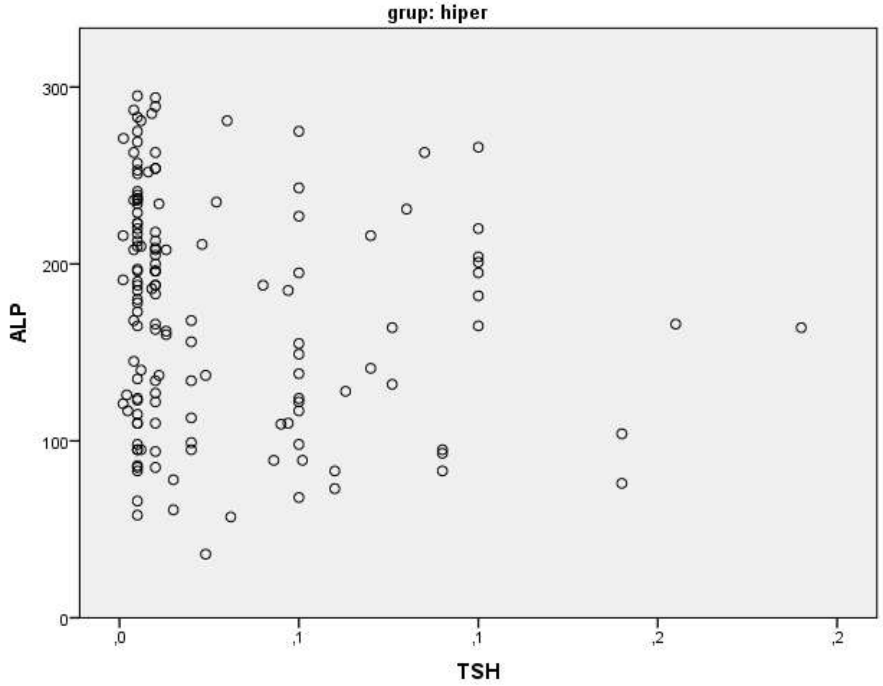

Figure 4.2. Correlation plots between TSH and ALP in patients with hyperthyroidism

5. DISCUSSION

The results of the study are presented in chapter four. It is important to note that the results of the study were obtained from the collected data which also included comprehensive statistical analysis. The study comprised a total of 450 participants. This sample size was categorized into three groups; control, hyperthyroidism, hypothyroidism with 150 participants equally distributed among the categories. However, only 445 samples were available for use in accordance to the aims and objectives of the study, therefore, 5 samples were discarded.

The main aim of the study was to find the association between the levels of thyroid hormones and liver function tests. Other biochemical parameters such as total and direct bilirubin, albumin, ferritin, magnesium and calcium as well as the levels of vitamins (D, B6, B9 and B12) were assessed among Libyan population. There were 85 female and 65 male participants in control group (total= 150), 134 females and 14 males in hypothyroidism group (total= 148) and 124 females and 23 males in hyperthyroidism group (total= 147). The number of the females (77%) is much higher than the number of the male participants (23%). Consistent with the findings of Dahiya et al., in both hypothyroidism and hyperthyroidism group majority of the patients were females. The reason of high prevalence of hypothyroidism among female population is due to the high level of estrogen which is responsible for anti-thyroid actions. In this case, females within the age range of reproduction (from puberty to perimenopause) are likely to have hypothyroidism (Dahiya et al., 2016). This is also confirmed in the study of He et al., where they indicated that thyroid disease is a common endocrine disorder prevalent in pregnant women (He et al., 2018).

In regards to age as a variable, the mean age of the participants in each category is also provided. In the control group, the mean age was 39,65 ± 10,36, in hyperthyroidism the mean age was 40,00 ± 11,96, while the mean age in hypothyroidism was 41,39 ± 10,70 as shown in Table 4.1. As noticed in the result, female participants in the study across all the categories appear to be much older compared to the male participants. Comparing the differences between the three groups (control, hypothyroidism and hyperthyroidism), age and gender as dependent factors do not reveal any significant difference among groups.

In the present study we assessed the relationship between thyroid hormones and liver function tests in a selected group of Libyan patients with thyroid disorders and healthy control subjects. When compared with controls, we observed significantly elevated ALT, AST, GGT, total and direct bilirubin and decreased ferritin levels in hypothyroid patients. We also observed that in these hypothyroid patients, the activities of liver function enzymes were negatively correlated with T3, showing that ALT and AST tend to increase as T3 decreases or hypothyroidism worsens. These findings are consistent with other previous reports Ambiger and Chincholikar (Ambiger and Chincholikar, 2019). Elevated GGT activity along with elevated total and direct bilirubin levels in the hypothyroid group reflect an impairment in the biliary and excretory activity of the liver under hypothyroid conditions.

Sahana and Kruthi reported a significant correlation between thyroid hormones (TSH, T3 and T4) and serum ferritin as ferritin levels was found to decrease significantly (Sahana and Kruthi, 2020). Ferritin is the main storage form of iron in the human and iron is required for the production of T4 and T3 in the thyroid gland, the conversion of T4 into the more active T3, and iron is required for the utilization of T3 inside the cells. (Wang et al., 2019) Thus our results suggest that low ferritin levels may be partly implicated in the pathogenesis of hypothyroidism in our patients. Our finding of low ferritin levels in hypothyroid patients are in accordance with the findings of others who reported an association between low ferritin/iron levels and decreased thyroid function (Beard et al., 1990; Smith et al., 1993; Das et al., 2012; Dahiya et al., 2016).

In patients with hyperthyroidism, TSH, FT4, FT3, T4, T3, ALT, AST, ALP, GGT, D-BIL, T-BIL, vitamin D and vitamin B9 levels were statistically different than controls ($p < 0,05$). Liver function enzymes (ALT, AST, ALP, GGT), direct and total bilirubin, vitamin D and vitamin B9 levels were elevated. Similarly, Ajala et al. also reported that total bilirubin together with liver enzymes were elevated in hyperthyroid patients (Ajala et al., 2013). Contrary to ALT, AST, GGT, D-BIL, T-BIL which were elevated in both hypo-and hyperthyroid patients, we noticed that ALP was elevated only in the hyperthyroid group. Furthermore, in these patients we found significant positive correlations between ALP and FT3 and T3, the physiologically active forms of the hormone and a significant negative correlation between ALP and TSH. These

data suggest that increased ALP activity reflects hepatobiliary injury in a hyperthyroid environment.

In patients with hypothyroidism, TSH levels were positively correlated with AST, ALP, GGT and negatively correlated with magnesium levels. This is also consistent with the findings of Ambiger and Chincholikar, hence confirming the relationship between liver function tests and thyroid gland (diseases). Similar to our study, Ambiger and Chincholikar evaluated liver function tests in thyroid disease patients to find any association between liver function tests and thyroid function. They showed a significant elevation in in the activities of liver enzymes in thyroid patients compared to the control group (Ambiger and Chincholikar, 2019). Kalita et al. and Yadav et al. also found similar results (Yadav et al., 2013; Kalita et al., 2016).

Alterations in magnesium metabolism in thyroid disease are reported (Dolev et al., 1988; Disashi et al., 1996). We demonstrated a negative correlation between TSH and Mg levels in hypothyroid patients. Similarly, Kolanu et al. reported a correlation between low magnesium and increased TSH levels (Kolanu et al., 2020).

In patients with hyperthyroidism, TSH showed a negative correlation only with ALP, while positive correlation was detected between FT4 and ALT; FT3 and ALT, ALP; T4 and ALT, AST, GGT; T3 and ALP, total bilirubin. These findings are in consistent with the findings of Khan et al (Khan et al., 2010). This confirms that an effect on the thyroid hormone function alters the function of liver enzymes.

Furthermore, the analysis of other biochemical parameters including albumin, vitamin D, vitamin B6, vitamin B12, calcium and magnesium did not reveal any differences among the control, hypothyroidism and hyperthyroidism groups. However, differences were significant in TSH, FT4, FT3, T4, T3, ALT, AST, ALP, GGT, D-BIL, T-BIL, Vitamin B9 and ferritin.

6. CONCLUSION

This study has successfully achieved the aim of investigating the link between the levels of thyroid hormones, liver function tests and the levels of vitamins (D, B6, B9 and B12) in Libyan population. As we emphasized in the study, the liver plays a pivotal role in the various processes of thyroid hormone metabolism. Similarly, thyroid dysfunction can have profound effects on hepatic function as reflected by increased liver function tests including ALT, AST, ALP, GGT, total and direct bilirubin. Most of the liver function enzymes were found to be correlated with thyroid hormones especially in hypothyroidism. It is clear that disruption in thyroid hormones causes alterations in many parameters in Libyan population. Patients with thyroid diseases should be periodically tested for liver function in order to prevent further complications.

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EK: 1036-2020

YAKIN DOĞU ÜNİVERSİTESİ
BİLİMSEL ARAŞTIRMALAR ETİK KURULU

ARAŞTIRMA PROJESİ DEĞERLENDİRME RAPORU

Toplantı Tarihi : 23.01.2020
Toplantı No : 2020/76
Proje No :943

Yakın Doğu Üniversitesi Tıp Fakültesi öğretim üyelerinden Prof. Dr. Özlem Dalmızrak'ın sorumlu araştırmacısı olduğu, YDU/2020/76-943 proje numaralı ve **"Association between thyroid hormone levels, liver function tests, vitamin D, B_6 and B_{12} levels in Libyan population"** başlıklı proje önerisi kurulumuzca değerlendirilmiş olup, etik olarak uygun bulunmuştur.

1. Prof. Dr. Rüştü Onur (BAŞKAN)
2. Prof. Dr. Nerin Bahçeciler Önder (ÜYE) KATILMADI
3. Prof. Dr. Tamer Yılmaz (ÜYE) KATILMADI
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