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Evaluation level of Adipokines, MicroRNA-590 and some physiological parameters in hypothyroidism obese women

A thesis

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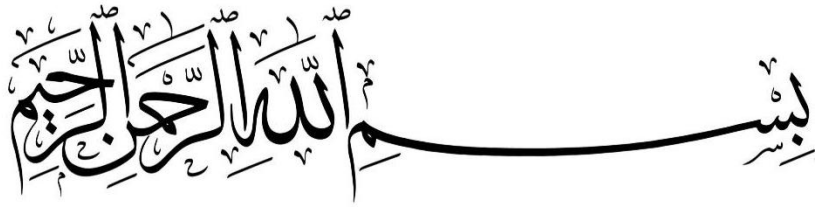

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Dedication

To Allah my Lord,

To the one who led the hearts and minds of humanity to safety, the first teacher of humanity, Muhammad (PBUH)

My homeland Iraq is the symbol of civilization …
To my dear father …and compassionate heart mother
My brothers and sisters.

To the soul of my martyr grandfather …

I dedicate this thesis to my determination that has not diminished, to my strength that appeared in moments of weakness, and to my Resoluteness that pushed me to continue despite all obstacles. I dedicate to myself this well-deserved success.

I dedicate this work, hoping to God that it will be a window of knowledge

With love and gratitude

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First, thanks to Allah he gave me the power, strength, and patience to Accomplish this work.

"There is a special time in every body's life, which one never forgets"

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Thanks to every hand and heart that walked with me on the path of Achievement to be

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Summary

Hypothyroidism is linked to elevated dyslipidemia, traditionally attributed to decreased thyroid hormone (TH). However, recent research suggests thyroid-stimulating hormone (TSH) independently influences lipid metabolism. On the other hand, miRNAs are small, single-stranded, non-coding molecules of RNA, which regulate gene expression at post-transcriptional level. The dysregulation of the expression of several miRNAs affects pathways involved in thyroid disorders pathogenesis.

This aims of this study were accomplished by the following objectives:To determine the levels of miRNA 590 in thyroid diseases. Also to assess the sensitivity and specificity of Preselection miRNA and its diagnostic utility in these circumstances. Then find the relationship between miRNA590 and certain physiological markers, such as Obestatin and Ghrelin.

A case-control study included 90 samples of women, 60 samples of women patients with thyroid disorders and 30 samples of healthy women. The demographic aspects of the patients were collected through technique (questionnaire). The patients were subjected to a complete clinical history, clinical examination, and laboratory tests, and the type of disorder was determined based on Evaluation of laboratory measurements for thyroid hormones. The enzyme-linked immunosorbent assay system (ELISA) was performed using the Competitive-ELISA principle to measure the concentrations of hormones ghrelin and obestatin in serum samples.

Statistical analysis was performed and the efficiency of the predicting value was assessed using the receiver operating characteristic (ROC).

The results indicated that serum hormone ghrelin concentrations was higher (35.26 ng /ml) in obese women of hypothyroidism, as compared with control subjects (26.92 ng/ml), on the other hand obese women of hypothyroidism

have higher obestatin levels (10.99 ng/ml) compared to the control group (7.22 ng/ml). In this study, the cycle threshold (Ct) of the microRNA590 was measured using a quantitative determination present in a sample.

A lower Ct value indicates a higher abundance of target microRNA. In this case, the patients group has a higher Ct value (29.94) compared to the control group (22.14), suggesting lower levels of microRNA590 in the hypothyroidism group. Folding change, which refers to the difference in gene expression between two conditions, was indicated to be upregulation, and higher in the patient group (13.46) compared to the control group (0.95). The results show that higher TSH levels are associated with lower microRNA590 levels.

ROC curve and Area under curve (AUC) analysis for the folding change of miRNA-590 among obese women patients with hypothyroidism compared to control groups were performed. The study concluded that The miRNA-590 folding change provides high predictive value for diagnosing hypothyroidism patients, according to the ROC curve study

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List of Abbreviations

Abbreviation	Full Form
ABCA1	ATP-binding cassette transporter A1
ABCG5/8	ATP binding cassette subfamily G member 5
ACC	Acetyl-CoA carboxylase
ACTH	Adrenocorticotropic Hormone
AITDs	Autoimmune thyroid disorders
ANGPTL3/8	Angiopoietin-like-protein 8
Apo A1	Apo lipoprotein A1
Apo B	Apo lipoprotein B
ApoC3	Apolipoprotein C-III
Auc	Area under curve
BMI	Body max index
CAMK11	Ca2+/calmodulin-dependent protein kinase II

cAMP/PKA/CREB	Cyclic adenosine monophosphate/ protein kinase A/r esponsive element binding protein
CAV1	Caveolin 1
cDNA	Complementary DNA
CETP	Cholesteryl ester transfer protein
c-jun	Transcription factor Jun
CM	chylomicron
CPT1A	Carnitine palmitoyltransferase I
CREB5-NF-kB	CAMP Responsive Element Binding Protein 5-nuclear factor-kappa B
CT	Cycle threshold
CVD	Cardio vascular disease
CYP7A1	Cholesterol 7 alpha-hydroxylase
DNL	De novo lipogenesis
EGFR	epidermal growth factor receptor
ERK1/2	Extracellular signal-regulated kinases
FAS	Fatty acid synthase
FFA	Free fatty acid
FGF19/21	Fibroblast growth factors 19/21
FT3	Free Triiodothyronine hormone
FT4	Free Thyroxin hormone
GH	Growth hormone
GHS_R	Ghrelin receptors
GOAT	Ghrelin O-acyltransferase
GPR39	G-protein coupled receptor 39
HDL-C	High- Density Lipoprotein Cholesterol
HL	Hepatic lipase
HMGCR	3-hydroxy-3-methyl-glutaryl-coenzyme A reductase
HNF4	Hepatocyte nuclear factor 4

HSL	Hormone-sensitive lipase
IR	Insulin resistance
LCAT	Lecithin cholesterol acyltransferase
LDL-C	Low-Density Lipoprotein Cholesterol
LDLR	LDL-receptor
LPL	Lipoprotein lipase
LPs	lipoproteins
LRP1	LDL receptor-related protein1
MCT8	Mon carboxylate transporter8
miRNAs	micro ribonucleic acid
NIS	The Sodium-Iodide Symporter
NPC1L1	Niemann-Pick C1-Like 1
NPY	Neuropeptide Y
PCSK9	Proprotein convertase subtilisin/kexin type 9
PI3K/ AKT	The phosphoinositide 3-OH kinase/a serine/threonine protein kinase
PLTP	Phospholipid transfer protein
Pre-β-HDL	Pre Beta High Density Lipoprotein
PRL	prolactin
(qPCR)	Quantitative polymerase chain reaction
qRT-PCR	Quantitative Reverse Transcription polymerase chain reaction
RCT	Reverse cholesterol transport
RLP	Remnant lipoprotein
ROC	Receiver Operating Curve
RT_PCR	Reverse transcription polymerase chain reaction
SCH	Subclinical hypothyroidism
SD	Standard Deviation
sdLDL	Small dense LDL

SRB1	Scavenger receptor b1
SRE	Sterol regulatory element
SREBP2	Sterol regulatory element binding protein2
T3	Triiodothyronine hormone
T4	Thyroxin hormone
TC	Total Cholesterol
TG	Triglycerides
TH	Thyroid hormone
TPO	Thyroid peroxidase antibodies
TPO	Thyroid peroxidase
TRE	Thyroid _responsive element
TRH	Thyrotropin-releasing hormone
TRLs	TG-rich lipoproteins
TSH	Thyroid stimulating hormone
TSHR	Thyroid stimulating hormone receptor
TSHRα	Thyroid stimulating hormone receptor α
TSHRβ	Thyroid stimulating hormone receptor β
U6	Rnu6b
VLDL-C	very Low-Density Lipoprotein Cholesterol
WAT	White adipose tissue

CHAPTER ONE

Introduction

1.1. Introduction

The thyroid gland is known as one of the most important glands in the body, Since it produces the hormones triiodothyronine (T3) and thyroxine (T4), which control various metabolic processes like growth, brain development, fetus development, heart function, and many other processes (Al-Mofarji *et al.*, 2023). Thyroid disorders are the most prevalent endocrine disorders worldwide, with diabetes mellitus coming in second. The majority of problems that influence thyroid function are those that affect the production of thyroid hormones (Azahir *et al.*, 2022).

About 200 million individuals worldwide suffer from thyroid disorders, along with a number of other illnesses. Additionally, it is predicted that 40% of the global population is susceptible to a dietary shortage in iodine, which is necessary for the synthesis of thyroid hormones, the primary cause of disease global (Aversano *et al.*, 2021) .

Thyroid dysfunction is typically due to of an imbalance between hypo- and hyperthyroid gland activity, which increases or decreases thyroid hormone production (Garg and Vanderpump, 2013).

Thyroid-stimulating hormone (TSH) levels are the most sensitive measure of thyroid function, and they are the primary basis for the diagnosis of thyroid dysfunction. As a result, TSH above and free thyroxine (fT4) within the reference range are considered subclinical hypothyroidism, but TSH above and fT4 below the reference range are considered clinical hypothyroidism (Taylor *et al.*, 2018).

While hyperthyroidism, which is characterized by low levels of thyrotropin (thyroid stimulating hormone) and increased levels of triiodothyronine (T3) and/or free thyroxine (FT4) (Chung, 2020).

Patients with hypothyroidism have symptoms such as weight gain and cold sensitivity due to inadequate energy metabolism and poor thermogenesis (Zekri *et al.*, 2021).

Patients with hypothyroidism also often have a higher frequency of cardiovascular risk factors and symptoms of the metabolic syndrome, including hypertension, expanded waist circumference, dyslipidemia, and raised cholesterol levels. Hypothyroidism also causes an increase in low-density lipoprotein, total cholesterol (Tiller et al., 2016).

The control of energy balance by ghrelin and the likely presence of obestatin in this process raise the possibility of a relationship between thyroid function and both peptides. It is commonly recognized that metabolic disturbances affect the release of thyroid hormones; conversely, hyper- and hypothyroidism

Cause the rate of metabolism to rise and decrease, respectively. It has previously been established that the thyroid gland produces ghrelin and its receptors (Karaoglu *et al*., 2009).

MicroRNAs are kind of endogenously synthesized, tiny, single-stranded, noncoding RNA molecules. Usually, they have between 19 and 24 nucleotides in length, which is essential for cell growth, differentiation, cell cycle control, and death. (Wang, 2021). as numerous miRNAs have been discovered to be altered in adipose tissue (white, brown, and beige) during obesity and are strongly linked to metabolic problems associated with obesity, their role in the pathogenesis of obesity is well known (Abente .,*et al* 2016).

Though the list of diet-related miRNAs is significant, a number of studies indicate that the most dysregulated miRNAs in obesity belong to the miR-21/590-5p family (Palmer *et al*., 2014). Thyroid follicular cells' proliferation and differentiation are regulated by miRNAs, and disorders including goiter and thyroid cancer are caused by abnormal expression of miRNAs (Fuziwara and Kimura ,2017).

In the thyroid glands of rats suffering from high iodine-induced hypothyroidism and chronic hypothyroidism, Zhang *et al*. discovered a number

of differently expressed miRNAs. These miRNAs have the ability to control the expression of critical genes that influence the production of thyroid hormones, including NIS, pendrin, TPO, MCT8, TSHR, TSHα, and TSHβ. This indicates that miRNAs are crucial for the thyroid gland's metabolism (Zhang *et al.*, 2024).

1.2. Aims of the study

Due to the high incidence of hypothyroidism in women and metabolic changes associated with it ,this study was designed to Study physiological parameters, biochemical and hormonal variables and their relationship to microRNA in hypothyroidism females.

This aim was conducted by the following objectives:

1. To estimate the levels of miRNA 590 in the thyroid disorders
2. To evaluate the sensitivity and specificity of the Preselection miRNA and their diagnostic value in such cases
3. To study the correlation of the miRNA590 with some physiological markers such as Obestatin and ghrelin.

CHAPTER TWO

Literatures Review

2. Literature Review

Thyroid function is vital for tissue metabolism and growth, making it one of the biggest glands in the human body. It produces thyroid hormones through synthesis and secretion, which are vital for all metabolic activities in living cells as well as for growth, neuronal and general development, reproduction, and energy metabolism management. Since thyroid-stimulating hormone (TSH), which is primarily controlled by the pituitary gland, governs its activity, the variables that govern the production and secretion of TSH also play a major role in regulating thyroid function in healthy individuals. The thyroid hormones themselves, thyroxine (T4) and triiodothyronine (T3), have the greatest impact on TSH production and secretion (Mariotti and Beck-Peccoz ,2021). The hypothalamic-pituitary-thyroid axis regulates the development and activity of the thyroid gland. Thyrotropin-releasing hormone (TRH) from the hypothalamus stimulates the anterior pituitary's thyrotrope cells to produce serum thyrotropin (TSH), as presented in Figure (2.1). This, in turn, encourages the growth of the thyroid gland and the thyroid gland's secretion of T3 and T4 hormones, both of which, via negative feedback, prevent the synthesis of TSH and TRH and so keep blood levels of T4 and T3 stable (Yeza *et al.*, 2021).

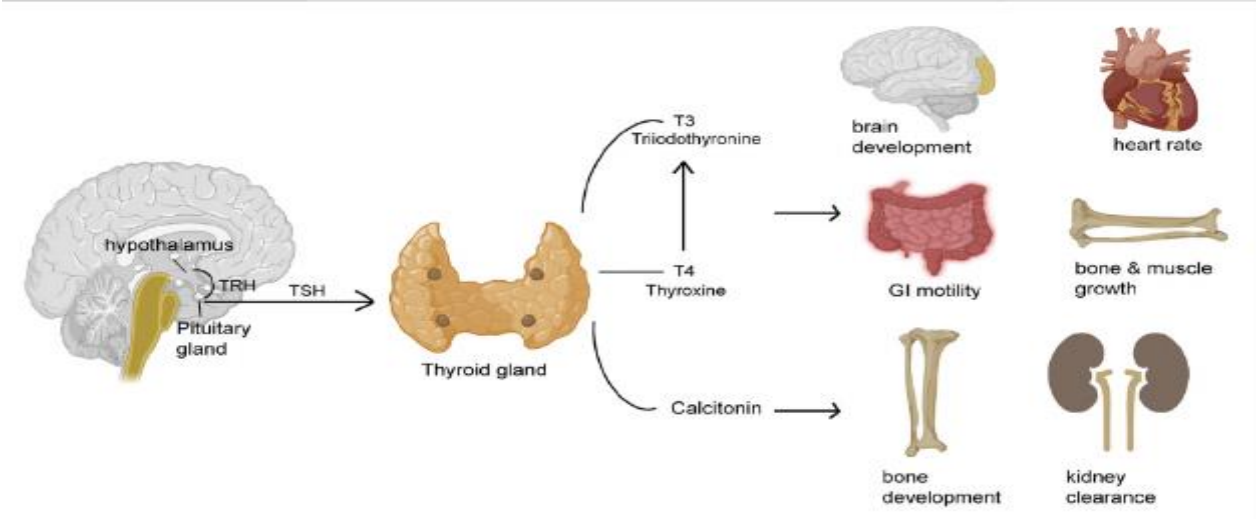


Figure (2.1) Several organ systems are regulated by thyroid hormones (Kyriacou *et al* ., 2015).

Thyroid hormones are essential for preserving energy equilibrium and controlling a variety of metabolic functions, such as the metabolism of fats and carbohydrates (Choi *et al.*, 2021). Hyperthyroidism and hypothyroidism are two frequent thyroid illnesses that are caused by unusual thyroid function.

The thyroid gland does not generate enough of a few key hormones when it is hypothyroid (low thyroid, underactive thyroid). Can lead to a number of health issues, including obesity, joint discomfort, infertility, and heart disease, if it is not well treated.

A disease known as hyperthyroidism, or hyperactive thyroid, occurs when the thyroid gland generates excessive amounts of the hormone thyroxin. There is a major rise in the body's metabolism, which results in abrupt weight loss, an erratic or fast heartbeat, perspiration, and anxiety or stress (Ioniţă *et al.*, 2016).

However, a wide range of other conditions can also affect thyroid function. Among these are demographic variables (age and sex) (Song *et al.*,2019), internal variables (microbiota)(Knezevic *et al.*,2020), tension (Helmreich *et al.*,2005) and the use of medications (Montanelli *et al.*,2010).

"Globally, women are typically five to eight times more likely than males to suffer thyroid diseases, and one in eight women will experience a thyroid condition at some point in her life (Hidayat, 2024). In addition, Reduced thermogenesis and a lower metabolic rate are linked to hypothyroidism; it has also been demonstrated to be correlated with a higher body mass index (BMI) and a higher incidence of obesity (Ambad *et al.*,2020).

Thyroid dysfunction was considered if patients' thyroid hormones fall outside the reference range: free T3 (FT3) (4.0–8.3 pmol/L), free T4 (FT4) (9.0–20.0 pmol/L), and thyrotropin (TSH) (0.25–5 mIU/L); overt hypothyroidism was defined as TSH >5 mIU/L and FT3 < 4.0 and FT4 < 9.0 pmol/L; subclinical

hypothyroidism was considered if TSH >5 mIU/L and FT3 and FT4 were within the reference range; subclinical hyperthyroidism was defined if TSH 8.3 pmol/L and FT4>20pmol/L (Walsh, 2016).

When free thyroxine (FT4) is low and thyroid stimulating hormone (TSH) is high, primary hypothyroidism is typically referred to be "overt." Normal levels of FT4 and free triiodothyronine (FT3) and high TSH characterize biochemically, subclinical hypothyroidism. (Biondi *et al*.,2019).

Patients with hypothyroidism also often have a higher frequency of cardiovascular risk factors and symptoms of the metabolic syndrome, including hypertension, expanded waist circumference, dyslipidemia, and raised cholesterol levels. Hypothyroidism also causes an increase in low-density lipoprotein, total cholesterol, and homocysteine levels. Patients with acute hypothyroidism experience a decline in their quality of life and mood while receiving treatment for thyroid cancer (Shin *et al*., 2016). Indeed, secondary dyslipidemia frequently results from hypothyroidism (Jin *et al*.,2014).

The majority of hypothyroidism patients are obese, and obesity contributes to some degree of insulin resistance (Bishop *et al*., 2010). A major worldwide health concern, thyroid problems are caused by an imbalance in the synthesis of thyroid hormones. Because these illnesses influence growth and cognition, they significantly worsen the physical and mental health of those who are afflicted, especially in early life. Hormonal imbalances are the cause of common thyroid conditions such as hypothyroidism, hyperthyroidism, thyroid nodules, goiter, and thyroid cancer. (Mariani *et al*., 2021).

2.1. Prevalence of thyroid dysfunction

The occurrence of hypothyroidism has grown to impact a noteworthy segment of the world's populace (Taylor *et al*., 2018). Over 200 million

individuals worldwide suffer from thyroid-related disorders as a result of iodine deficiency, which affects over 40% of the world's population (Aversano *et al*., 2023).

Women are up to 8–9 times more likely than males to have primary hypothyroidism, and the condition is increasingly frequent as people age, peaking between the ages of 30 and 50 (Al-Hindawi *et al*., 2017). The general population has an overt hypothyroidism prevalence of 0–3% to 3-7%. (Chaker *et al*., 2022). In addition, about 0.2% to 1.4% of people have hyperthyroidism (Chung, 2020).

Subclinical thyroid illnesses are quite prevalent in a variety of populations, according to several studies, and over 50% of subclinical thyroid diseases eventually advance to overt thyroid diseases over a 20-year period. Screening for subclinical thyroid disorders and tracking their progression to overt thyroid illnesses are essential if we hope to reduce the clinical effect of these ailments that are predicted in the future (Salman, 2023).

2.2. Etiology.

Researchers are still having difficulty determining the primary etiology of thyroid illness. A few studies used a comprehensive literature review to find possible risk variables that could be associated with the illness. Radiation, depression, obesity, hormonal variables, and genetic inheritance are among the causes that have been found (Fiore *et al*., 2019).

Hypothyroidism can have both primary and secondary causes. One of the main causes is an illness that directly affects the thyroid and causes it to generate inadequate levels of thyroid hormones. A further cause is the pituitary gland's dysfunction, which stops it from delivering thyroid-stimulating hormone (TSH) to the thyroid to control thyroid hormones (Hasan *et al*., 2024)

Hypothyroidism can be caused by a number of conditions, such as autoimmune illnesses, thyroid cancer, hypothalamic or pituitary gland damage, inadequate iodine intake, and thyroid dysfunction (Sunil and Leena , 2022).

For many years, iodine deficiency has been one of the main causes of thyroid illness worldwide. Long-term, uncontrolled use of iodized salt above recommended limits proved problematic since it not only corrected the deficit but also caused issues such thyroid autoimmunity and hypo/hyperthyroidism. (Qureshi, 2020).

Primary hypothyroidism has a number of common causes, all of which include different degrees of thyroid tissue damage. The most notable characteristic of Hashimoto's thyroiditis is likely the various degrees of residual thyroid function which can range from the slight reduction in thyroid function associated with subclinical hypothyroidism to the total absence of thyroid function that results from the thyroid gland being destroyed by the immune system (Lee *et al.*, 2010).

Although it is uncommon, hypothyroidism (secondary hypothyroidism) can also result from pituitary gland failure. Rare instances include iodine metabolism problems caused by innate enzymes, an increase in thyroid-stimulating hormone (TSH) production, defective thyroid hormone receptors, thyroid gland autoimmune, and cancer. A shortage of iodine was discovered in almost 90% of individuals with goiter. If left untreated, thyroid disorders can have a major negative impact on the body (Vanderpump , 2011).

The autoimmune disorders of the thyroid gland, which induce excessively high discharges from thyroid hormone-producing cells, are among the endogenous causes of hyperthyroidism. Graves' disease and thyroid gland adenomas are two examples of endogenous causes. Excessive thyroid hormone consumption or high iodine intake can also result in hyperthyroidism. The word "thyrotoxicosis" is often used to refer to hyperthyroidism; however, as was

previously indicated, not all instances of hyperthyroidism showed signs of thyrotoxicosis. Typically, it developed in the middle ages (Leung and Braverman, 2014).

2.3. Risk Factor

One of the greatest risk factors for the development of numerous disorders, including hypothyroidism, is obesity (Mullur *et al*., 2014). obesity, even in moderate cases (BMI greater than 23 kg/m^2) is thought to increase the incidence of thyroid problems, particularly in regions with low iodine levels (Gowachirapant *et al*., 2014).

Uncontrolled hypothyroidism causes high blood pressure, abnormalities in the neuromuscular system, lipid metabolism, cognitive decline, and infertility (Boucai *et al*.,2011). Numerous additional studies have suggested risk factors for thyroid illness, including vitamin D insufficiency (Zhao *et al*.,2019). Diabetes (Ogbonna *et al*., 2019). Radiation (Wiltshire *et al*.,2016). hormonal variables (Horn-Ross *et al*., 2011). and hereditary factors (Pak *et al*., 2015).

As presented in Figure (2.2) markedly elevated risk of hypothyroidism was linked to either elevated blood TSH or positive thyroid antibodies, either separately or in combination. The annual risk of spontaneous overt hypothyroidism in the surviving women was 4% in those with high serum TSH and anti-thyroid antibody concentrations, 3% in those with only high serum TSH concentrations, and 2% in those with only high serum thyroid antibody concentrations; at the time of follow-up, the corresponding rates of hypothyroidism were 55, 33, and 27%. Women with serum TSH values had an increased chance of getting hypothyroidism (McGrogan *et al*., 2008).

Currently, the focus is on detecting cases among women who are considered to be at high risk, meaning they have a history of thyroid problems in

their family or themselves, as well as a history of pregnancy problems (Vaidya *et a*l., 2007).

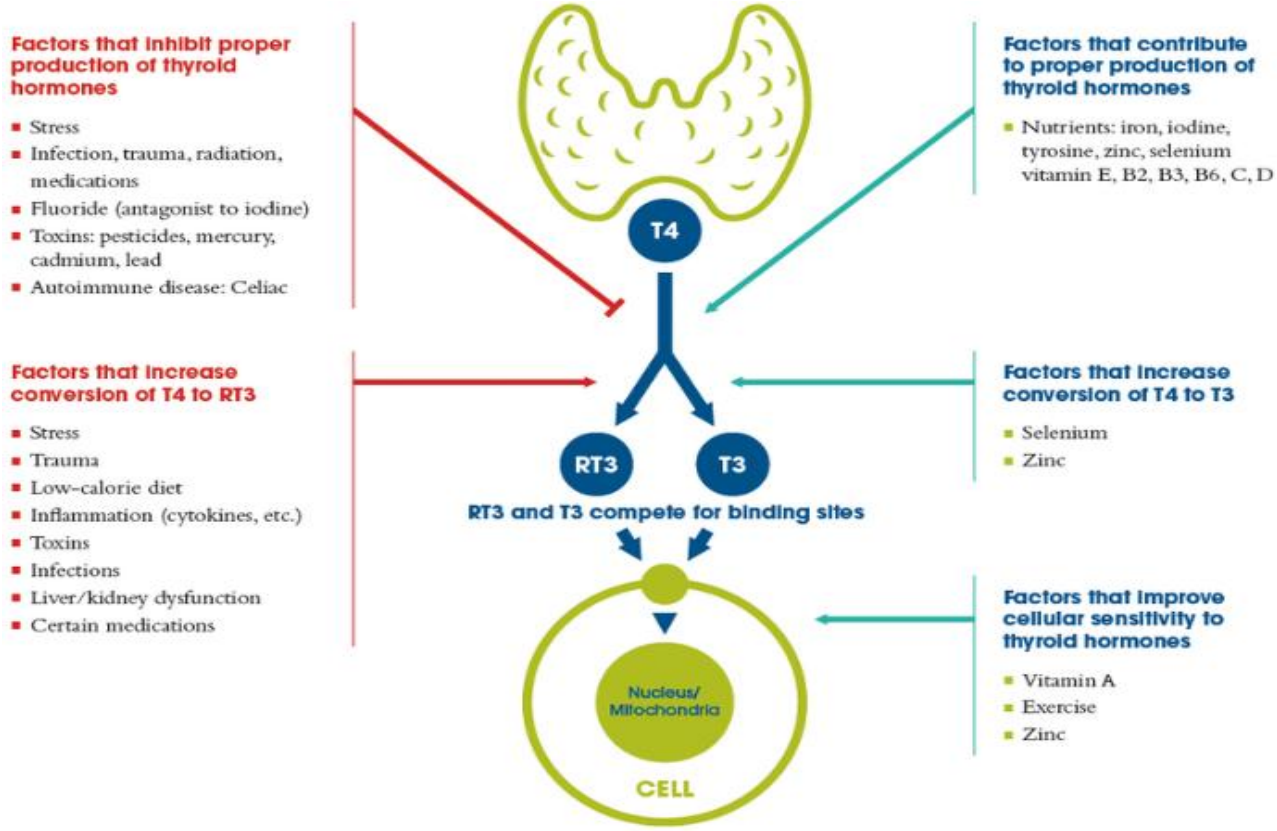


Figure (2.2) Factors that Affect Thyroid Function (Ioniţă *et al*., 2016).

2.4. The mechanism of dyslipidemia in hypothyroidism

Many disorders, including depression, bradyarrhythmia, and cretinism, have been linked to hypothyroidism as a common cause or significant risk factor. Increased blood levels of thyroid-stimulating hormone and decreased serum levels of free peripheral thyroid hormones are the main indicators of clinical hypothyroidism (Lauffer *et al*., 2021).

Based on epidemiological research, hypothyroidism has emerged as a major global health concern in the last several years, with grave implications for human health in the future and high global death rates.

Dyslipidemia, which is defined as elevated blood levels of triglycerides (TG), very LDL-C (VLDL-C), and low-density lipoprotein cholesterol (LDL-C), is one of the many concurrent symptoms of hypothyroidism that has lately been reported to be closely connected with the condition (Ejaz *et al*., 2021).

Increasing data supports this theory by showing that hypothyroidism may increase the risk of dyslipidemia and its pathological progression. It has been shown that patients with elevated blood levels of total cholesterol (TC) had a comparatively greater incidence of both subclinical and clinical hypothyroidism in contrast to the general population (Willard *et al*., 2014).

Previous research has demonstrated that hypothyroidism serves a variety of purposes in adjusting the serum lipid profiles (Gao *et al*., 2015).More specifically, it has been reported that individuals with subclinical hypothyroidism had increased blood TC and LDL-C values (Song *et al*., 2016).

Similar to these results, a different study found that patients with subclinical hypothyroidism had significantly higher serum concentrations of TC than did the control group. This finding also suggested that thyroid-stimulating hormone may play a physiological role in the regulation of serum lipid profile metabolism in subclinical hypothyroidism patients (Zhao *et al*., 2016).

However, a number of clinical studies have demonstrated that lipoprotein cholesterols, such as TG/HDL-C and LDL-C/HDL-C, that include apolipoprotein B to apolipoprotein A1 (ApoB/ApoA1) were much higher in these ratios (Liu *et al*., 2018).

Furthermore, individuals with hypothyroidism are verified to have an increased risk of developing postprandial hypertriglyceridemia, as evidenced by raised blood levels of TG, residual lipoprotein (RLP), and TG-rich lipoproteins (TRLs) (Arikan *et al*.,2012).

However, it is yet unclear if the onset of hypothyroidism might affect blood levels and metabolism of lipoprotein cholesterol that contains ApoA1, such

HDL-C. Therefore, more extensive clinical studies must be carried out to investigate the changes in the metabolism of serum ApoA1-containing lipoprotein cholesterol in hypothyroid individuals. It's also important to note that people with subclinical or clinical hypothyroidism have notable differences in their altered serum lipid profiles. For example, Dong *et al*. demonstrated that serum LDL-C values were considerably greater in hypothyroidism patients than in the control group (Dong *et al*., 2016).

Similar variations were seen in TG, suggesting that hypothyroidism is linked to the pathological development of dyslipidemia, although moderate chemical hypothyroidism has a less degree of dyslipidemia (Tognini *et al*., 2012).

Notably, it has been demonstrated that there is a positive correlation between the levels of thyroid-stimulating hormone and ApoB-containing lipoprotein cholesterol in the blood, regardless of whether thyroid function is normal (Luxia *et al*., 2021).

Therefore, the risk of dyslipidemia increases with blood concentrations of thyroid-stimulating hormones. When considered collectively, we may be able to conjecture that there is a substantial correlation between the development of dyslipidemia and thyroid dysfunction. Thyroid-stimulating hormone has a crucial role in controlling the metabolism of serum lipid profiles in addition to the modulatory impact of thyroid hormones.

Lipid profiles are mostly affected by hypothyroidism via TH. Thyroid hormone receptorβ (THRβ), which is mostly expressed in the liver, may bind to TH in order to control the expression of target genes downstream. Both the direct and indirect effects of TSH on TC level are present in the overall effect (TH) (Geng *et al*., 2015). In individuals who had their thyroidectomies treated with levothyroxine, multiple regression analysis revealed a strong correlation between the rise in cholesterol and the decrease in TH levels following TSH injection (Beukhof *et al*., 2018).

In cardiovascular disease (CVD) patients, TSH alone can raise TC levels without the help of TH (Xu *et al.*,2012). According to reports, TSH controls the metabolism of cholesterol by attaching itself to TSH receptors (TSHRs) on the surface of adipocytes and hepatocytes (Santini *et al.*, 2010).

As a result, available data points to the possibility that TSH and TH influence cholesterol metabolism. The following regulatory factors are involved in the regulation of cholesterol metabolism. Figure (2.3) summarizes the main impact of TH and TSH on lipid metabolism in hypothyroidism.

Regarding thyroid hormones, hypothyroidism results in a drop in TH, de novo lipogenesis (DNL) and HMG-COA reductase (HMGCR) activity, which reduces cholesterol formation. However, there is also a decrease in free fatty acid (FFA) β-oxidation. In order to decrease cholesterol clearance, TH lowering lowers the activity of ATP-binding cassette transporter G5/8 (ABCG5/8) and cholesterol 7α-hydroxylase (CYP7A1). Triglyceride-rich very low-density lipoprotein (VLDL) levels are often up in hypothyroidism, and TG-rich chylomicron (CM) is elevated as a result of an elevation in Niemann-Pick C1-like 1 protein (NPC1L1) concentration.

The decreased function of lipoprotein lipase (LPL), which hydrolyzes CM and VLDL, is brought on by a drop in TH. Additionally, LDL receptor (LDLR) and LDL receptor-related protein 1 (LRP1) are unable to remove as much LDL and residual lipoprotein (RLP). TG level rises as a result.

The net HDL concentration is not constant, though. Proprotein convertase subtilisin/kexin type 9 (PCSK9), HMGCR, and hormone-sensitive lipase (HSL) levels rise in response to TSH, but CYP7A1 levels fall. RLP stands for remnant lipoprotein; ANGPTL3/8, angiogenin-like protein3/8; ApoC3, apolipoprotein C3; CETP, cholesterol transport protein transporter; HL, hepatic lipase; PLTP, phospholipid transfer protein; LCAT, lecithin cholesterol acyltransferase; ABCA1, ATP-binding cassette transporter A1; SRB1, scavenger

receptor b1; FGF19/21, fibroblast growth factors 19/21; HMG-COA, 3-hydroxy-3-methyl glutaryl coenzyme A; ACC, acetyl-CoA carboxylase; FAS, fatty acid synthase; CPT1A, carnitine palmitoyltransferase Iα; WAT, white adipose tissue (Liu *et al.*, 2022).

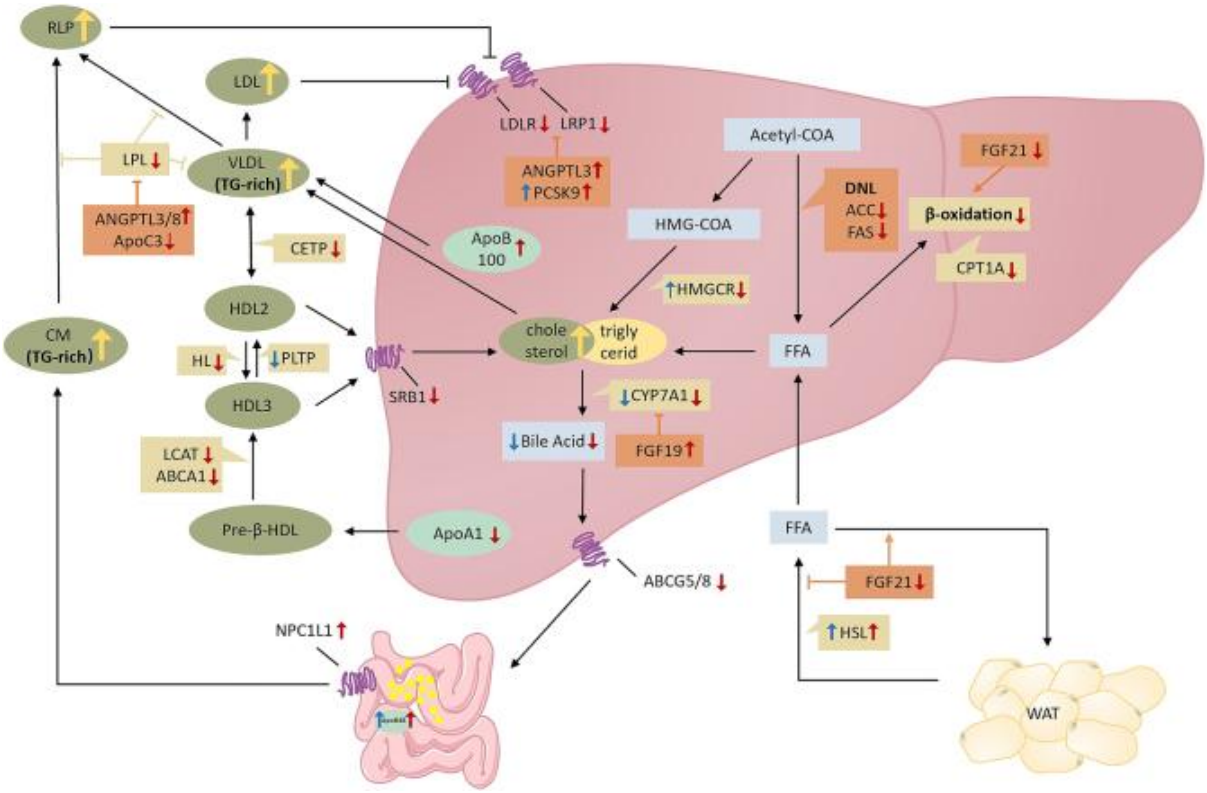


Figure (2.3) Effects on lipid metabolism in hypothyroidism of elevated TSH and low TH. In the event that hypothyroidism is present, the changed functions are marked. Blue arrows indicate activities of increased TSH, and red arrows indicate actions of declining TH (Liu *et al.*, 2022).

2.4.1 Hypothyroidism and LDL-C metabolism

Hypothyroidism affects the production and clearance of LDL-C

The effects of TH on the synthesis and absorption of cholesterol are inconsistent. The rate-limiting enzyme in the production of cholesterol, liver HMG-COA reductase (HMGCR), can be directly induced by TH (Zhang *et al*., 2015) Increasing the production of cholesterol.

In addition to binding to THR, TH has the ability to activate a significant transcription factor called sterol regulatory element-binding protein 2 (SREBP2), which in turn can drive the transcription of the HMGCR gene. Therefore, by influencing HMGCR, hypothyroidism can result in decreased cholesterol production. Nevertheless, in hypothyroidism, TH's impact on the intestinal Niemann-Pick C1-like 1 protein (NPC1L1) results in higher cholesterol absorption. Free fatty acid (FFA) oxidation is also decreased in hypothyroidism, which increases the liver's output of very low-density lipoprotein (VLDL). TH may promote the β-oxidation of FFA via boosting hepatocyte autophagy (Sinha *et al*., 2012).

Additionally, carnitine palmitoyltransferase Iα (CPT1A), an enzyme that limits the rate of β-oxidation, may be stimulated. As a result, CPT1A is suppressed in hypothyroidism mice whereas CPT1A mRNA and enzyme activity rise dramatically in the livers of hyperthyroidism animals. Additionally, TH can lower ApoB48 and ApoB100 synthesis, which in turn lowers VLDL and chylomicron (CM) production. Additionally, TH and ApoB48 levels have a negative correlation (Mugii *et al*., 2012).

Overall, TH lowering limits the production of cholesterol via HMGCR, but increases cholesterol absorption through NPC1L1 and decreases catabolism through β-oxidation.

On the surface of hepatocytes, the LDL receptor (LDLR) is a trans membrane glycoprotein that identifies lipoproteins carrying ApoB and facilitates the removal of cholesterol. By attaching itself to the thyroid-responsive element (TRE) of the LDLR gene on the surface of hepatocytes, TH can increase the production of LDLR mRNA. In the meanwhile, SREBP2 promotes the transcription of the LDLR gene by binding to the sterol regulatory element (SRE) on the LDLR promoter (Dong *et al.*,2014).

As a result, in hypothyroidism, the number of LDLR and LDL-C clearance rates dropped. In hypothyroidism, the combined effects of TH on cholesterol synthesis and clearance result in a net build-up of serum LDL-C.

TSH can, on the one hand, directly impact the production of cholesterol. Mice lacking the liver TSHR exhibited decreased TC levels, particularly in serum LDL-C (Zhou *et al.*, 2018).

Through the cAMP/PKA/CREB signaling pathway, the binding of TSH to the TSHR of the hepatocyte membrane increases the production and activity of HMGCR. Moreover, TSH can increase SREBP2 expression to control HMGCR (Liu *et al.*, 2015). TSHβ increases HMGCR mRNA levels in adipocytes and TSHβ expression levels in mice's serum. Adipocytes and blood cholesterol levels are intimately correlated (Moreno-Navarrete *et al.*, 2017).

Fatty acid mobilization expression and TSHβ gene expression have a favorable correlation as well (CAV1, ENGL1) (Comas *et al.*, 2019). In vitro experiments, it was discovered that TSH injection greatly raised circulation free fatty acid (FFA), phosphorylated perilipin, and hormone-sensitive lipase (HSL)

to enhance lipolysis. TSH may also promote the rise of ApoB (Beukhof *et al*., 2018). TSH, on the other hand, is crucial for the removal of LDL. In order to prevent the production of hepatic bile acids through TSHR, TSH triggers the signaling pathways for PI3K/AKT/SREBP2 and SREBP2/HNF4/Cholesterol 7α-hydroxylase (CYP7A1) (Song *et al*., 2015).

Serum TSH, regardless of TH, showed a substantial negative correlation with bile acid levels in individuals with hypercholesterolemia and hypothyroidism. It's interesting to note that patients under 65 had a greater correlation between TSH and total serum bile acid (Liu *et al*., 2014). Similar pathogenic processes of LDL-C buildup in hypothyroidism are shared by the rise in TSH and reduction in TH, both of which may stimulate the creation of cholesterol and impede clearance. Figure (2.4) illustrates how TH and TSH affect LDL-C in hypothyroidism.

While cholesterol production is inhibited by HMGCR when TH is reduced, cholesterol is still absorbed through NPC1L1, catabolism occurs through β-oxidation, and LDL-C clearance occurs through LDL-R. TSH may promote lipolysis and cholesterol production, but it also prevents cholesterol from being cleared on its own. LDL-C buildup results from the net synthesis of LDL-C and the inhibition of its clearance caused by the rise in TSH and reduction in TH.

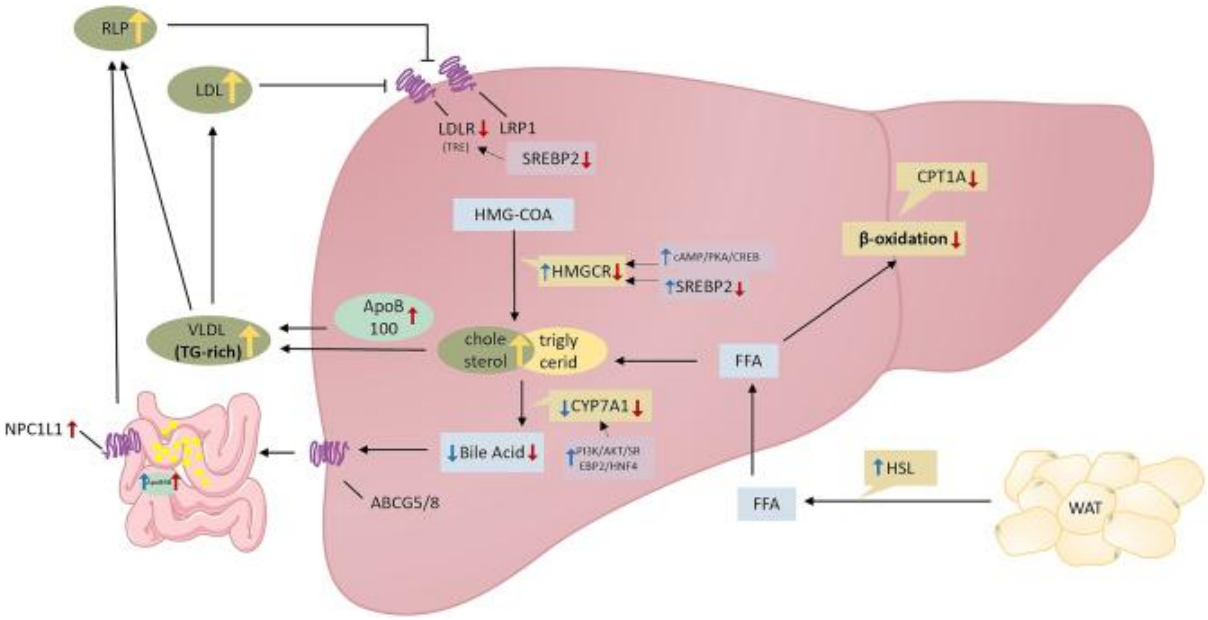


Figure (2.4) shows how elevated TSH affects LDL-C metabolism in hypothyroidism and lowered TH. When hypothyroidism is present, the changed functions are indicated (Liu *et al.*, 2022).

While cholesterol production is inhibited by HMGCR when TH is reduced, cholesterol is still absorbed through NPC1L1, catabolism occurs through β-oxidation, and LDL-C clearance occurs through LDL-R. TSH may promote lipolysis and cholesterol production, but it also prevents cholesterol from being cleared on its own. LDL-C accumulates as a result of the net synthesis of LDL-C and the inhibition of its clearance caused by the rise in TSH and reduction in TH (Liu *et al.*, 2022).

2.4.2 Hypothyroidism and hypertriglyceridemia

Hypothyroidism affects TG production and transformation

The source of TG is fat and circulating exogenous or intracellular FFAs from glycolysis. TH may decrease the liver's synthesis of VLDL-TG. Hypothyroidism will result in enhanced TG and reduced lipid oxidation rates when the rate of lipolysis is constant (Gjedde *et al.*, 2010).

Additionally, TH may raise hepatocyte ApoA5 mRNA and protein levels, which would cause TG to decrease. The primary action of TH is to increase the

activity of lipoprotein lipase (LPL), which may cause TRLs, such as CM and VLDL, to be lipolyzed. The reduction of TH attenuates these actions, which in turn encourages the elevation of serum TG in hypothyroidism. Research has indicated that the build-up of TRL in hypothyroidism patients may also be associated with decreased hepatic lipase (HL) activity (Mugii *et al.*, 2012).

TG to HDL translocation is impaired in individuals with subclinical hypothyroidism (Sigal *et al.*, 2011). ApoE-rich smaller particles, cholesterol, and CE make up remnant lipoprotein (RLP). TRL particles are digested by LPL, which causes them to gradually lose TG, phospholipid, ApoA, and ApoC before transferring to RLP.

Previous research has demonstrated a correlation between elevated blood RLP levels and hypothyroidism. One may argue that people with hypothyroidism have higher RLP levels because of the liver's overproduction of TRL particles. Conversely, LRP1 is surface-expressed on hepatocytes and binds to ApoE to internalize TRL and aid in the clearance of RLP. As shown in Figure (2.5), TH affects the lipid profile in both people and mice via upregulating LRP1 transcription (Moon *et al.*, 2013).

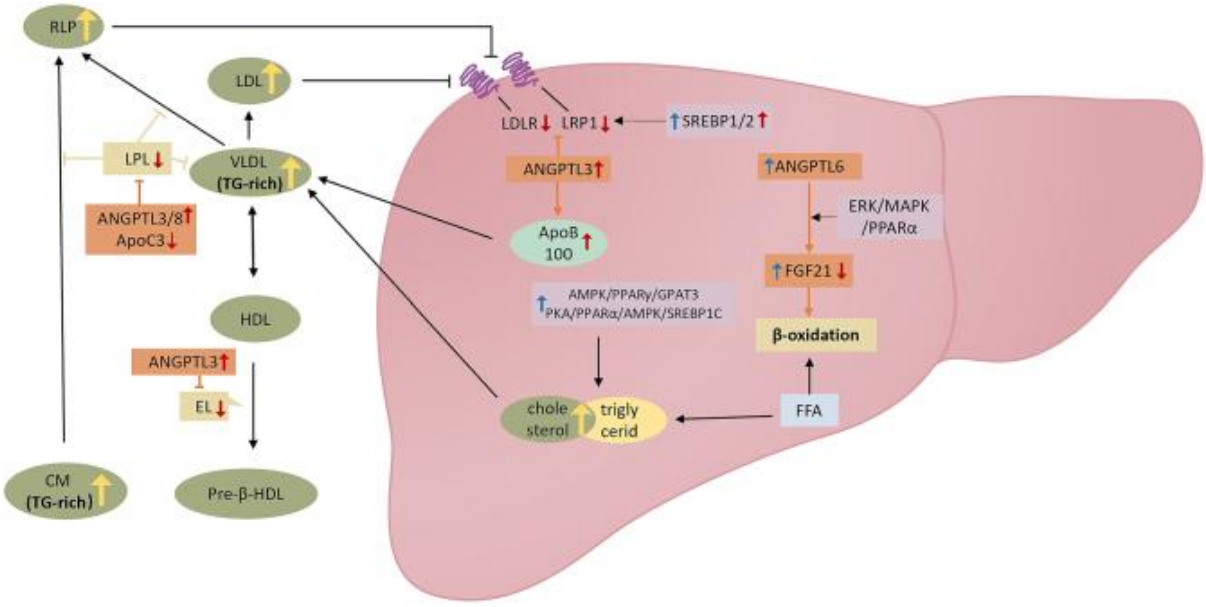


Figure (2.5) shows how elevated TSH affects TG metabolism in hypothyroidism and reduced TH (Moon *et al.*, 2013).

2.4.3 Hypothyroidism and HDL-C metabolism

Hypothyroidism causes a reduction in HDL production. In individuals with type 2 diabetes, a research found a favorable correlation between FT4 and plasma pre-β-HDL production (van Tienhoven-Wind *et al.*, 2016). The ApoA1 gene and protein expression are substantially induced by TH (Boone *et al.*, 2011).

Reverse cholesterol transport (RCT) increases cholesterol efflux from peripheral tissues to HDL as a result. In mice with hypothyroidism, homocysteine levels rise dramatically. Additionally, homocysteine can lower circulating HDL-C by blocking the formation of ApoA1, which in turn prevents RCT (Yang *et al.*, 2016).Nonetheless, following a thyroidectomy, ApoA1 levels rise in hypothyroidism patients (van der Boom *et al.*, 2020).

The contradicting outcome may have unclear causes at this point. Through the transporter protein ATP-binding cassette transporter A1, TH may also promote the efflux of cholesterol from macrophages to HDL (ABCA1) (Boone *et al.*, 2011).

In hypothyroidism, the HDL clearance and transformation process are reduced. By stimulating HL, TH encourages HDL breakdown and modifies the components of HDL. In hypothyroidism, plasma concentrations of the cholesterol transport protein transporter (CETP) drop, leading to higher levels of plasma HDL-C (McGowan *et al.*, 2016).

Thyroid Hormone may accelerate the conversion of cholesterol to bile acid by increasing the transcription of CYP7A1, an enzyme that limits the pace of RCT. Additionally, TH may increase the production of bile acid in the liver and intestine by inducing the last stage of an RCT in rats: the transcription of the ATP-binding cassette transporter G5/8 (ABCG5/ABCG8) (Bonde *et al.*, 2012).

Because hypothyroidism reduces scavenger receptor b1, it may hinder the elimination of cholesterol. The HDL-C level is not constant because of these TH-induced counteracting effects on HDL production and clearance. HDL2 and HDL3 levels were higher in patients with a modest elevation in TSH due to diminished CETP and phospholipid transfer protein activity (Skoczyńska *et al.*,2016).

Nonetheless, TSH and CETP have a positive association in T2DM patients, suggesting that hyperglycemia may have a significant impact on VLDL's capacity to absorb cholesteryl esters from HDL (Triolo *et al.*, 2013).

HDL-C levels, therefore, may be impacted by several factors. Figure (2.6) displays the precise effects of TSH and TH on HDL levels.

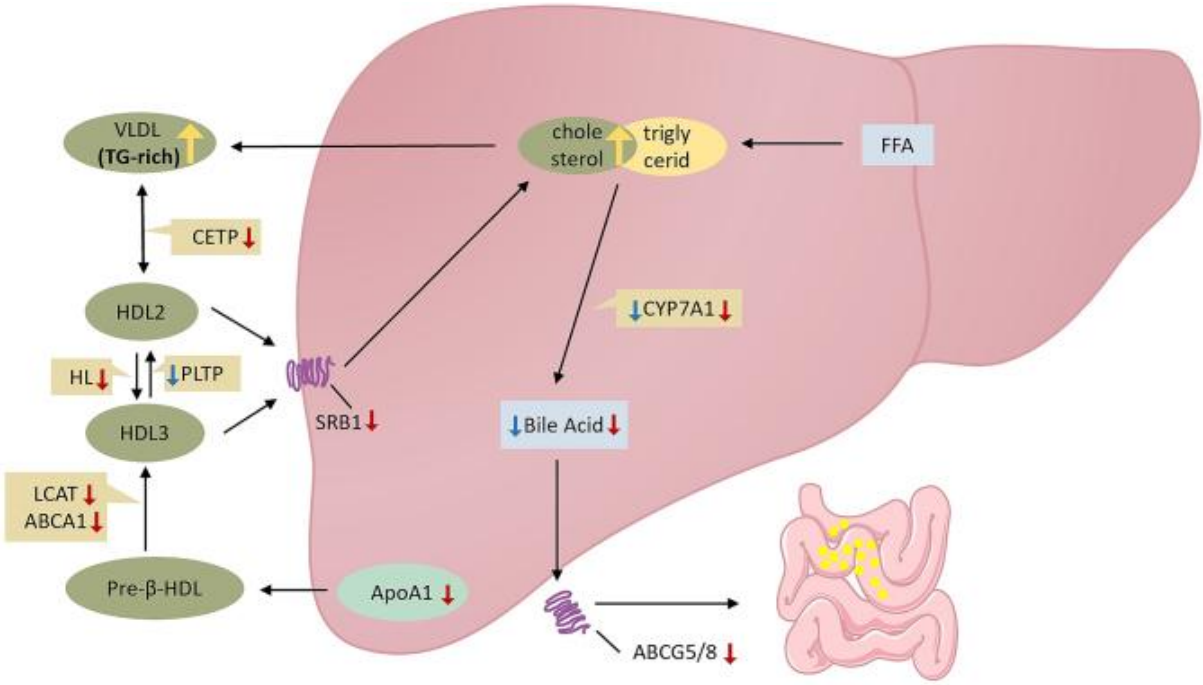


Figure (2.6) Effects on HDL metabolism in hypothyroidism of elevated TSH and reduced TH (Bonde *et al.*, 2012).

2.5 Diagnostic markers for hypothyroidism

To determine the thyroid gland's anatomical and functional condition, a variety of clinical, instrumental, and laboratory research techniques are

employed. These techniques make it possible to identify thyroid gland disorders and assess the efficacy (dynamics) of therapy. (Soyka *et al*., 2024).

The best laboratory test for assessing thyroid function in an outpatient setting is the serum TSH test. If TSH levels are high, serum free thyroxine (T4) levels should be measured. Overt primary hypothyroidism is recognized when serum free T4 levels are low and TSH levels are high. If a patient's blood TSH levels are elevated yet their serum free T4 levels are normal (often > 4.0 mIU/l), they are diagnosed with subclinical hypothyroidism (SH). TSH screening should frequently be performed again 1-3 months prior to a hypothyroidism diagnosis in situations of subclinical hypothyroidism. More study on hypothalamic-pituitary insufficiency is necessary since low blood TSH and serum free T4 levels are suggestive of secondary hypothyroidism. If there is a clearly low serum T4 level and an incorrectly normal or even slightly but disproportionately elevated TSH, it should be investigated further. Under these conditions, hypothyroidism cannot be detected by a simple blood TSH measurement (Hasan *et al*., 2024)

Thyroid-stimulating hormone (TSH) rises in primary hypothyroidism (PHT) because of the thyroid gland producing insufficient thyroid hormone. (Pappan *et al*., 2024).

According to guidelines, people with normal thyroid function fall between the 0.4–4.0 mIU/mL reference and therapeutic goal range for TSH (Jonklaas *et al*., 2014). TSH levels above the reference range and normal thyroid hormone levels are indicative of subclinical hypothyroidism; on the other hand, low TSH and normal thyroid hormone levels are indicative of subclinical hyperthyroidism. The majority of laboratories only detect FT4 in cases where TSH is outside of normal range.

Restricting measures of free T4 (FT4) to individuals whose blood TSH was obviously outside its reference range (<0.2 mIU/L or >6 mIU/L) had little to no

effect on the TSH test's diagnostic value, according to a large observational research from Australia (Henze *et al.*, 2017).

While testing for the thyroid peroxidase (TPO) antibodies may not aid in the diagnosis of hypothyroidism, a positive test result may indicate an autoimmune cause (Godbole *et al.*, 2023). Thyroid cancer and structural alterations can be detected early using ultrasound. (Yang *et al.*, 2023).

2.6 Proposed biomarker for diagnostic

2.6.1 Micro RNAs-590

miRNAs are 20–24 nucleotide short, non-coding RNA molecules that are involved in a variety of biological activities(Benavides-Aguilar *et al.*,2023).

MiR-590 belongs to the microRNA family. Its gene is localized at 7q11.23, as shown in Figure (2.7). The body contains mature versions of mir-590-3p and mir-590-5p, which combine various target genes to execute distinct tasks (Yang Dong *et al.*,2017).

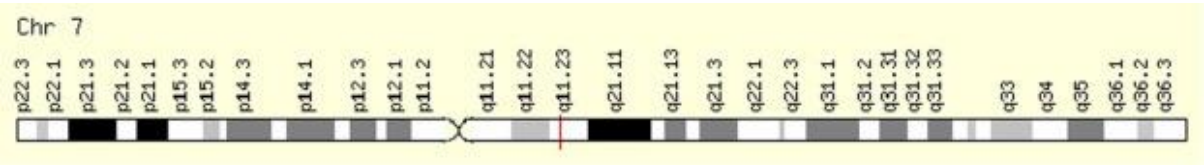


Figure (2.7). The gene is Micro RNAs-590 location (Yang Dong *et al.*,2017).

According to reports, miR-590 is crucial for cell division, proliferation, and tumor development. This suggests that miR-590 is involved in a variety of physiological processes, such as the control of health and the occurrence of illness.

By targeting LPL, miR-590 can decrease the buildup of lipids in human THP-1 macrophages and increase the release of inflammatory cytokines (He *et al.*, 2014).

Additional research has demonstrated that miR-582-5p and miR-590-5p, which target the CREB1/CREB5-NF-κB signaling pathway in monocytes, are essential factors in immunosuppression induced by opioids (Long *et al.*, 2016).

Research has shown that there are differences in miRNA expression between thyroid cancer and healthy thyroid tissue, as well as between different subtypes of thyroid cancer. For thyroid cancer, a number of miRNAs have been identified as possible prognostic indicators and therapeutic targets (Agarwal *et al.*, 2021).

Future innovative approaches to diagnosis and treatment may be based on a deeper comprehension of the biological function that microRNAs play in the development of thyroid disorders. Since miRNAs are a unique type of noncoding RNAs that negatively, affect gene expression post-transcriptionally. It is predicted that miRNAs will be extremely useful for both diagnosis and therapy.

2.6.2 Ghrelin

The hormone ghrelin is peptide consist of 28-amino acid, is primarily secreted by the stomach oxyntic glands and functions via the ghrelin receptor (GhrR), a G-protein coupled receptor (Sivertsen *et al.*, 2013).

There are two different types of ghrelin: des-acyl ghrelin (nonoctanoylated form) and acyl ghrelin (octanoylated form). 20% of ghrelin is discovered to be octanoylated at the third carbon, and octa-noylation is essential for its physiological activities, which rely on ghrelin O-acyltransferase (GOAT) catalyzed activity (Du *et al.*, 2018). as in Figure (2.8)

The anonoctanoylated, inactive form of des-acyl ghrelin does not trigger the growth hormone secretagogue receptor, which is the receptor's target for the release of growth hormone (GH) in the acyl form. It is recently established that des-acyl ghrelin is has separate physiological functioning (Sominsky *et al.*, 2019).

Functioning ghrelin receptors (GHS-R) are found in many different tissues, including lymphoid organs. This shows that the immune system and systemic metabolism are linked in response to various physiological and pathological situations (Pereira *et al*., 2017).

The ghrelin gene on the third chromosome encodes it. Genes that code for ghrelin and its receptors may have similar genetic variants that impact polygenic obesity sensitivity (Hosoda , 2022).

The only peptide hormone that has been modulated by a fatty acid is ghrelin. Endocrine X/A-like cells of the fundus mucosa, comprising about 20% of human stomach mucosal cells, produce ghrelin (Rindi *et al*., 2002).

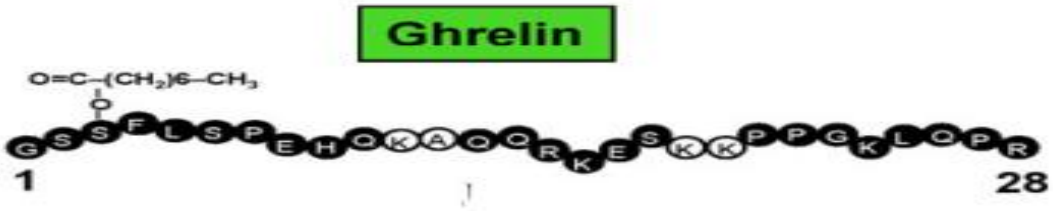


Figure (2.8) The structure of ghrelin. Ghrelin is a 28-amino-acid peptide with an n-octanoyl group attached to serine at position 3 that is critical for the majority of its activity (Yang *et al*., 2008).

Before meals, ghrelin (hunger hormone) is released by the enteroendocrine cells in the gastrointestinal system, mainly the stomach, to promote food intake and gastric acid secretion. It serves a variety of physiological operations, including as promoting hunger, causing fat to buildup, and releasing growth hormone (Mhaibes *et al*., 2021) as presented in Figure (2.9)

The blood-brain barrier enables ghrelin, which gets released by the stomach, to pass through the brain (Venkova *et al*., 2008). And the vagal nerve is another pathway by which ghrelin sends its signal (Date ,2012).

Therefore, ghrelin contributes to regulating energy balance, enhancing food intake, and decreasing energy expenditure (Emami *et al*., 2014).

In the paraventricular nucleus, ghrelin has inhibitory effects on thyroid hormone control (Ahangarpour *et al.*, 2016). In vivo studies conducted on rats demonstrating a decrease in thyrotropin-releasing hormone (TRH), TSH, triiodothyronine (T3) hormone, and T4 following ghrelin injection (Barington *et al.*, 2017).

Analogous research conducted on people proves the ghrelin's suppressive effect on TSH plasma levels (Kordi *et al.*, 2015).

It is currently believed that ghrelin regulates lipid accumulation in white adipose tissue (WAT) in a major way. Prolonged exposure to ghrelin results in increased fat mass even if acute ghrelin exposure also causes GH production. It has been shown that ghrelin increases adipogenesis, increases the activity of fat storage enzymes, increases the level of triglycerides, and decreases fat utilisation/lipolysis (Perez-Tilve *et al.*, 2011).

In addition, different tissues including the kidneys, adrenal glands, thyroid, breast, ovary, placenta, testis, prostate, liver, gallbladder, lung, skeletal muscles, myocardium, skin, and bone have ghrelin (Ghelardoni *et al.*, 2006)

Given that variations in hunger and body weight are characteristic of thyroid problems, it makes essential to evaluate how serum ghrelin levels vary between thyroid dysfunction stages and investigate the potential involvement of ghrelin in hunger changes in these individuals (Sadegh *et al.*, 2007).

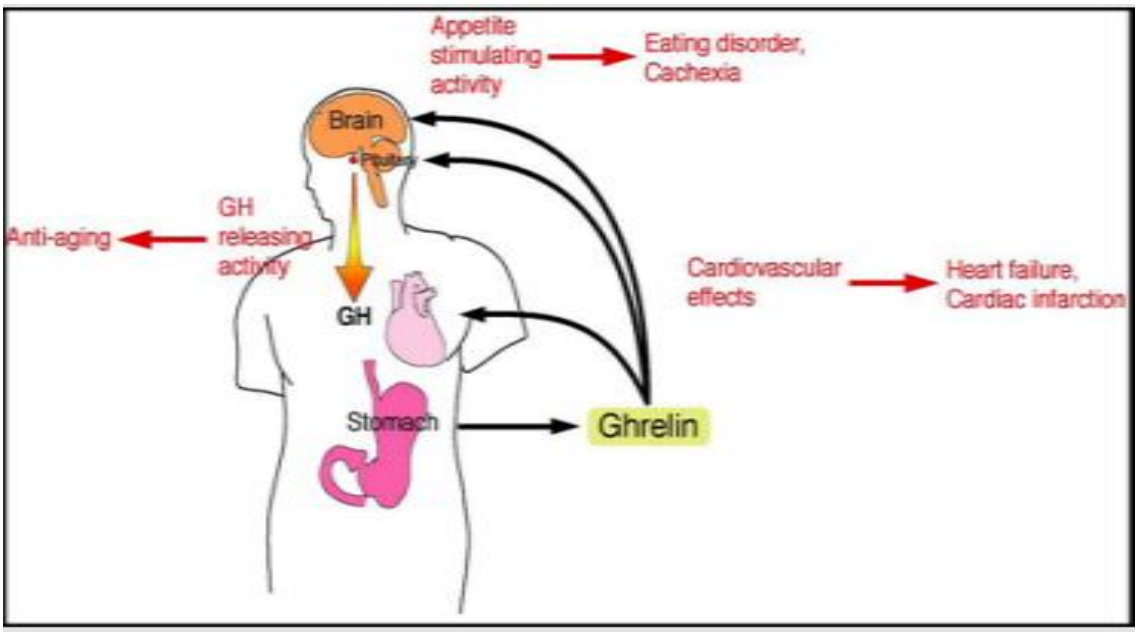


Figure (2.9). The several roles of ghrelin (Basuny *et al*,. 2020)

2.6.3 Obestatin

Obestatin is a 23-amino acid that is C-terminally amidated as in figure (2.10). Despite its short biological half-life and quick degradation, obestatin may have a variety of pathogenic effects (Cowan *et al*., 2016)

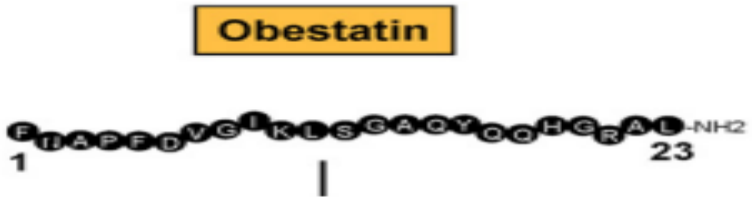


Figure (2.10) Peptide structure of human obestatin (Zhang et al ,.2005)

According to Volante *et al*., obestatin has been found in the thyroid, pituitary, lung, pancreas, and gastrointestinal tract in embryonic human tissue samples. The expression of the obestatin protein in adult human tissues is limited to the pancreas, gastrointestinal system, lung, and pituitary. On the other hand, obestatin is expressed in a tiny percentage of pancreatic, thyroid, parathyroid, and gastrointestinal neoplasms in endocrine tumors (Volante *et al*., 2009).

According to reports, obesatin promotes cell survival, gastrointestinal motility, and body weight increase. It also inhibits the intake of food and fluids and prevents apoptosis. By upregulating genes linked to beta cell regeneration, adipogenesis, and insulin synthesis, obestatin promoted adipogenesis, increased beta cell mass, and improved lipid metabolism (Yu *et al*., 2020).

Obestatin is a physiological competitor of ghrelin and is encoded by the same gene as ghrelin. It has been demonstrated that ghrelin, an endogenous growth hormone secretagogue receptor, plays a role in controlling pituitary hormone secretion and maintaining energy balance. (Wu *et al*., 2018).

A summary of the reported pathophysiological effects of obestatin was shown in Figure (2.11).

The selectivity and efficacy of the signals are regulated by the biological mechanisms of obestatin, which link the activated GPR39 receptor with different sets of effector proteins through both G-protein-dependent and G-protein-independent pathways. The complex pathways involving Extracellular signal –regulated kinases (ERK1/2) and JunD axis are activated in a G-protein-dependent manner, which determines the obestatin-associated mitogenic effect. The initiation and advancement of obestatin-dependent differentiation via a kinase hierarchy regulated by the Akt, CAMKII, c-Jun, and p38 axis are dictated by the transactivation of epidermal growth factor receptor (EGFR) via the β-arrestin signal complex (Santos-Zas *et al*., 2016).

As the thyroid gland function is linked to the regulation of energy balance, it is important to take into account the interaction between ghrelin, obestatin, and thyroid function (Ruchala *et al*., 2014).

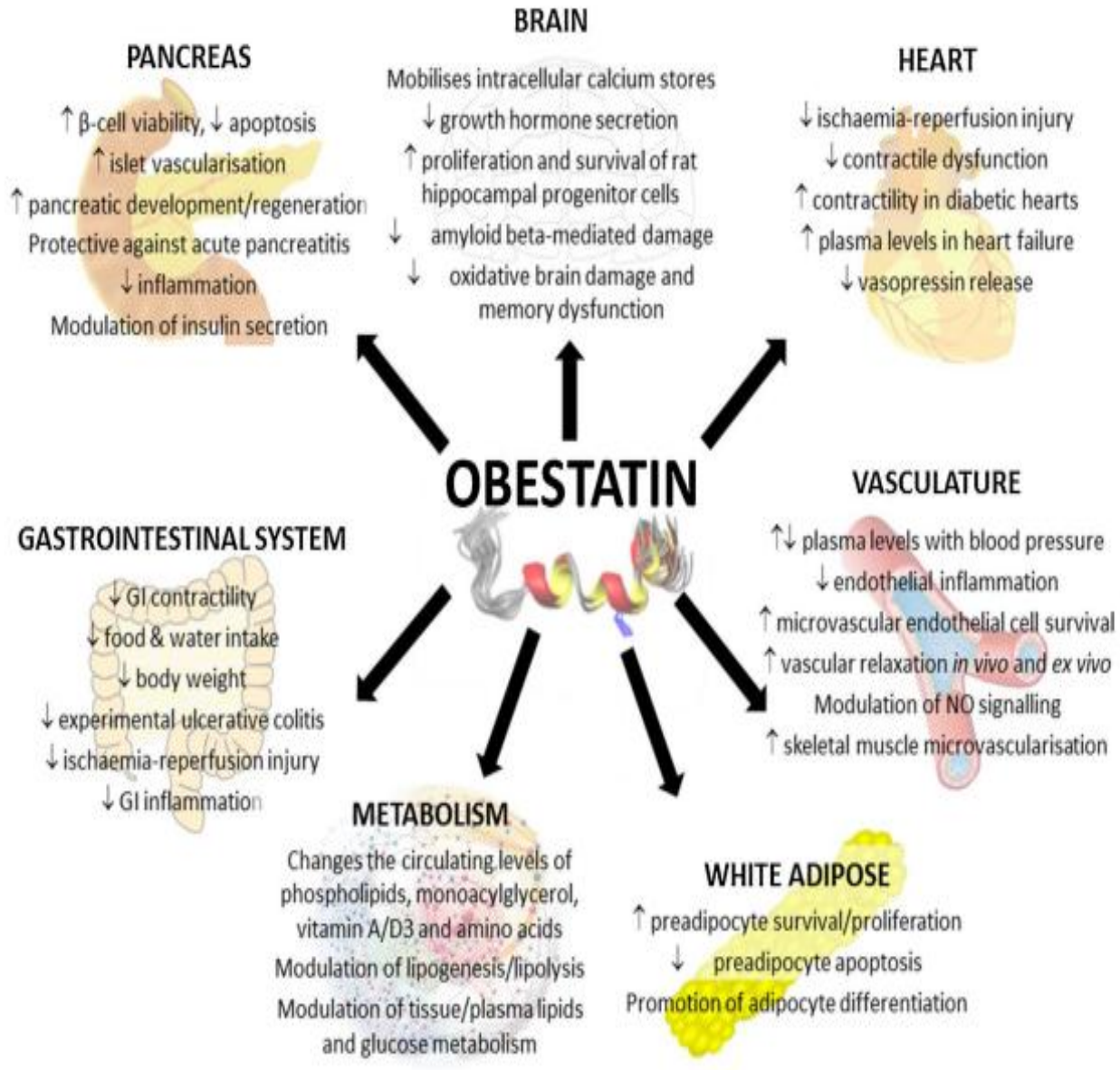


Figure (2.11) A summary of the reported pathophysiological effects of obestatin. The effects of obestatin on biology seem to be varied. It is stated that obestatin affects metabolism generally as well as the heart, vascular, brain, pancreas, white adipose tissue, and gastrointestinal tract (Green *et al.*, 2018)

Chapter Three
Materials and Methods

3. Material &Methods

Includes:

3.1. Study design

The present study included a case control study for a group of (90) samples as presented in figure(3.1) .Patients with hypothyroidism were selected from Hillah teaching hospital and Marjan Hospital, Health Directorates / Babylon – Iraq.

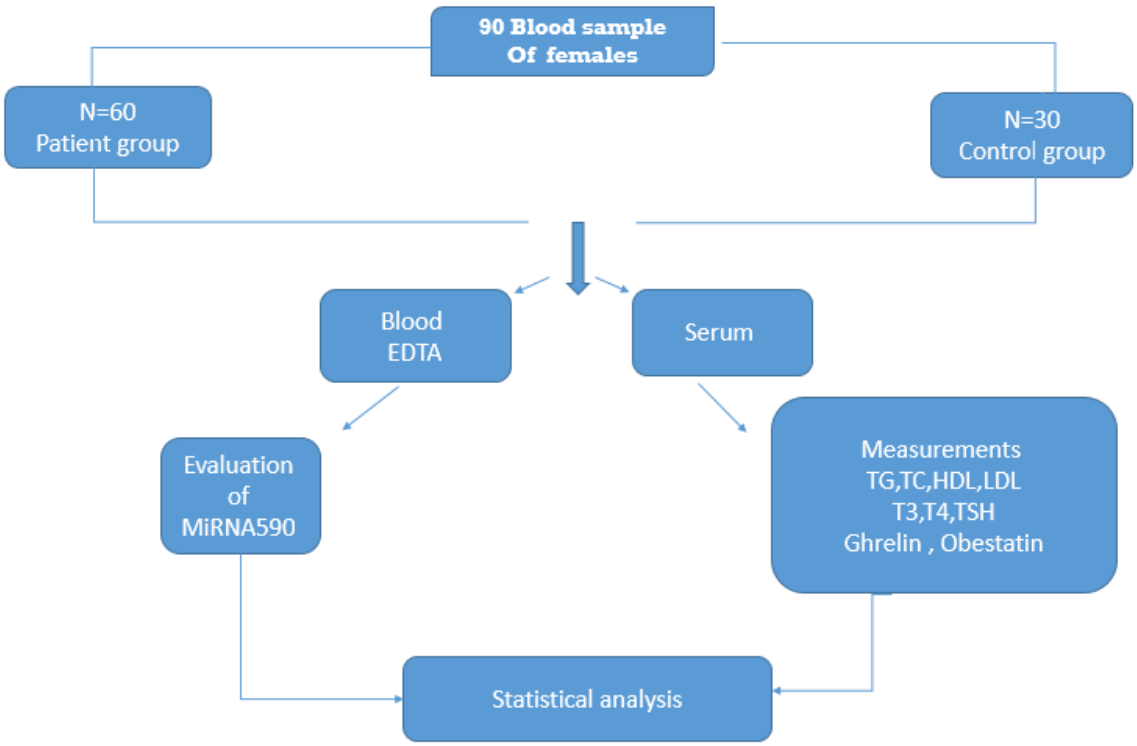


Figure (3.1) study design

3.2. Data collection: Demographic information, including age, disease duration, concurrent diseases, combined medication, and detailed medical history, was collected. All the subjects were carefully evaluated by endocrinology specialist.

3.3. Instruments

In this chapter, materials, and instruments were described and listed in Tables (3-1) and (3-2)

Table (3-1): The Tools & instruments used in the study:

NO	equipment	Company	Suppliers
1	gel tube	CHINA MHECO	CHINA
2	EDTA tube	CHINA MHECO	CHINA
3	Pipette(100-1000µl)	MICROLIT	UNITED STATES
4	Micropipette(10-100 µl)	MICROLIT	UNITED STATES
5	Eppendroff tubes	CHINA MHECO	CHINA
6	Tips, 100µl -1000µl (blue)	CHINA MHECO	CHINA
7	Centrifuge	HETTICH	GERMANY
8	Deep freeze	BADDA	LEBANON
9	Real time PCR	ANALYTIK JENA	GERMANY
10	ELISA system	LABTECH	GERMANY

Table (3-2): The materials used in the study:

NO	Materials	company
1	Human ghrelin Elisa kit	Elabscience\USA
2	Human obestatin Elisa kit	Elabscience\USA
3	Micro RNA kit	Macrogen /Seoul Korea

3.4 Patients Criteria

All patients were subjected to a complete clinical history and relevant laboratory tests that diagnose clinical cases of thyroid disorders. They were also subjected to the latest clinical guidelines by the WHO and the type of disorder was determined based on laboratory values and clinical evaluation measurements of the disorders

Exclusion criteria:

- **Pregnancy:** Pregnancy can significantly alter thyroid hormone levels and their interpretation.
- **Recent iodine exposure:** Recent iodine exposure, such as from contrast agents used in medical imaging, can interfere with thyroid function tests.
- **Acute illness:** Acute illnesses can temporarily affect thyroid hormone Levels, making it difficult to accurately assess baseline thyroid status.
- **History of thyroid surgery or radioactive iodine treatment:** These

individuals may have altered thyroid function due to the treatment itself, making it difficult to interpret results.

- **History of other endocrine disorders:** Other endocrine disorders can affect thyroid hormone levels
- **Inability to provide informed consent:** Individuals who are unable to understand or provide informed consent for the study should be excluded.

3.5 Control Criteria

Blood samples were obtained from 30 healthy individuals without thyroid disorder and chronic diseases, considering the age and gender matching with the patients' group, all of them underwent questionnaires, laboratory investigations.

3.6 Study variables

Includes

3.6.1. Dependent Variable

MicroRNA590, Ghrelin, Obestatin, Thyroid hormones (T3, T4), TSH

3.6.2. Independent Variable

Age, BMI

3.7. Approval of the Ethical Committee

A valid written, signed consent was achieved from the hospitals administration and a valid verbal consent from each patient and control subject before their inclusion in the study. The procedure had been informed before the samples were collected, making absolutely sure that they understood the procedure that was to be carried out. The subjects were sentient that they had the right to reject to be included in the study without any detrimental effects. Permission was taken from all subjects of the control group after they were told about the aim and advantages of this study. The Ethical Committee of Kerbala science College approved the protocol of the study.

3.8. Measurement and Data Collection

3.8.1. Data collection

A structured questionnaire was specifically design to obtained information which helps to select individuals according to the selection criteria of the study. Sociodemographic aspects of the subjects (patients and control) were also collected through the self-reported technique (questionnaire) which including: age, BMI, chronic disease, duration of disease (for patient), family history of disease.

3.8.2. Blood collection and storage

Five ml of blood was taken and 1ml of it was placed in EDTA tube to store it in deep freeze (-20c) and other 4ml was placed in gel tube to centrifugate for 10minutes at approximately 4000xg. After the serum is separated divided into two Eppendorf tubes and stored until it is used.

3.9. Methods

3.9.1 Measurement of Body Mass Index

The equation determined the body mass index (BMI): a person's weight in kilograms (*Kg*)was divided by the square of the person's height in meters (m^2) using appropriate balance as in the equation below:

BMI = Kg/m^2

The body mass index was classified according to the world health organization (WHO) in Table 3.3. (Seo *et al.*, 2019)

Table (3.3) BMI Status

N	BMI	Unit	Classify by BMI status
1	Below 18.5	Kg/m^2	Underweight
2	18.5-24.9	Kg/m^2	Normal Weight
3	25.0-29.9	Kg/m^2	Pre-obesity
4	30.0-34.9	Kg/m^2	Obesity Class-1
5	35.0-39.9	Kg/m^2	Obesity Class-2

6	Above 40	Kg/m^2	Obesity Class-3

3.10. Measurement of Ghrelin level

Enzyme-linked immunosorbent assay system (ELISA) method was used to measure concentration of serum GHRL.

Test principle

This ELISA kit uses the Competitive-ELISA principle. The micro ELISA plate provided in this kit has been pre-coated with Human GHRL. During the reaction, Human GHRL in samples or Standard competes with a fixed amount of Human GHRL on the solid phase supporter for sites on the Biotinylated Detection Ab specific to Human GHRL. Excess conjugate and unbound sample or standard are washed from the plate, and Avidin conjugated to Horseradish Peroxidase(HRP) are added to each microplate well and incubated. Then a TMB substrate solution is added to each well. The enzyme-substrate reaction is terminated by the addition of stop solution and the color change is measured spectrophotometrically at a wavelength of 450±2 nm. The concentration of Human GHRL in the samples is then determined by comparing the OD of the samples to the standard curve

Kit components & Storage. An unopened kit can be stored at 2-8°C for 1 week. If the kit is not supposed to be used within 1 week, store the items separately according to the following conditions once the kit is received

Table (3-4): Reagents of determination ghrelin concentration.

Item	specification
Micro ELISA Plate (Dismountable)	96T: 8 wells ×12 strips 48T: 8 wells ×6 strips 24T: 8 wells ×3 strips 96T*5: 5 plates, 96T
Reference Standard	96T: 2 vials 48T/24T: 1 vial 96T*5: 10 vials
Concentrated Biotinylated Detection Ab)100(×	96T: 1 vial, 120 µL 48T/24T: 1 vial, 60 µL 96T*5:5 vials, 120µl
Concentrated HRP Conjugate)100(×	96T: 1 vial, 120 µL 48T/24T: 1 vial, 60 µL 96T*5: 5 vials, 120 µL
Reference Standard & Sample Diluent	96T/48T/24T: 1 vial, 20mL 96T*5: 5 vials, 20 mL
Biotinylated Detection Ab Diluent	96T/48T/24T: 1 vial, 14mL 96T*5: 5 vials, 14 mL
HRP Conjugate Diluent	T/48T/24T: 1 vial, 14mL 96 96T*5: 5 vials, 14 mL
Concentrated Wash Buffer) 25(×	96T/48T/24T: 1 vial, 30mL 96T*5: 5 vials, 30 mL
Substrate Reagent	96T/48T/24T: 1 vial, 10mL 96T*5: 5 vials, 10 mL
Stop Solution	96T/48T/24T: 1 vial, 10mL 96T*5: 5 vials, 10 mL
Plate Sealer	96T/48T/24T: 5 pieces 96T*5: 25 pieces
Product Description	1copy
Certificate of Analysis	1copy

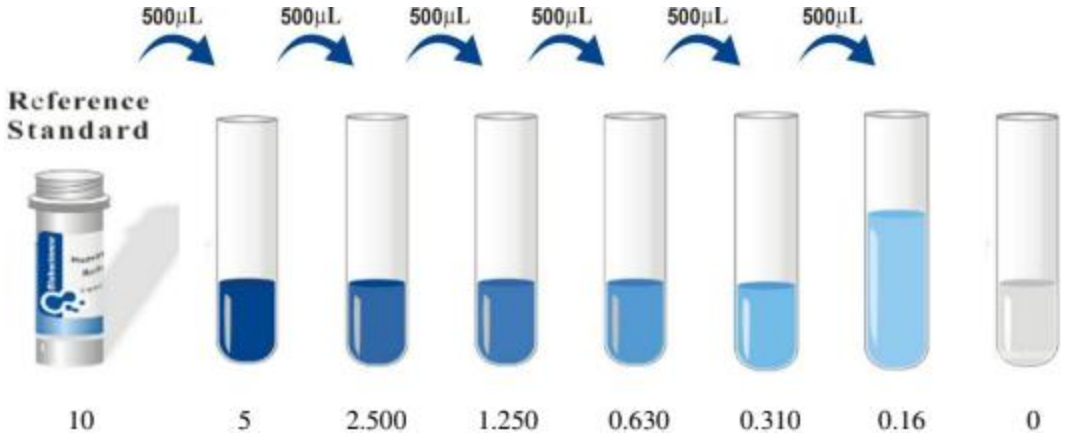
3.10.1 Reagent preparation

Bring all reagents to room temperature (18-25°C) before use. If the kit will not be used up in one assay, please only take out the necessary strips and reagents for present experiment, and store the remaining strips and reagents at required condition

Dilution method: Take 7 EP tubes, add 500uL of Reference Standard & Sample Diluent to each tube. Pipette 500uL of the 10 ng/mL working solution to the first tube and mix up to produce a 5 ng/mL working solution. Pipette 500uL of

the solution from the former tube into the latter one according to this step. The illustration below is for reference.

Note: the last tube is regarded as a blank. Don't pipette solution into it from the former tube. Gradient diluted standard working solution should be prepared just before use



Assay procedure

1-Determine wells for diluted standard, blank and sample. Add 50 μL each dilution of standard, blank and sample into the appropriate wells (It is recommended that all samples and standards be assayed in duplicate. It is recommended to determine the dilution ratio of samples through preliminary experiments or technical support recommendations).Immediately add 50 μL of Biotinylated Detection Ab working solution to each well. Cover the plate with the sealer provided in the kit. Incubate for 45 min at 37°C. Note: solutions should be added to the bottom of the micro ELISA plate well, avoid touching the inside wall and causing foaming as much as possible

2-Decant the solution from each well, add 350 μL of wash buffer to each well. Soak for 1 min and aspirate or decant the solution from each well and pat it dry against clean absorbent paper. Repeat this wash step 3 times. Note: a microplate washer can be used in this step and other wash steps. Make the tested strips in use immediately after the wash step. Do not allow wells to be dry

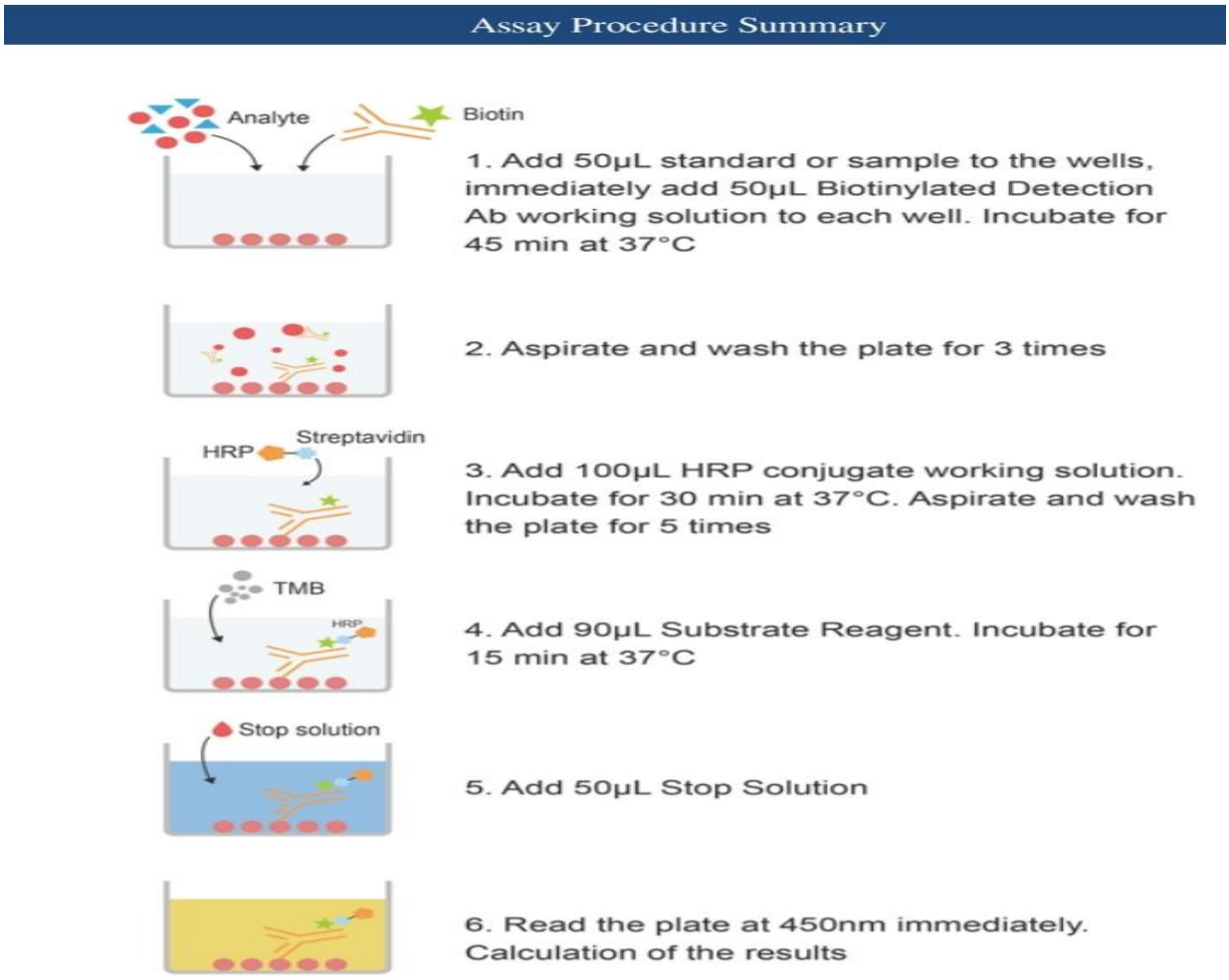
3-Add 100 μL of HRP Conjugate working solution to each well. Cover the plate with a new sealer. Incubate for 30 min at 37c

4-Decant the solution from each well, repeat the wash process for 5 times as conducted in step 2

5-Add 90 µL of Substrate Reagent to each well. Cover the plate with a new sealer. Incubate for about 15 min at 37C. Protect the plate from light. Note: the reaction time can be shortened or extended according to the actual color change, but not more than 30 min. Preheat the Microplate Reader for about 15 min before OD measurement

6-Add 50 µL of Stop Solution to each well. Note: adding the stop solution should be done in the same order as the substrate solution

7-Determine the optical density (OD value) of each well at once with a micro-plate reader set to 450 nm



3.10.2 Calculation of result

Average the duplicate readings for each standard and samples. Plot a four parameter logistic curve on log-log graph paper, with standard concentration on the x-axis and OD values on the y-axis If the OD of the sample under the lowest limit of the standard curve, you should re-test it with an appropriate dilution.The actual concentration is the calculated concentration multiplied by the dilution factor

3.11 Measurement of obestatin level

This ELISA kit applies to the in vitro quantitative determination of Human OB concentrations in serum, plasma and other biological fluids

Test principle

This ELISA kit uses the Competitive-ELISA principle. The micro ELISA plate provided in this kit has been pre-coated with Human OB. During the reaction, Human OB in samples or Standard competes with a fixed amount of Human OB on the solid phase supporter for sites on the Biotinylated Detection Ab specific to Human OB.

Excess conjugate and unbound sample or standard are washed from the plate, and Avidin conjugated to Horseradish Peroxidase (HRP) are added to each microplate well and incubated. Then a TMB substrate solution is added to each well. The enzyme-substrate reaction is terminated by the addition of stop solution and the color change is measured spectrophotometrically at a wavelength of 450±2 nm. The concentration of Human OB in the samples is then determined by comparing the OD of the samples to the standard curve

Kit components & Storage

An unopened kit can be stored at 2-8°C for 1 week. If the kit is not supposed to be used within 1 week, store the items separately according to the following conditions once the kit is received

Table (3-5): Reagents of determination obestatin concentration .

item	specification
Micro ELISA Plate (Dismountable)	96T: 8 wells ×12 strips 48T: 8 wells ×6 strips 24T: 8 wells ×3 strips 96T*5: 5 plates, 96T
Reference Standard	96T: 2 vials 48T/24T: 1 vial 96T*5: 10 vials
Concentrated Biotinylated Detection Ab)100(×	96T: 1 vial, 120 µL 48T/24T: 1 vial, 60 µL 96T*5: 5 vials, 120 µL
Concentrated HRP Conjugate) 100(×	96T: 1 vial, 120 µL 48T/24T: 1 vial, 60 µL 96T*5: 5 vials, 120 µL
Reference Standard & Sample Diluent	96T/48T/24T: 1 vial, 20mL 96T*5: 5 vials, 20 mL
Biotinylated Detection Ab Diluent	96T/48T/24T: 1 vial, 14 96T*5: 5 vials, 14 mL
HRP Conjugate Diluent	96T/48T/24T: 1 vial, 14mL 96T*5: 5 vials, 14 mL
Concentrated Wash Buffer) 25(×	96T/48T/24T: 1 vial, 30mL 96T*5: 5 vials, 30 mL
Substrate Reagent	96T/48T/24T: 1 vial, 10mL 96T*5: 5 vials, 10 mL
Stop solution	96T/48T/24T: 1 vial, 10mL 96T*5: 5 vials, 10 mL
Plate Sealer	96T/48T/24T: 5 pieces 96T*5: 25 pieces
Product Description	1copy
Certificate of Analysis	1copy

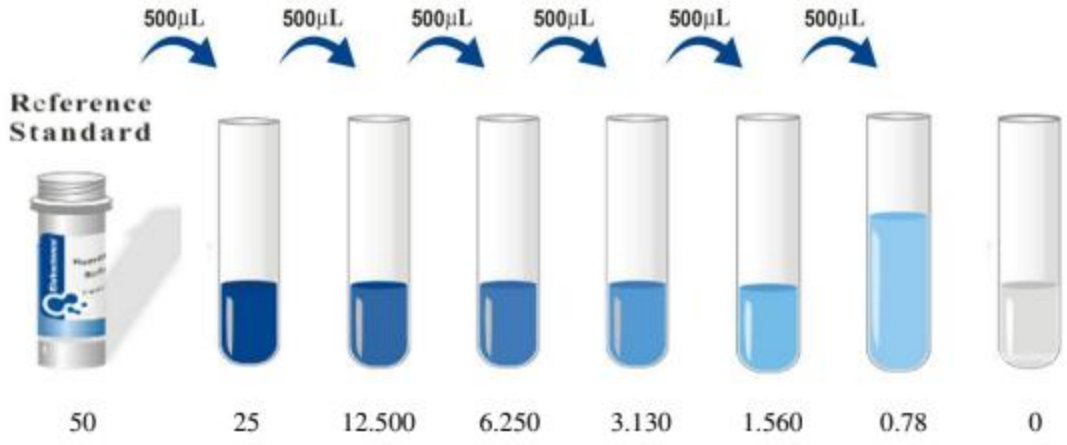
3.11.1 Reagent preparation

Bring all reagents to room temperature (18-25°C) before use. If the kit will not be used up in one assay, please only take out the necessary strips and reagents for present experiment, and store the remaining strips and reagents at required condition

Dilution method: Take 7 EP tubes, add 500uL of Reference Standard & Sample Diluent to each tube. Pipette 500uL of the 50 ng/mL working solution to

the first tube and mix up to produce a 25 ng/mL working solution. Pipette 500uL of the solution from the former tube into the latter one according to this step. The illustration below is for reference.

Note: the last tube is regarded as a blank. Don't pipette solution into it from the former tube. Gradient diluted standard working solution should be prepared just before use



3.11.2 Assay procedure

1-Determine wells for diluted standard, blank and sample. Add 50 μL each dilution of standard, blank and sample into the appropriate wells (It is recommended that all samples and standards be assayed in duplicate. It is recommended to determine the dilution ratio of samples through preliminary experiments or technical support recommendations).Immediately add 50 μL of Biotinylated Detection Ab working solution to each well. Cover the plate with the sealer provided in the kit. Incubate for 45 min at 37°C. Note: solutions should be added to the bottom of the micro ELISA plate well, avoid touching the inside wall and causing foaming as much as possible

2-Decant the solution from each well, add 350 μL of wash buffer to each well. Soak for 1 min and aspirate or decant the solution from each well and pat it dry against clean absorbent paper. Repeat this wash step 3 times. Note: a

microplate washer can be used in this step and other wash steps. Make the tested strips in use immediately after the Wash step. Do not allow wells to be dry

3-Add 100 µL of HRP Conjugate working solution to each well. Cover the plate with a new sealer. Incubate for 30 min at 37°C

4- Decant the solution from each well, repeat the wash process for 5 times as conducted in step 2

5- Add 90 µL of Substrate Reagent to each well. Cover the plate with a new sealer. Incubate for about 15 min at 37C. Protect the plate from light. Note: the reaction time can be shortened or extended according to the actual color change, but not more than 30 min. Preheat the Microplate Reader for about 15 min before OD measurement

6- Add 50 µL of Stop Solution to each well. Note: adding the stop solution should be done in the same order as the substrate solution

7- Determine the optical density (OD value) of each well at once with a micro-plate reader set to 450 nm.

3.11.3 Calculation of results

Average the duplicate readings for each standard and samples. Plot a four parameter logistic curve on log-log axis, with standard concentration on the x-axis and OD values on the y-axis If the OD of the sample under the lowest limit of the standard curve, you should re-test it with an appropriate dilution

The actual concentration is the calculated concentration multiplied by the dilution factor

3.12 Molecular Analyses

Includes:

3.12.1 Micro RNA analysis

The TransZol Up Plus RNA Kit (Cat.NO.ET101-01) was used for total RNA extraction according to the manufacturer's instructions. Total RNA was purified using an RNAClean XP Kit and RNase-Free DNase Set.

TransZol lyses cell with guanidine isothiocynate.in the process of sample lysis,TransZol can maintain the integrity of RNA.after adding RNA extraction agent,the solution is divided into a colorless aqueous phase and a pink organic phase.RNA is in the aqueous phase. RNA can be recovered by precipitation with isopropanol.isopropyl alcohol recovers protein.suitable for rapid extraction of total RNA from a variety of tissue and cells.

Table (3.6) RNA analysis Kit contents

compenent	ET101-01
TransZol up	100ml
RNA Extraction Agent	20ml
RNA Dissolving Solution	15ml

3.12.2 Extraction of microRNAs from the blood

All reagents were brought to the room temperature (25ºC) for 30 minutes before each run. For protecting the samples from contamination, workplace was sterilized, disposable gloves (free of powder) were changed at each miRNAs extraction step and work was done quickly during all steps. The analysis steps

were modified according to our lab conditions to get the required results. Optimization was done for each step to meet a good yield.

1- Lysis was done by using 1mL RNAzol RT per (0.5) mL of blood, letting it stand at room temperature for (30) min and centrifuged.

2. The aqueous(upper) phase is transferred to a fresh tube. Centrifuged a second time then aqueous phase is transferred to a clean tube.

3. A volume of 0.4 mL of RNase-free water is added per mL of RNAzol RT used for homogenization. The sample is covered tightly, and shacked vigorously for (60) seconds, and allow to stand for (30) minutes at room temperature.

4. The resulting mixture is centrifuged at 12,000 x*g* for (15) minutes at 4 °C. Centrifugation separates the mixture into a semisolid pellet (containing DNA, proteins and polysaccharides) and an upper supernatant (containing RNA). Supernatant is transferred to a new tube, leaving a layer of the supernatant above the DNA/protein pellet.

5. The supernatant is transferred to a fresh tube and a volume of 0.4 mL of 75% ethanol is added to precipitate mRNA.

6. The sample is allowed to stand for 10 minutes at room temperature.

7. Centrifugation is done at 12,000 x*g* for 8 minutes. The mRNA precipitate will form a white pellet on the side and bottom of the tube.

8. The supernatant was transferred to a clean tube, while being sure not disturbing the pellet.

9. A volume of 0.8ml of 100% isopropanol was added to the supernatant.

10. Sample was allowed to stand for (24 hrs).

11.Centrifugation was done at 12,000 x*g* for 15 minutes. The micro RNA precipitate will form a white pellet on the bottom of the tube.

12.microRNA pellet was washed **twice** with 0.6 mL 75% ethanol (v/v) and 70% isopropanol (v/v), respectively, per 1mL of supernatant used for

precipitation. Then centrifugation at 8,000 x*g* for 3minutes at room temperature. Alcohol solution was removed with a micropipette.

13.The RNA pellets were solubilized, with drying, in RNase-free water. The samples were Vortexed at room temperature for 5 minutes.

3.12.3 Gene Expression Analysis

RNA Measurement kit:

The assay is highly selective for miRNA over other types of RNA and is accurate for initial sample concentrations from 10 pg/µL to 100 ng/µL. The assay is performed at room temperature, and the signal is stable for 3 hours. Common contaminants such as salts, free nucleotides, solvents, detergents, or protein are well tolerated in the assay. The standard and short procedure showing in Figure (3-2).

- The Qubit working solution was prepared by diluting the Qubit miRNA HS Reagent 1:200 in Qubit miRNA HS buffer.
- The volume 190 µL from Qubit working solution has been added to each tube designed to be as a standard, then 10 µl from each provided standard solution has been added into same tubes, then vortexed.
- The Qubit® working solution as 197 µL has been added to each tube prepared for sample and then 3 µL of sample has been added individually.
- All composition has been vortexed and incubated at room temperature for 3 minutes.
- Standards tubes have been inserted in Qubit instrument for creating concentration curve.
- Tubes for samples have been added one by one to read the concentration for miRNA in each sample.

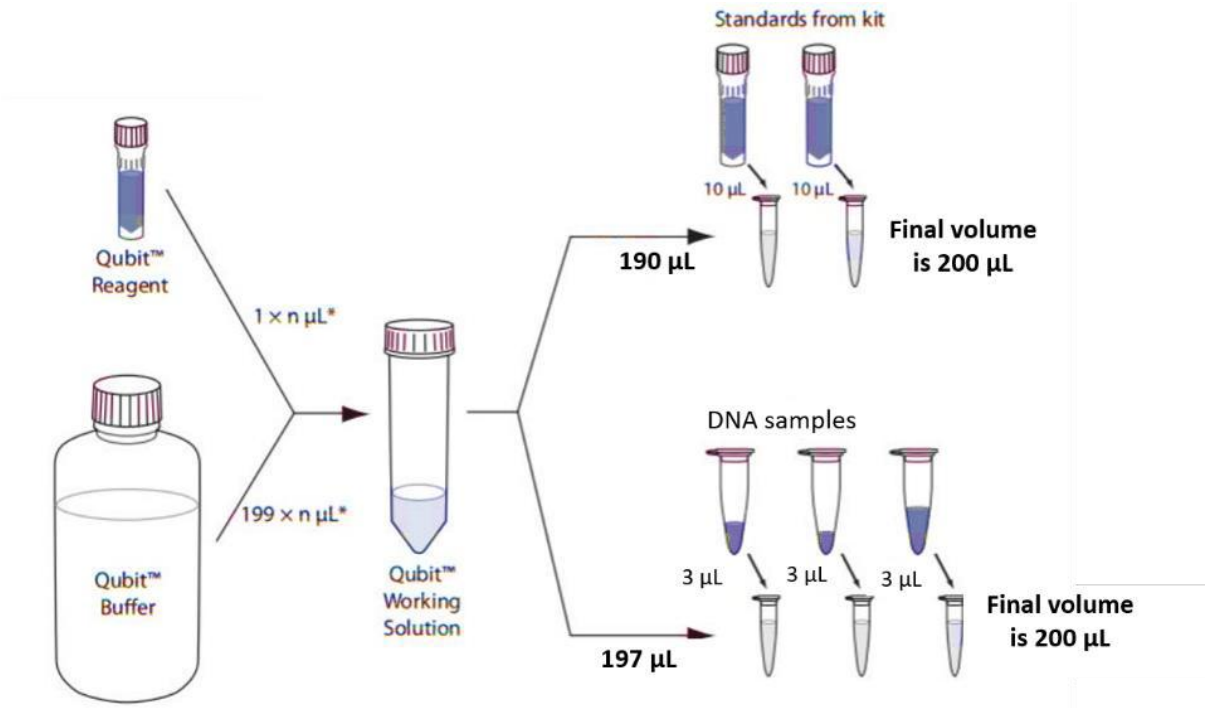


Figure (3.2): Standard procedure for nucleic acid quantification

3.12.4 Real-Time Quantitative PCR (qPCR)

The real time polymerase chain reaction (qPCR) system is based on fluorescent light measurement to determine the amount of complementary DNA (cDNA) of a specific gene. Isolation of total RNA from samples in and Reverse Transcription with the High-Capacity complementary (cDNA) Kit which done according to the target-specific primers and the choice of primers can cause marked variation in calculated mRNA copy

3.12.5 RNA Reverse Transcription

To test the expression of PCR target RNA, the method of reverse transcription involves the conversion of RNA to cDNA. All RNA species were converted into cDNA, using oligo-dT primers they were reverse transcribed into cDNA. The oligo dT primers carry a universal tag sequence on the 5' end to allow amplification of mature miRNA in the step of real-time PCR.

Total RNA containing miRNA was used as row material for reverse-transcription reaction. The PCR tube microfuge was used and reverse transcription master was added then gently mixed, the master mix for reverse transcription contains all materials required in cDNA first-strand synthesis except template RNA.

Template RNA was added to each tube containing reverse transcription master mix, gently mixed, briefly centrifuged, by using thermal cycler the tubes were incubated for 60 min at 37oC, then 5 min at95oC to inactivate reverse transcriptase**.**

3.12.6 Housekeeping Gene

Quantitative reverse transcriptase polymerase chain reaction (qRT-PCR) is a rapid and sensitive approach to identify mRNA and protein-coding gene expression. However, because of the specially designated reverse transcription and shorter PCR products, very few reference genes have been identified for the quantitative analysis of mRNA expression, and different internal reference genes are needed to normalize the expression of mRNA genes. Therefore, it is particularly important to select the suitable common reference genes for normalization of quantitative PCR of mRNA.Glyceraldehyde-3-phosphate dehydrogenase (gapdh) is one of the most commonly used housekeeping genes used in comparisons of gene expression data.

3.12.7 Detectation of MiRNA by qPCR

The total RNA containing miRNA was the starting material in RT-PCR reaction which was performed in one step.due to its short sequence the quantification of miRNAs by qRT-PCR requires extending the length of the mature miRNAs using stem loop or adding poly (A)-tai**l.** GoTaq® 1-Step RT-qPCR System(a) combines GoScript™ Reverse Transcriptase and GoTaq® qPCR Master Mix in a single-step real-time amplification reaction.

The system, which is optimized for RT-qPCR, contains a proprietary fluorescent DNA-binding dye, BRYT Green® Dye. The system enables detection of RNA expression levels using a one-step RT-qPCR method, combining GoScript™ Reverse Transcriptase and GoTaq® qPCR Master Mix in a single-step real-time amplification reaction. An overview of the protocol is shown in Figure (3.3).

The GoScript™ RT Mix for 1-Step RT-qPCR (50X) includes optimized amounts of GoScript™ Reverse Transcriptase, RNasin® Plus RNase Inhibitor and additives to enhance single-step reactions.

The GoTaq® qPCR Master Mix is a simple-to-use, stabilized 2X formulation that includes all components for qPCR except template, primers and water. This formulation, which includes a proprietary dsDNA-binding dye, a low level of carboxy-X-rhodamine (CXR) reference dye (identical to ROX™ dye), GoTaq® Hot Start Polymerase, $MgCl_2$, dNTPs and a proprietary reaction buffer, produces optimal results in qPCR experiments. A separate tube of CXR Reference Dye is included for use with instruments that require a higher level of reference dye than that in the GoTaq® qPCR Master Mix.

3.12.7.1 Description (continued)

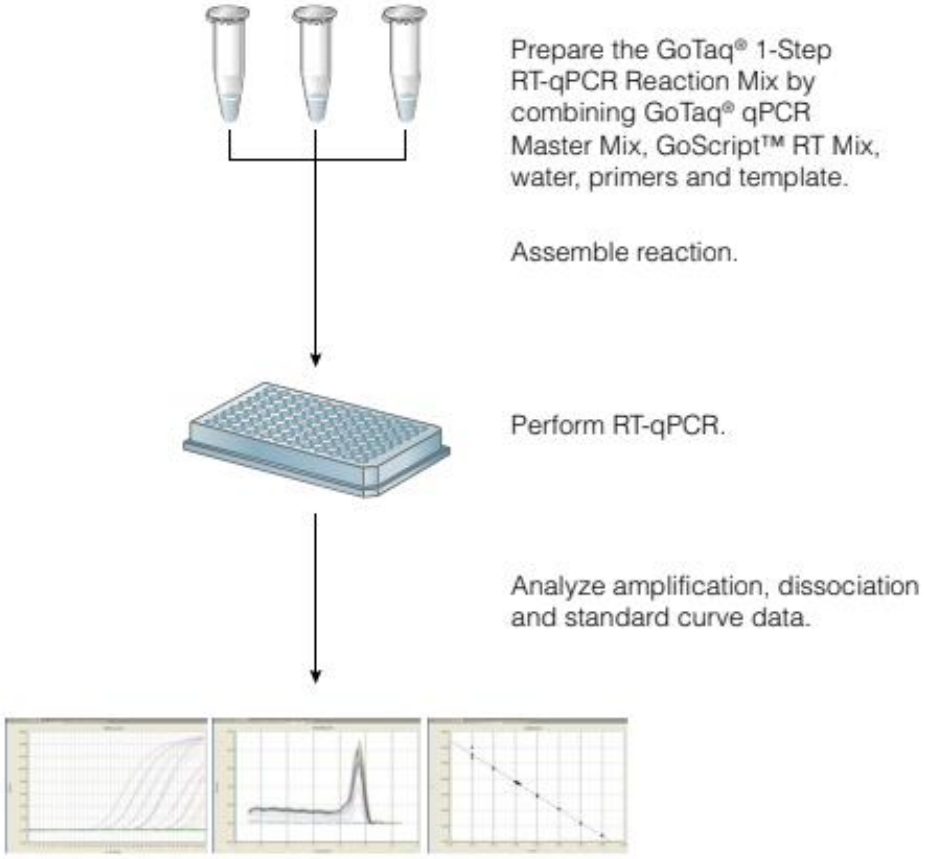


Figure (3.3). Overview of the GoTaq®1-Step RT qPCR Protocol

3.12.7.2 GoTaq® 1-Step RT-qPCR Protocol (continued)

1- Assembling the GoTaq® 1-Step RT-qPCR Reaction Mix-

Program a real-time instrument for standard or fast mode one-step RT-qPCR (Table 2-6).

2-Thaw the components of the GoTaq® 1-Step RT-qPCR System, the RNA templates and the primer pair on ice, at room temperature or at 37°C. Immediately mix each thawed component thoroughly. If using a vortex mixer, mix at low speed to minimize aeration. Keep thawed reagents on ice

3-Prepare:

a. RNA samples (total RNA, mRNA, viral RNA or transcript RNA [500fg–100ng]) in water or other qPCR compatible diluent

b. Standards and controls

c. Primer pair: 1X concentration is approximately 50–300nM.

4-Combine reaction components (Table 2-7) in a nonstick, sterile tube on ice. Mix gently after each addition. Carefully pipet reaction volumes to plate on ice.

5-Transfer plate from ice into the preprogrammed instrument. Seal the tubes or optical plate, and centrifuge briefly to collect the contents of the wells at the bottom. Protect from extended light exposure or elevated temperatures. The samples are ready for thermal cycling Start the run immediately.

6-When the run is complete, collect the data and analyze the results

Table (3-7) General Thermocycler Program

stage	#of Cycles	Program in Standard or Fast Mode
1-Reverse transcription	1	≤37°C for 15 minutes
RT inactivation/Hot-start activation	1	95°C for 10 minutes
-Step qPCR:3- a. Denature b. Anneal/Collect data c. Extend	40	° 95 C for 10 second 60C for 30 seconds ° 72C for 30 seconds
4-dissociation	1	60-95c

Table (3-8). GoTaq® 1-Step RT-qPCR Reaction Mix

Compenent	Volume per 20µl Reaction	Volume per 50µl Reaction	Final Concentration in Reaction
GoTaq® qPCR Master Mix, 2X	10µl	25µl	1x
Forward Primer, 10X	2µl	5µl	50-300nM
Reverse Primer, 10X	2µl	5µl	50-300nM
GoScriptTM RT Mix for 1-Step RT-qPCR, 50X or Nuclease-Free Water for Minus-RT Control	0.4µl	1.0µl	1x
RNA Template (500fg–100ng) or Nuclease-Free Water for No-Template Control	4µl	10µl	variable
Optional: MgCl2, 25mM*	__µl	__µl	>2mM
Optional: CXR Reference Dye, Mm** 30	__µl	__µl	>33nM
Nuclease-Free Water	to 20µl	to 50µl	------

.

*To supplement the MgCl2 provided in Master Mix.

**Guidelines for addition of CXR Reference Dye (30µM) to the reaction mix to achieve a final concentration of 0.5µM:

31µl per 100-reaction batch for 20µl reactions or 87µl per 100-reaction batch for 50µl reactions

3.12.7.3 One step RT-PCR

The miRNA gene miRNA590 and U6 (Housekeeping gene) expression was done by using specific primers as shown in the table (3.9)

Table (3.9) primer Sequence for miRNA Gene Expression (Designed by NCBI)

Gene	Specific primers
rnu6	**F 5`- (CTCGCTTCGGCAGCACA) 3`** **R 5`- (AACGCTTCACGAATTTGCGT) 3`**
miRNA-590 primer	**F,5-TAATTTTATGTATAAGCTAGT-3** **R,5-TGGTGTCGTGGAGTCG-3**

3.12.8 Gene Expression Calculation

The result was collected and analyzed by Livak formula (Livak and Schmittgen, 2001)

Folding =2-ΔΔCT

ΔΔCT =ΔCT patients - ΔCT control

ΔCT =CT Gene - CT House Keeping gene

3.13 Statistical Analysis:

Information from the questionnaire and all test results from patients and control samples were entered a data sheet.

The data analysis for this work was generated using the Real Statistics Resource Pack software for Mac (Release 7.2) of the resource pack for Excel 2016. Copy-right (2013 – 2020).

Descriptive statistics was performed on the participants' data of each group. Values were presented as a median for abnormal distribution, and n (%) for categorical variables, respectively. The distribution of the data was checked for normality using the Box plot test.

T- test was used to adjust other risk factors including: age, BMI. The 95% confidence intervals (95%CI) were also determined for all variables.

Significant differences in continuous variables among the parameters were confirmed through analytical statistical tests. The biomarker were compared to evaluate the relationship between parameters. Results of all hypothesis tests with p-values <0.05 (two-side) were considered to be statistically significant.

Receiver operating characteristics (ROC) curves was also used to test the markers' diagnostic performance in both hypothyroid patients and control groups.

CHAPTER Four

Results and Discussion

4.1 Demographic and clinical data

This study included 60 patients with hypothyroidism along with 30 adjacent healthy control samples. The age range of the participants was (26-51) Years, the mean body mass index BMI was (31.8) Kg/m2

Figure (4.1) shows the distribution levels of lipid profile panel components in patients with hypothyroidism compared to a control group.The results were shown that Patients with hypothyroidism have markedly higher Triglycerides levels (199.55±83.3 mg/dL) compared to the control group (86.6±33.39 mg/dL). Similar to TG, the hypothyroidism group shows elevated Total Cholesterol TC levels (186.91±40.88 mg/dL) compared to the control group (132.15±26.48 mg/dL), as presented in Table (4.1). In term of HDL Cholesterol, the "good" cholesterol, was demonstrated to be lower in the hypothyroidism group (37.57±8.25 mg/dL) compared to the control group (56.44±7.49 mg/dL). While LDL Cholesterol the "bad" cholesterol, was demonstrated to be also higher in the hypothyroid group (108.12±29.66 mg/dL) compared to control group (37±16.34 mg/dL). Furthermore, VLDL was elevated in the hypothyroidism group (40.57±16.63 mg/dL) compared to the control group (17.71±6.98 mg/dL)

Table (4.1) Mean level± 2SD of the Lipid profile panel in obese women patients of hypothyroidism compared to control group

Variable		Patients group	Control group	P value
Lipid Profile	TG mg/dL	199.55±83.3	86.6±33.39	0.003
	TC mg/dL	186.91±40.88	132.15±26.48	0.02
	HDL mg/dL	37.57±8.25	56.44±7.49	<0.001
	LDL mg/dL	108.12±29.66	37±16.34	<0.001
	VLDL mg/dL	40.57±16.63	17.71±6.98	<0.001

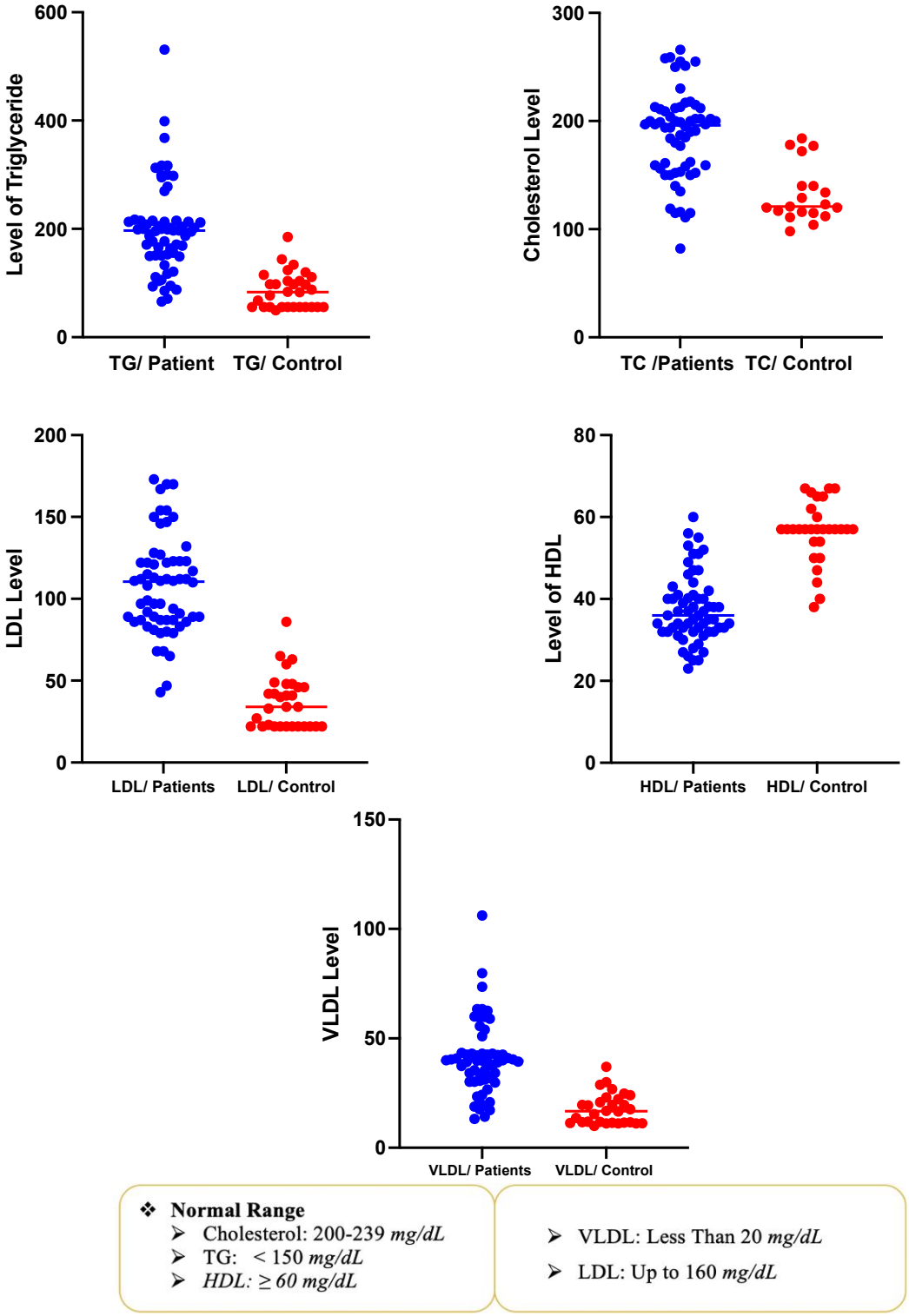


Figure (4.1) Lipid profile Level in obese women patients of hypothyroidism compared to control group

t- test was *: significant at $p \leq 0.05$, : significant at $p \leq 0.01$, *: significant at $p \leq 0.001$, ****: significant at $p \leq 0.0001$)

The findings presented in Figure 4.1 and Table 4.1 highlight significant alterations in lipid profile components among individuals with hypothyroidism compared to a control group. These results underscore the intricate relationship between thyroid hormone function and lipid metabolism

Triglyceride levels were significantly elevated in the hypothyroidism group, which is a typical finding in thyroid diseases. Reduced lipolysis, or the breakdown of lipids for energy, is linked to hypothyroidism. Triglycerides build up in the blood as a result of this decrease in fat breakdown (Duntas and Brenta,2018)

The raised triglyceride levels in the hypothyroid group most likely directly contributed to the rise in total cholesterol levels in that group. Triglycerides contribute considerably to total cholesterol. Additionally, hypothyroidism may affect the processes involved in the production and elimination of cholesterol (Liu *et al* ,.2022)

One troubling finding in hypothyroidism is the reduction in HDL cholesterol. Transporting extra cholesterol from the tissues back to the liver for excretion is a critical function of HDL cholesterol. The chance of developing cardiovascular disease may rise with lower HDL levels (Pearce, 2012)

Another cardiovascular disease risk factor in the hypothyroid group is an increase in LDL cholesterol. When cholesterol enters the tissues through LDL cholesterol, it can build up in the artery walls and cause atherosclerosis.

The higher LDL readings are consistent with a rise in VLDL cholesterol, which is a precursor to LDL cholesterol. VLDL is primarily responsible for transporting triglycerides from the liver to the tissues (Mavromati and Jornayvaz, 2021)

Patients with hypothyroidism were shown to have a greater risk of cardiovascular illnesses, indicating that the primary risk factor for lipid metabolic disorders may be faulty thyroid hormone metabolism. (Bekkering *et al*,. 2019)

Research continuously shows that people with hypothyroidism have higher blood levels of lipoprotein (a), apolipoprotein B, total cholesterol (TC), low-density lipoprotein (LDL-C), and maybe triglycerides (TGs) (Pearce , 2004).

Proatherogenic alterations, such as decreased cardio protective high-density lipoprotein (HDL) and the production of small dense LDL (sdLDL), are linked to elevated TG levels (Moon *et al*,. 2013)

It is commonly recognized that thyroid dysfunction significantly affects lipid levels, and several research have shown this link. The primary cause of dyslipidemia in people with overt hypothyroidism is a change to higher synthesis rate, which raises total cholesterol and low-density lipoprotein (LDL) levels (Duntas and Brenta, 2018)

Moreover, the reduced activation of LDL-receptors leading to a reduction in LDL catabolism, In addition, because lipoprotein lipase activity is decreased in this group, overt hypothyroidism is associated with higher TG and VLDL levels (Xiao *et al*,. 2016)

A significant study conducted in Korea revealed elevated levels of cholesterol and low-density lipoprotein (LDL) in individuals with overt hypothyroidism as compared to the normal group. In contrast to the normal group,

another research revealed that those with overt hypothyroidism had higher blood levels of total cholesterol, LDL, and TG (Alamdari *et al*,. 2016)

Figure (4.2) shows the mean levels and standard deviations (SD) of thyroid function tests in patients with hypothyroidism compared to a control group.

Thyroid Stimulating Hormone (TSH) was shown to be elevated in patients with hypothyroidism, the mean levels were (8.37±3.6 mU/L) compared to the control group (1.17±1.004 mU/L). TSH is a pituitary hormone that stimulates the thyroid gland to produce thyroid hormones. In hypothyroidism, the body attempts to compensate for low thyroid hormone levels by increasing TSH production.

In this study, the results were demonstrated that Free Triiodothyronine (FT3), the major active thyroid hormone, was lower in the hypothyroidism group (60.98±17.01 ng/mL) compared to the control group (80.55±14.76 ng/mL). This might be due to reduced conversion of FT4 (thyroxine) to FT3 in such patients, as presented in Table (4.2)

Similar to FT3, Free Thyroxine **(**FT4) levels were also lower in the hypothyroidism group (1.22±0.45 mcg/dL) compared to the control group (1.57±0.26 mcg/dL). FT4 is the main storage form of thyroid hormone and gets converted to FT3 in the body

Table (4.2) Mean level± 2SD of the thyroid function test in patients group of hypothyroidism compared to control group

Variable		Patients group	Control group	P value
Hormones	TSH mU/L	8.37±3.6	1.17±1.004	0.006
	FT3 ng/mL	60.98±17.01	80.55±14.76	0.003
	FT4 mcg/dL	1.22±0.45	1.57±0.26	<0.001

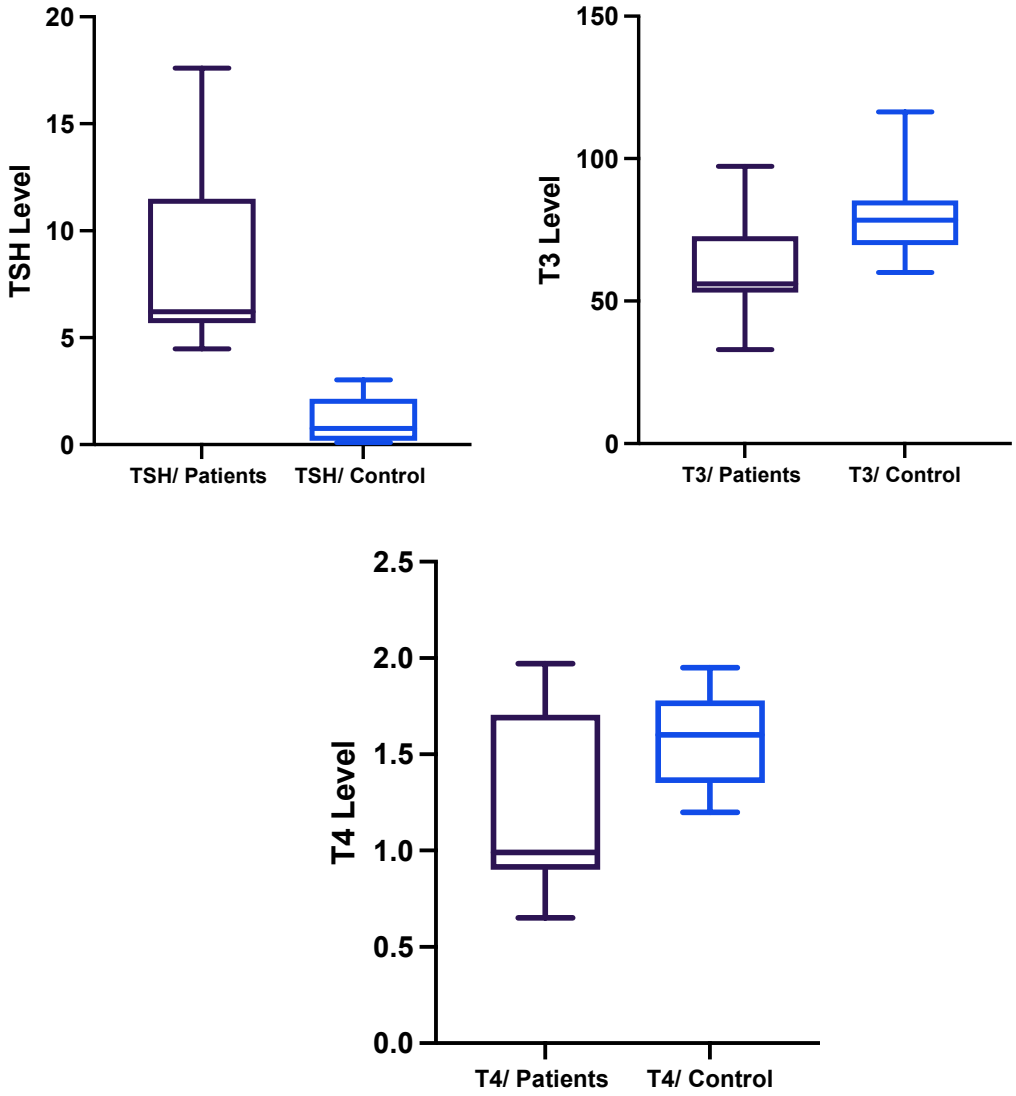


Figure (4.2) Thyroid function Hormones in patients group of hypothyroidism compared to control group
t- Test was *: significant at $p \leq 0.05$, : significant at $p \leq 0.01$, *: significant at $p \leq 0.001$, ****: significant at $p \leq 0.0001$)

Table (4.2) elucidate the pathophysiology of hypothyroidism and provide valuable insights into the hormonal imbalances associated with this condition.

The results were showed a significantly elevated TSH levels in the hypothyroid group which are a hallmark of this disorder. The pituitary gland attempts to compensate for the low circulating thyroid hormone levels by increasing TSH production, stimulating increase thyroid hormones levels

Results were also shown a lower FT3 levels in the hypothyroid group are consistent with the diagnosis of hypothyroidism. FT3 is the primary active form of thyroid hormone and is responsible for most of the metabolic effects. The reduction in FT3 levels can lead to a wide range of symptoms, including fatigue, weight gain, and cold intolerance.

While the decrease in FT4 levels in the hypothyroidism group is also expected, as FT4 is the main storage form of thyroid hormone and a precursor to FT3. The reduced availability of FT4 limits the conversion to FT3, further contributing to the low FT3 levels. This conversion process occurs primarily in the liver and other tissues. Factors such as inflammation, medications, and underlying medical conditions can impair this conversion.

Figure (4.2) presented the characteristic hormonal imbalances observed in hypothyroidism. The elevated TSH levels, coupled with reduced FT3 and FT4 levels, confirm the diagnosis of hypothyroidism. The reduced conversion of FT4 to FT3 may play a role in the development of hypothyroidism and contribute to the severity of its symptoms.

The interaction between hormones ghrelin, obestatin, and thyroid function is important to understand since the thyroid gland's function is linked to the maintenance of energy balance. Adipokines are hormones generated by adipose tissue that control hunger, metabolism, and inflammation. (Ruchala *et al*., 2014)

In this study, both biomarkers were potentially differences and reflect their influence on hypothyroidism cases, as presented in Figure (4.3). Results were indicated that serum ghrelin concentrations was higher 35.26±12.10 ng / ml in hypothyroidism patients, as compared with control subjects 26.92±7.58 ng/ml. on the other hand; Patients with hypothyroidism have higher obestatin levels

10.99 ±4.23 compared to the control group (7.22± 1.32) as presented in Table (4.3)

Table (4.3) Mean level± 2SD of the Adipokines in patients group of hypothyroidism compared to control group

Variable		Patients group	Control group	P value
Adipokines	Obestatin ng/mL	10.99 ±4.23	7.22± 1.32	<0.001
	Ghrelin ng/mL	35.26±12.10	26.92±7.58	<0.001

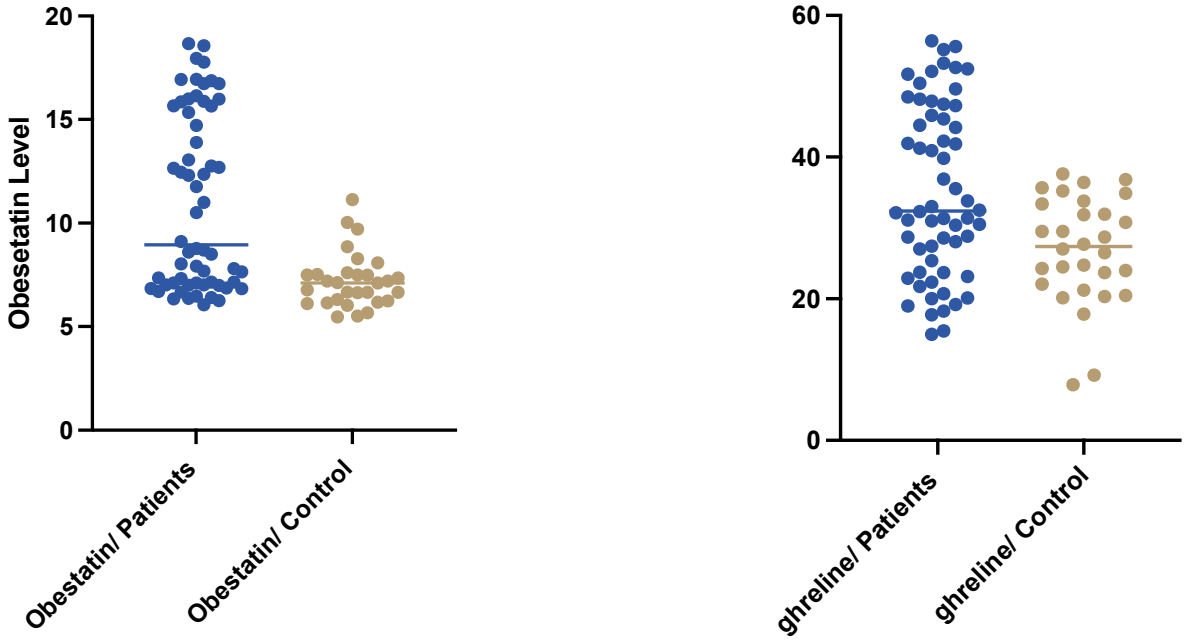


Figure (4.3) Adipokines Level in obese women patients of hypothyroidism compared to control group
t- test was *: significant at $p \leq 0.05$, : significant at $p \leq 0.01$, *: significant at $p \leq 0.001$, ****: significant at $p \leq 0.0001$)

Table (4.3) presented and highlighted the intriguing relationship between thyroid function and adipokines, specifically ghrelin and obestatin. These results suggest that alterations in adipokine levels may play a role in the pathophysiology of hypothyroidism.

The elevation in ghrelin levels in the hypothyroidism group is a noteworthy observation. Ghrelin, often referred to as the "hunger hormone," is primarily produced by the stomach and stimulates appetite. The increased ghrelin levels in hypothyroidism might contribute to weight gain, a common symptom of this condition. It is possible that the body's attempt to compensate for reduced energy expenditure in hypothyroidism leads to increased hunger and food intake (Ruchala *et al*., 2014).

The higher obestatin levels in the hypothyroidism group are also of interest. Obestatin is primarily produced by the stomach and has been implicated in satiety and weight regulation. The elevated obestatin levels in hypothyroidism could potentially reflect the body's attempt to counteract the effects of increased ghrelin and reduce appetite.

The interplay between thyroid function and adipokines is complex and not fully understood. It is possible that alterations in thyroid hormone levels influence the production and secretion of adipokines, leading to changes in appetite, metabolism, and energy balance. Conversely, adipokines may also exert effects on thyroid function, potentially contributing to the development or progression of hypothyroidism.

This study indicated a potential association between hypothyroidism and alterations in ghrelin and obestatin levels. These findings contribute to a growing body of evidence highlighting the intricate relationship between thyroid function and adipokine signaling (Emami *et al*,. 2014).

The body's nutritional and energy status is observed by the peripheral hormones thyroid, obestatin, and ghrelin, which also have integrated regulatory functions. These hormones affect how much food is consumed, how much energy is expended, and how well energy balance is maintained via modulating central brain circuits, particularly the hypothalamus. Furthermore, because there are few studies that are accessible and the results are inconsistent, it is unclear if changes

in thyroid status and alterations in gut hormone levels are related (Emami *et al*,. 2014).

Adipokines have a major impact on immunological responses, body weight, insulin sensitivity, food intake, and the reproductive axis in addition to their function in inflammation (Richard *et al*,. 2020).

Thyroid hormones interact with gut hormones and adipocytokines to affect insulin sensitivity and energy metabolism (Aydogan and Sahin ,2013)

Individuals with thyroid dysfunction have altered body weight and insulin sensitivity, as well as altered metabolism of adipose tissue and adipocytokine production. Ghrelin levels in the blood can change, which affects appetite and energy balance as well as contributing to the development of IR.

Ghrelin is an intestinal hormone that exerts its effects on hunger stimulation, energy balance, and, presumably, IR. According to a number of studies, individuals with hyperthyroidism had lower blood ghrelin concentrations than control groups, however patients with hypothyroidism show variable outcomes, either unchanged or increased (Kim *et al*,. 2015) .

In this study, the cycle threshold (Ct) of the microRNA590 was measured using a quantitative determination present in a sample. A lower Ct value indicates a higher abundance of target microRNA. In this case, the patients group has a higher Ct value (29.94) compared to the control group (22.14), suggesting lower levels of microRNA590 in the hypothyroidism group.

Folding change which refers to the difference in gene expression between two conditions was indicated to be up regulation, and higher in the patient group (13.46) compared to the control group (0.95), as presented in Table (4.4)

Table (4.4) Mean level± 2SD of the microRNA590 and folding change in obese women patients of hypothyroidism compared to control group

Variable		Patients group	Control group	P value
microRNA590	Average Ct microRNA	29.94±2.78	22.14±1.59	<0.001
	Folding change	13.46±2.73	0.95±0.88	<0.001

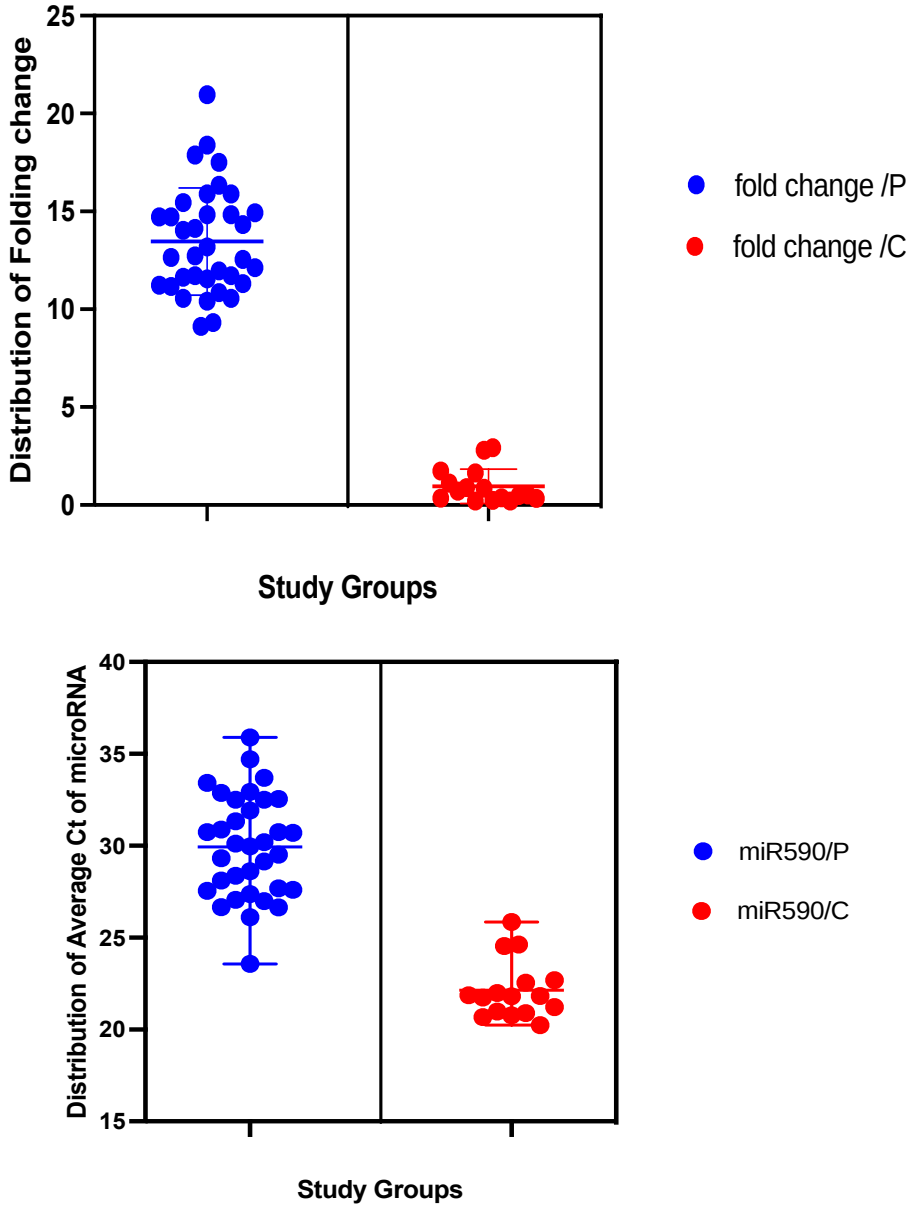


Figure (4.4) the microRNA590 and folding change distribution in obese women patients of hypothyroidism compared to control group
t- test was *: significant at $p \leq 0.05$, : significant at $p \leq 0.01$, *: significant at $p \leq 0.001$, ****: significant at $p \leq 0.0001$)

Quantitative analysis revealed a significant difference in microRNA-590 expression between the hypothyroidism and control groups. The cycle threshold (Ct) values, a measure of microRNA abundance, were significantly higher in the hypothyroidism group (Ct = 29.94) compared to the control group (Ct = 22.14). This indicates a lower level of microRNA-590 in the hypothyroidism patients.

Furthermore, the folding change analysis demonstrated a substantial up regulation of microRNA-590 in the hypothyroidism group compared to the control group. The folding change value of 13.46 in the hypothyroid group indicated an increase in microRNA-590 expression relative to the control group.

Unfortunately, the amount of microRNA-590 in hypothyroidism patients and healthy controls has never been compared in a research. Few studies have established a connection between a number of miRNAs that have been discovered to control the biology and function of adipose tissue, and as a result, their imbalance may contribute to the emergence of obesity and associated metabolic problems (Derghal *et al*,. 2016).

Studying the function of miRNAs in the context of obesity and associated disorders is interesting. In particular, the shift in miRNA expression may result in modifications to the gene patterns governing a variety of biological processes, including as lipid metabolism, inflammation of the adipose tissue, and adipogenesis (Landrier *et al*,. 2019)

The regulation of genes involved in the synthesis of thyroid hormone has been connected to miR-590. Studies have shown that miR-590 can affect the expression of thyroid peroxidase (TPO) and thyroglobulin (TG), two essential proteins needed for the production of thyroid hormones. MiR-590's interference with these genes' regulatory mechanisms may affect thyroid hormone levels and worsen hypothyroidism (Zou *et al*,. 2018)

Low levels of thyroid hormones (T3 and T4) result in higher amounts of thyroid-stimulating hormone (TSH) in hypothyroidism due to a negative feedback loop. This hormonal imbalance may affect the expression of several microRNAs, including miR-590. Thyroid hormone deficiency in particular can downregulate the production of miR-590, perhaps as a compensatory strategy to maintain thyroid homeostasis (Liu *et al*,. 2020)

The expression of miR-590 is directly regulated by thyroid hormones. Thyroid hormones have been shown to influence the expression of several microRNAs, including miR-590. In hypothyroidism, a disease where thyroid hormone levels are insufficient, the thyroid hormones' diminished regulatory impact on miR-590 may lead to decreased levels of miR-590 (Huang *et al*,. 2019)

MiR-590 may potentially have an effect on the regulation of thyroid-stimulating hormone (TSH), which is necessary to maintain thyroid function. Since elevated TSH levels are common in hypothyroidism, miR-590 may affect TSH signaling pathways and the thyroid gland's sensitivity to hormone signals.

The low levels of miR-590 in hypothyroidism can be attributed to a variety of factors, including altered cellular and molecular pathways, direct regulation by thyroid hormones, the negative feedback mechanism of thyroid hormones, and modifications in metabolic processes (Xu *et al*,. 2021)

4.2 Correlation Coefficients between Biomarkers in hypothyroidism patients group

Considering the important role of the measured parameters in hypothyroidism patients, the Pearson analysis was used to show the response relationship between microRNA590 and folding change and the studied parameters. The correlation study demonstrated many significant relationships among the measured parameters. P values were (< 0.05). Thyroid hormone included TSH and free FT3 were indicated a similar trend as a weak negative correlation ($r = -0.43, 0.42$ respectively) suggests that higher TSH levels are

associated with lower microRNA590 levels. While, Moderate positive correlation (r = 0.6285) was illustrated with FT4, indicate that levels FT4 highly associated with the level of microRNA590, as presented in Figure (4.5)

On the other hand, both TSH and FT4 were show a weak negative correlation with folding change. Both Obestatin and Ghrelin were shown weak to moderated negative correlation (r = -0.36, -0.612 respectively) link with microRNA590

Lipid profile namely triglycerides and Total Cholesterol were show a weak negative correlation (r = -0.48, -0.41) respectively.

Interestingly, only Obestatin was shown an opposing effect compared to microRNA590, since Obestatin demonstrated a weak positive correlation (r = 0.4391) to the folding change, as presented in Figure (4.5)

Given that it has already been established that severely defective HDL particles may be seen in hypothyroidism (Su *et al*., 2022) results were indicated a significant negative correlation between HDL and folding change, as presented in Figure (4.6)

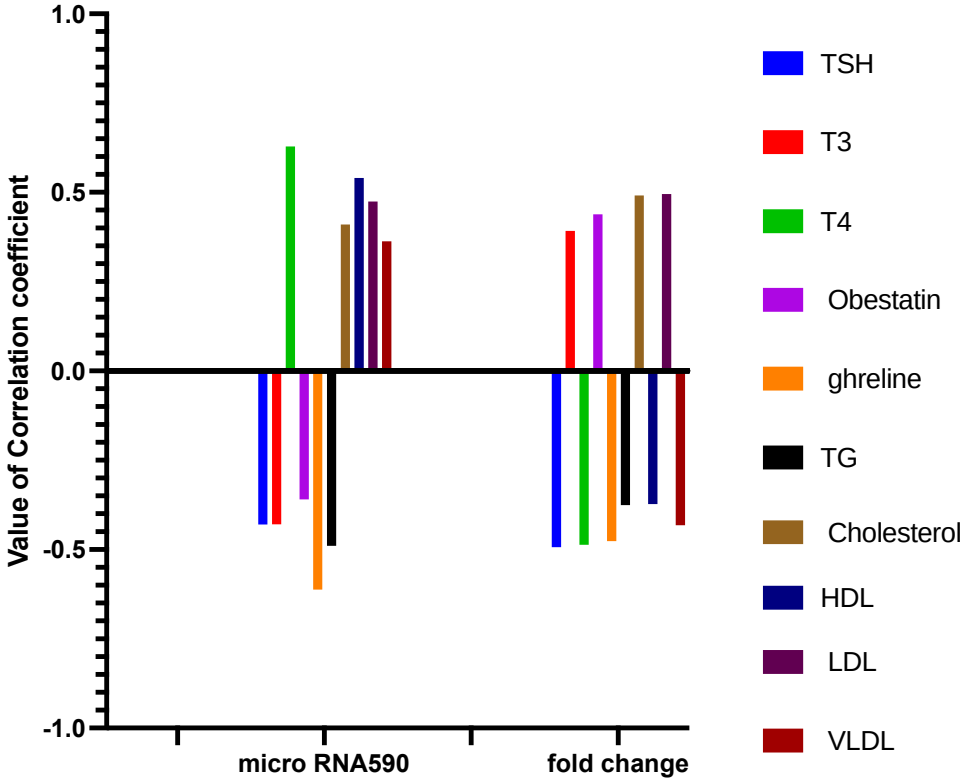


Figure (4.5) The correlation coefficient (r) between microRNA590 and folding change and the studied biomarkers among obese women patients of hypothyroidism

t- test was *: significant at $p \leq 0.05$, : significant at $p \leq 0.01$, *: significant at $p \leq 0.001$, ****: significant at $p \leq 0.0001$)

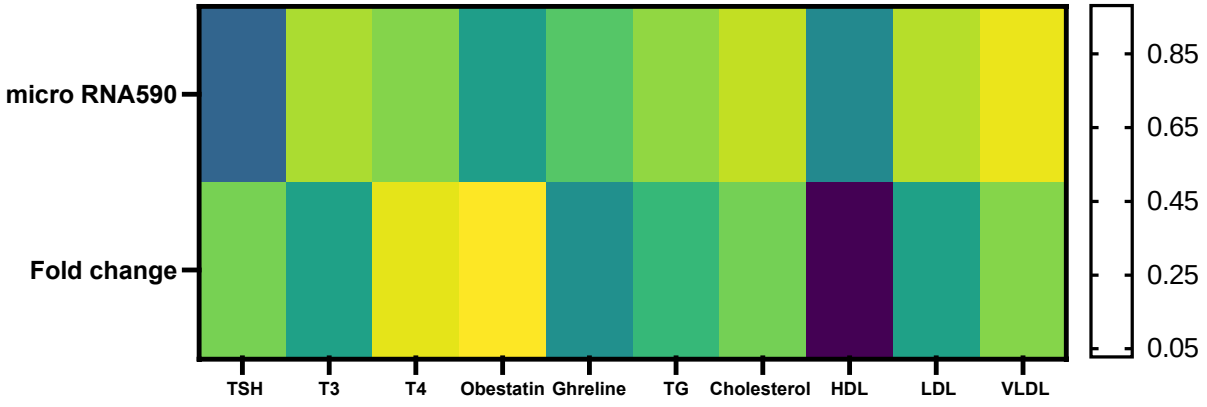


Figure (4.6) The P values of the correlation between microRNA590 and folding change and the studied biomarkers among obese women patients of hypothyroidism

t- test was *: significant at $p \leq 0.05$, : significant at $p \leq 0.01$, *: significant at $p \leq 0.001$, ****: significant at $p \leq 0.0001$)

Both obestatin and ghrelin show weak to moderate correlations with various biomarkers, as presented in Figure (4.7). Obestatin exhibits a positive correlation with TSH, TG, TC, LDL and VLDL, while ghrelin shows a negative correlation with FT3, FT4 and the level of HDL. The findings suggest possible interactions between these hormones and cholesterol metabolism in hypothyroidism, a significant correlation was found between both adipokines and cholesterol between obestatin and FT3, as presented in Figure (4.8)

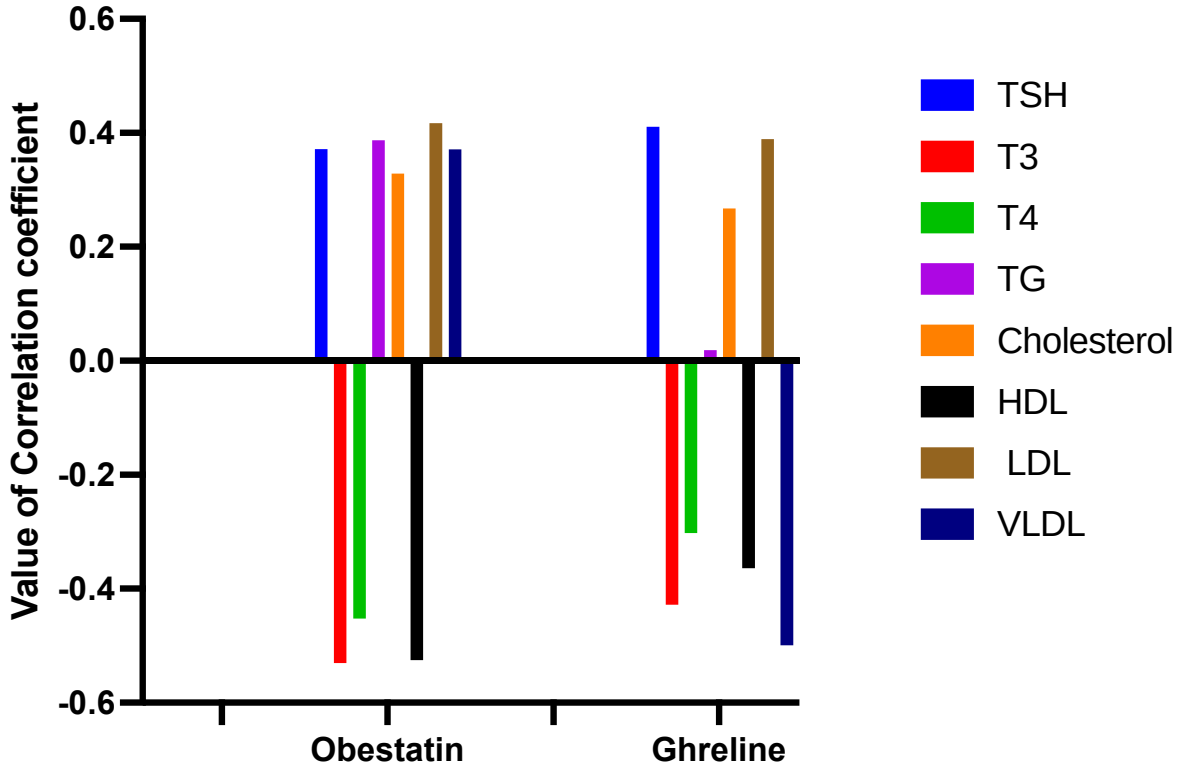


Figure (4.7) The correlation coefficient (r) between obestatin, ghreline and the studied biomarkers among obese women patients of hypothyroidism
t- test was *: significant at $p \leq 0.05$, : significant at $p \leq 0.01$, *: significant at $p \leq 0.001$, ****: significant at $p \leq 0.0001$)

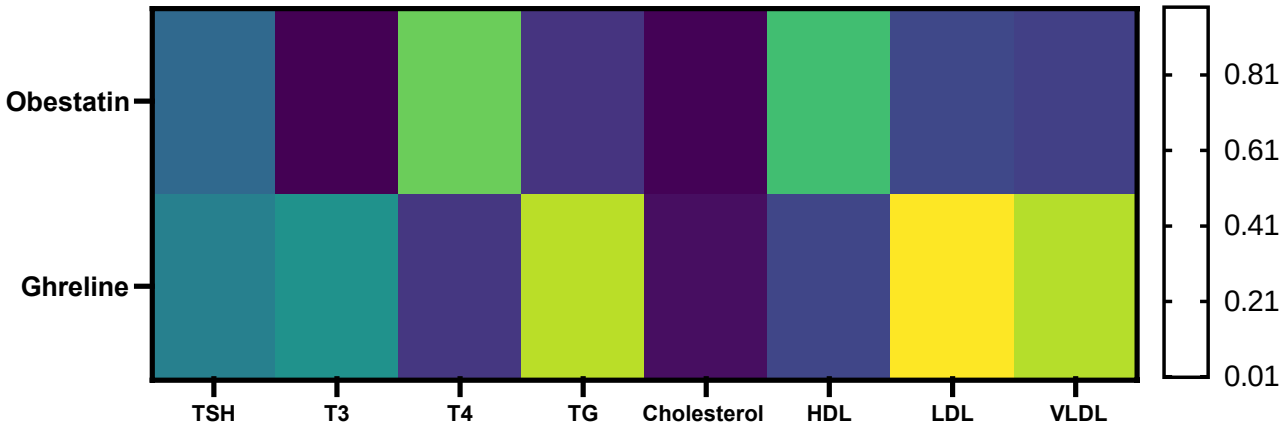


Figure (4.8) The P values of the correlation between obestatin, ghrelin and the studied biomarkers among obese women patients of hypothyroidism
t- test was *: significant at $p \leq 0.05$, : significant at $p \leq 0.01$, *: significant at $p \leq 0.001$, ****: significant at $p \leq 0.0001$)

The increased levels of TSH hormone seen in hypothyroidism are consistent with the positive association between obestatin and TSH. This implies that obestatin and the thyroid hormone axis may be related (Sanyal and Raychaudhuri, 2016)

However, it is still unknown how obestatin will affect body composition. The connection between thyroid conditions and obestatin levels has seldom been studied. Kosowicz and colleagues have demonstrated a connection between high levels of obestatin with hypothyroidism (Kosowicz *et al*,. 2011)

Gurgul *et al*. found a positive relationship between ghrelin, obestatin, and TSH levels in hypothyroidism and postulated that obestatin could be a modulatory molecule (Gurgul *et al*,. 2012)

To the best of our knowledge, not much study has been done on the connection between serum obestatin levels and thyroid dysfunction. In people with hypothyroidism, who often have altered lipid profiles (e.g., increased triglyceride and cholesterol levels), elevated ghrelin can exacerbate dyslipidemia by increasing lipogenesis and lowering lipolysis, which can lead to further metabolic issues (Dixon *et al* ,.2002)

It is common knowledge that being obese reduces appetite. It works on the central nervous system, namely the hypothalamus, to reduce appetite. Elevated levels of obestatin may also affect appetite control in hypothyroidism, a disease in which there is already a decreased metabolic rate and potential for impaired hunger control. The balance between these hormones determines the overall impact, while excess obestatin may counteract some of the effects of high ghrelin on hunger (Stengel and Tache ,2013).

4.3 ROC curve and AUC analysis for the folding change of miRNA-590 in patients with hypothyroidism compared to control groups

ROC curve and AUC analysis for the folding change of miRNA-590 among patients with hypothyroidism compared to control groups were performed. Results of the receiver operating curve (ROC) was shown that folding change of miRNA-590 has good performance for predicting such cases, data are presented in Table (4.5), Figure (4.9)

For Aspersion folding change: (sensitivity 95%, specificity 92%) at a level = 2.32, the p-value of the AUC was <0.001 and highly statistically significant. results of the Sensitivity & Specificity were confirmed using Youden's J statistics to the parameters.

Table (4.5) Receiver operating characteristic curve showing sensitivity and specificity of folding change of miRNA-590 in obese womem patients with hypothyroidism compared to control groups.

Test Result Variable(s)	Folding change of miRNA-590
AUC	97.4%
Sensitivity %	95%
Specificity %	92%
Youden index	0.94
Cut-off points	2.32
CI (95%)	(0. 944-1. 001)
P value	<0.001[S]
S= Significant, PPV= Positive protective value, NPV= Negative predictive value, AUC= Area under curve, CI= confidence interval	

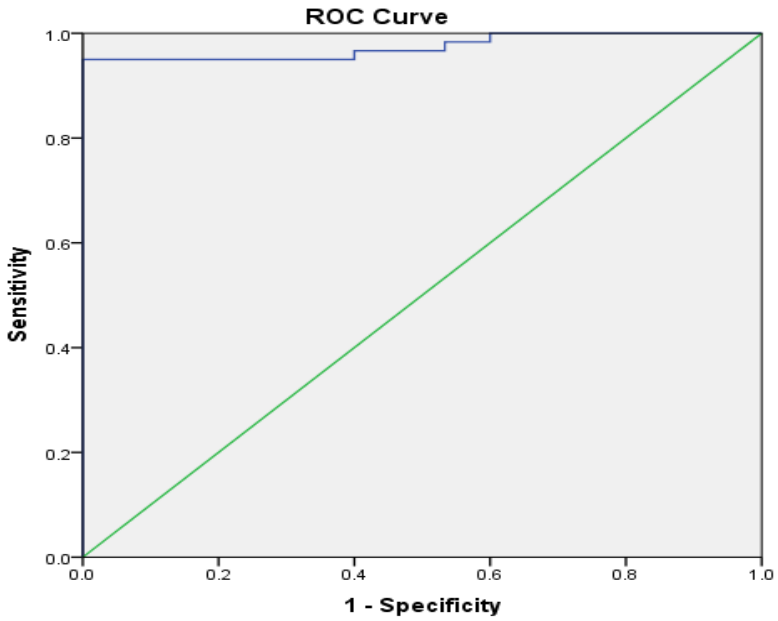


Figure (4.9) Receiver operating characteristics (ROC) curve analysis of folding change miRNA-590 levels

t- test was *: significant at $p \leq 0.05$, : significant at $p \leq 0.01$, *: significant at $p \leq 0.001$, ****: significant at $p \leq 0.0001$)

Many researches were confirmed the needed to validate the role of these markers and determine if a threshold level or changes in serum levels which can be utilized clinically in the assessment and follow up of patients with thyroid disorder. These findings were established the cut-offs and indicated the reference ranges of such cases to get a broader context on their levels. No previous studies were found to compare these results.

The ROC curve analysis and AUC calculation in this study demonstrate the potential of microRNA-590 as a valuable biomarker for predicting hypothyroidism. The high sensitivity and specificity of microRNA-590 folding change at a cutoff value of 2.32 suggest that it can accurately differentiate between individuals with and without hypothyroidism.

The p-value of the AUC, which was significantly less than 0.001, provides strong statistical evidence for the discriminatory power of microRNA-590 in predicting hypothyroidism. This indicates that the model developed using microRNA-590 folding change is highly accurate in distinguishing between the two groups.

The confirmation of sensitivity and specificity using Youden's J statistics further supports the validity of the results. Youden's J index is a measure of diagnostic test performance that combines sensitivity and specificity into a single metric. A higher Youden's J value indicates better overall diagnostic accuracy.

These findings suggest that microRNA-590 could be a promising non-invasive biomarker for the early diagnosis of hypothyroidism. However, further research is needed to validate these results in larger and more diverse patient populations. Additionally, investigating the biological mechanisms underlying the association between microRNA-590 and hypothyroidism could provide valuable insights into the pathophysiology of this condition.

Conclusions and Recommendations

Conclusions

The results revealed significant alterations in lipid profile, thyroid hormone levels, and adipokine concentrations in the hypothyroidism group compared to the control group.

1. Hypothyroid patients exhibited elevated levels of triglycerides, total cholesterol, LDL cholesterol, and VLDL cholesterol, while HDL cholesterol levels were reduced. These lipid abnormalities are associated with an increased risk of cardiovascular disease.
2. Hypothyroid patients had significantly elevated TSH levels and decreased levels of FT3 and FT4. These hormonal imbalances are characteristic of hypothyroidism and can lead to various metabolic disturbances.
3. Increased levels of ghrelin and obestatin were observed in the hypothyroid group. These alterations in adipokine levels may contribute to the metabolic changes associated with hypothyroidism, including weight gain and insulin resistance.
4. A significant downregulation of microRNA-590 was observed in the hypothyroid group. This finding suggests a potential role of microRNA-590 in the pathogenesis of hypothyroidism.
5. Significant correlations were observed between microRNA-590, thyroid hormones, lipid profile parameters, and adipokines. These findings suggest a complex interplay between these factors in the development and progression of hypothyroidism.
6. The ROC curve analysis demonstrated the potential of microRNA-590 as a non-invasive biomarker for the early diagnosis of hypothyroidism.

Recommendations

While this study has provided valuable insights into the association between hypothyroidism, lipid profile, thyroid function, adipokines, and microRNA-590, further research is necessary to elucidate the underlying mechanisms, included:

1. A larger sample size would increase the statistical power of the study and enhance the generalizability of the findings.
2. Longitudinal studies can track the progression of hypothyroidism and the associated changes in lipid profile, thyroid function, adipokines, and microRNA-590 over time.
3. Exploring potential therapeutic interventions targeting microRNA-590 or other identified biomarkers could offer novel approaches for the management of hypothyroidism. Evaluating this biomarker in a population –based study to confirm its role as a direction for early diagnosis and accurate treatment of hypothyroidism.
4. Genetic studies can identify genetic variations associated with hypothyroidism and its complications, providing insights into personalized treatment strategies.
5. Based on this study, recommend using miRNA-590 as a potential sensitive, non-invasive marker for diagnosis and screening hypothyroidism in order to confirm the results.

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236.

APPENDIX

RESEARCHER TEEBA HAIDER

Thyroid Disorder Questionnaire

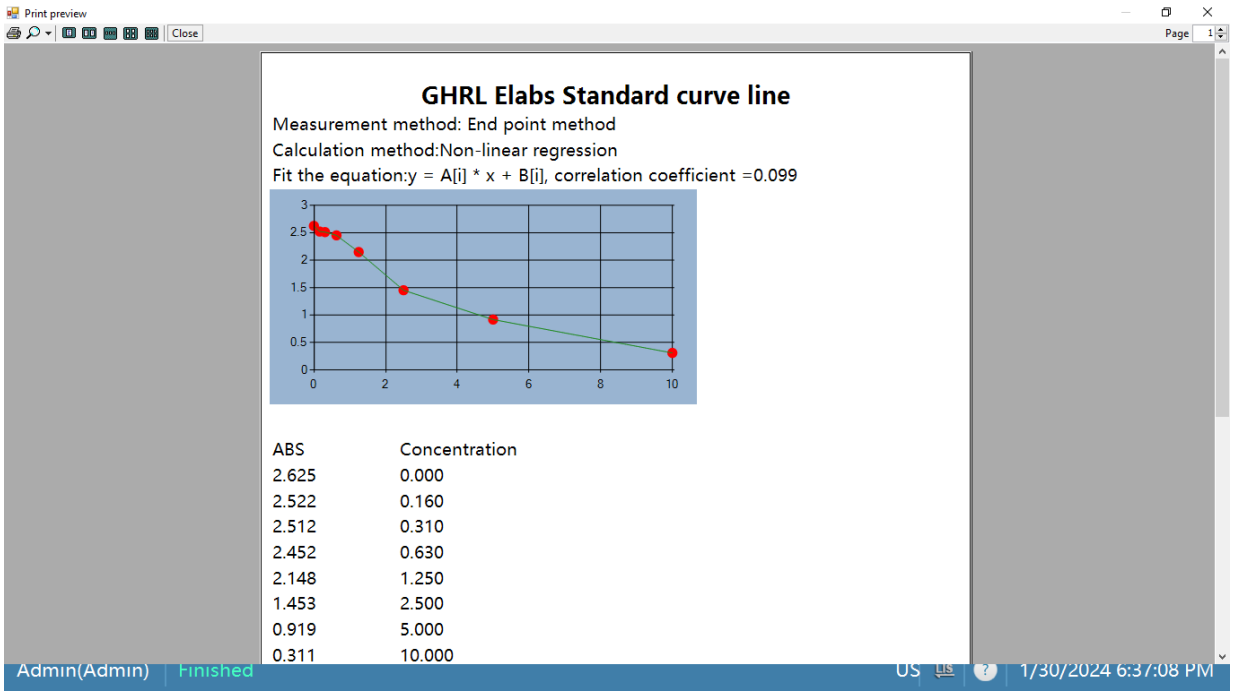
Patient information:

Name	Age	Weight	High	BMI	Chronic disease/Diabetes, pressure,others.	Family history of disease with thyroid	Duration of disease

Required tests:

Thyroid Hormones		Lipid Profile	
TSH		TG	
		TC	
T3			
		HDL	
T4		LDL	
		VLDL	

Biological treatment. Yes ☐ No ☐


Print preview
Close
Page 1
GHRL Elabs Standard curve line
Measurement method: End point method
Calculation method:Non-linear regression
Fit the equation:y = A[i] * x + B[i], correlation coefficient =0.099
ABS Concentration
2.625 0.000
2.522 0.160
2.512 0.310
2.452 0.630
2.148 1.250
1.453 2.500
0.919 5.000
0.311 10.000
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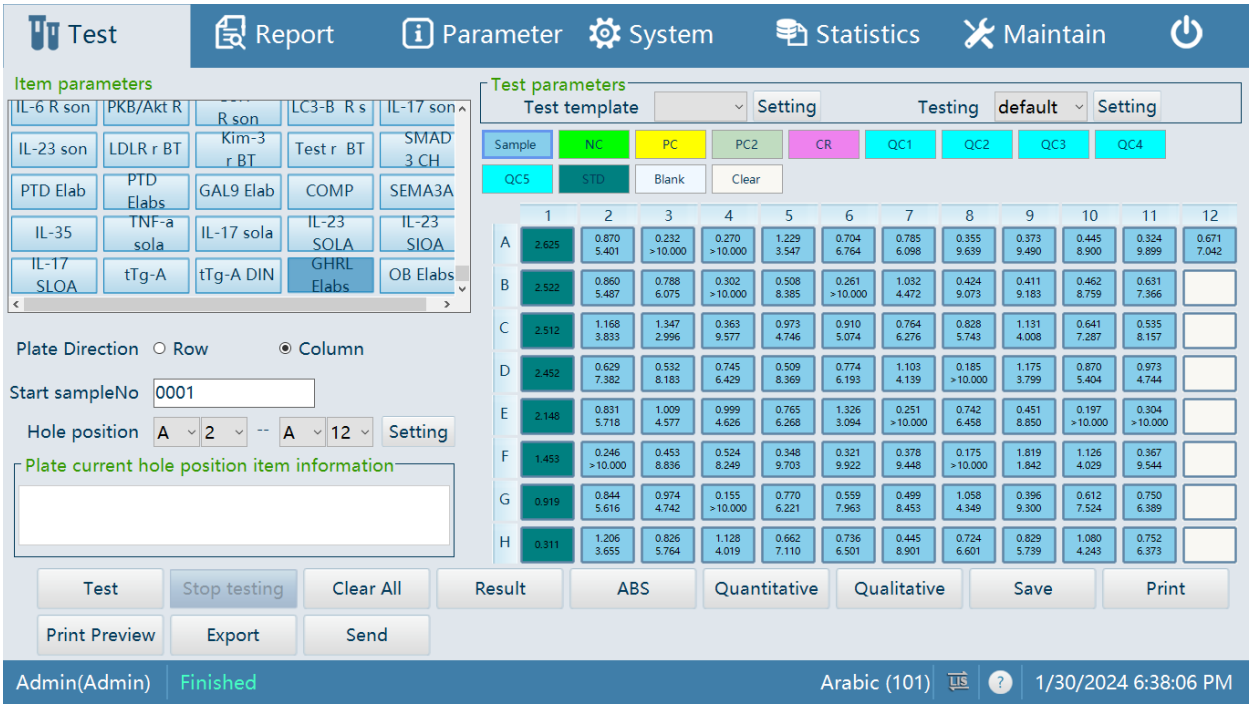
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Report
Parameter
System
Statistics
Maintain
Item parameters
IL-6 R son
PKB/Akt R
R son
LC3-B R s
IL-17 son
IL-23 son
LDLR r BT
Kim-3 r BT
Test r BT
SMAD 3 CH
PTD Elab
PTD Elabs
GAL9 Elab
COMP
SEMA3A
IL-35
TNF-a sola
IL-17 sola
IL-23 SOLA
IL-23 SIOA
IL-17 SLOA
tTg-A
tTg-A DIN
GHRL Elabs
OB Elabs
Test parameters
Test template
Setting
Testing
default
Setting
Sample
NC
PC
PC2
CR
QC1
QC2
QC3
QC4
QC5
STD
Blank
Clear
Plate Direction
Row
Column
Start sampleNo
0001
Hole position
A
2
A
12
Setting
Plate current hole position item information
Test
Stop testing
Clear All
Result
ABS
Quantitative
Qualitative
Save
Print
Print Preview
Export
Send
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Finished
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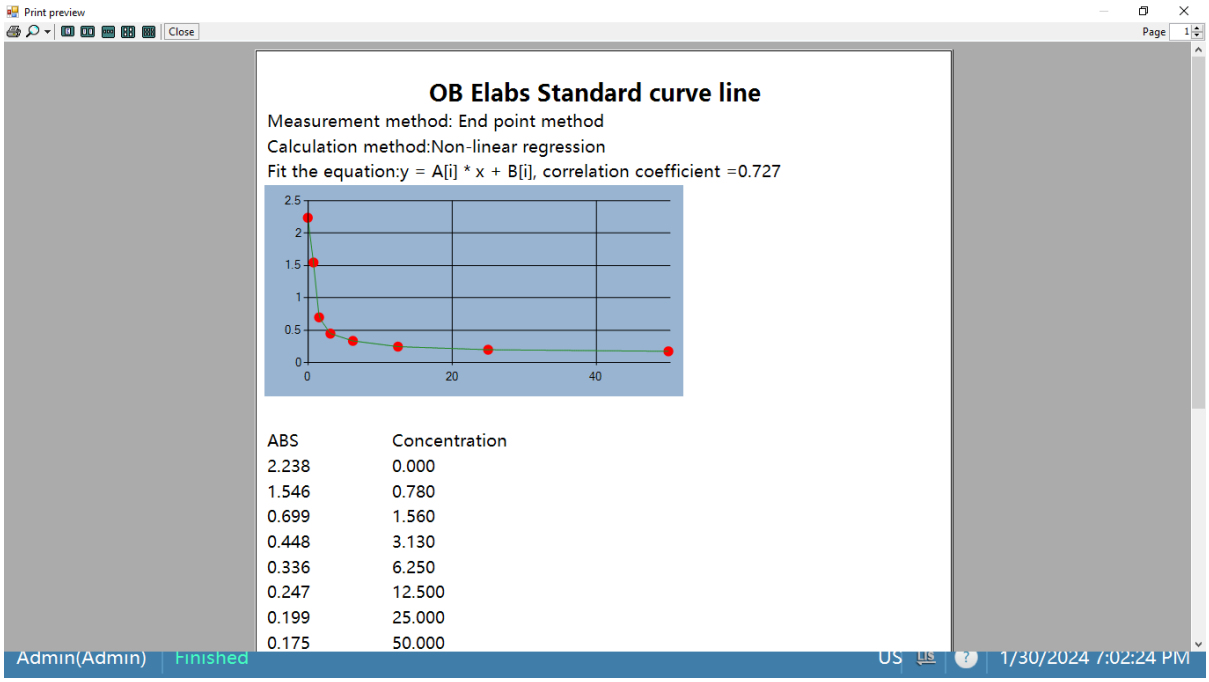
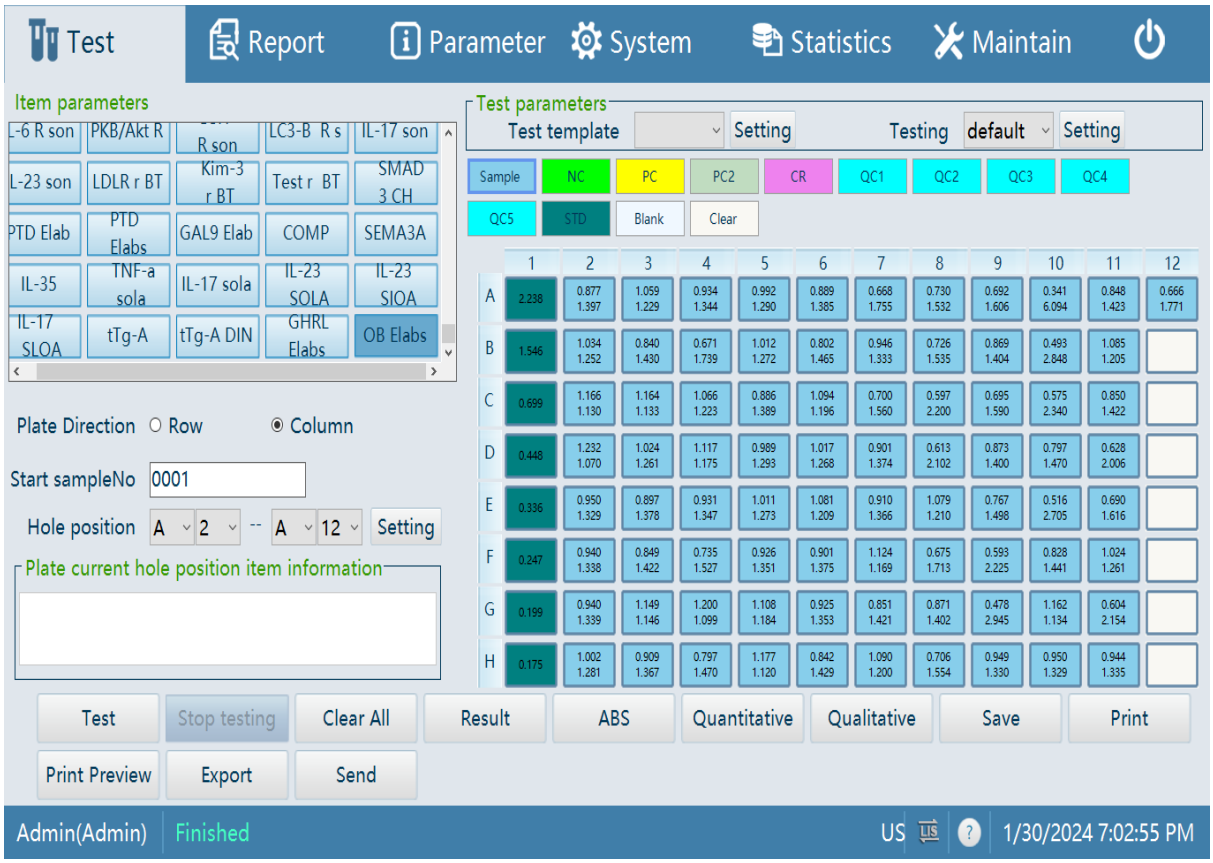
1/30/2024 6:38:06 PM


Print preview
Close
Page 1
OB Elabs Standard curve line
Measurement method: End point method
Calculation method:Non-linear regression
Fit the equation:y = A[i] * x + B[i], correlation coefficient =0.727
2.5
2
1.5
1
0.5
0
0
20
40
ABS Concentration
2.238 0.000
1.546 0.780
0.699 1.560
0.448 3.130
0.336 6.250
0.247 12.500
0.199 25.000
0.175 50.000
Admin(Admin) Finished US 1/30/2024 7:02:24 PM


Test
Report
Parameter
System
Statistics
Maintain
Item parameters
L-6 R son
PKB/Akt R
R son
LC3-B R s
IL-17 son
L-23 son
LDLR r BT
Kim-3 r BT
Test r BT
SMAD 3 CH
PTD Elab
PTD Elabs
GAL9 Elab
COMP
SEMA3A
IL-35
TNF-a sola
IL-17 sola
IL-23 SOLA
IL-23 SIOA
IL-17 SLOA
tTg-A
tTg-A DIN
GHRL Elabs
OB Elabs
Test parameters
Test template
Setting
Testing
default
Setting
Sample
NC
PC
PC2
CR
QC1
QC2
QC3
QC4
QC5
STD
Blank
Clear
Plate Direction Row Column
Start sampleNo 0001
Hole position A 2 -- A 12 Setting
Plate current hole position item information
Test
Stop testing
Clear All
Result
ABS
Quantitative
Qualitative
Save
Print
Print Preview
Export
Send
Admin(Admin) Finished US 1/30/2024 7:02:55 PM

جمهورية العراق

وزارة الصحة
دائرة صحة محافظة بابـــل
المديـــر العـــام
مركـــز التدريب والتنمية البشرية
وحدة أدارة البحوث

Ministry Of Health
Babylon Health Directorate
Email:-
Babel_Healthmoh@yahoo.com

لأجل عراق اخضر مستدام .. سنعمل معا لترشيد استهلاك الطاقة الكهربائية والمحافظة على البيئة من التلوث

العدد : ١٤٥٩
التاريخ: ٢٠٢٣/٩/٢٦

١٥-١٤

إلى / مستشفى الأمام الصادق (ع)
مستشفى مرجان التعليمي
مستشفى الحلة التعليمي
م / تسهيل مهمة

وزارة الصحة والبيئة
الوارده
مستشفى الحلة التعليمي العام
دائرة صحة بابل

تحية طيبة ...

استناداً إلى كتـــاب جامعه كربلاء/ كلية العلوم /شعبة الدراسات العليا المرقم ٣٠٨٨ في ٢٠٢٣/٩/٢٥

نرفق لكم ربطا استمارات الموافقة المبدئية لمشروع البحث العائد للباحثة طالبة الماجستير (طيبة حيدر عبد الامير)

بالاطلاع وتسهيل مهمة الموما أليه من خلال توقيع وختم استمارات أجراء البحث المرفقة في مؤسساتكم وحسب الضوابط والإمكانات لاستحصال الموافقة المبدئية ليتسنى لنا أجراء اللازم على أن لا تتحمل مؤسساتكم أية تبعات مادية وقانونية .... مع الاحترام

المرفقات :
استمارة عدد /٢

لا مانع من تسهيل مهمة الباحثة طيبة حيدر عبد الامير
على ان لا تتحمل المستشفى اي تبعات
مدير المستشفى
فهيم رحمن الجبوري
مدير شعبة المختبر

وزارة الصحة
دائرة صحة بابل
مركز التدريب والتنمية البشرية

الدكتور
م/ محمد عبد الله عجرش
مدير مركز التدريب والتنمية البشرية
/ / ٢٠٢٣

نسخة منه إلى :
- مركز التدريب والتنمية البشرية / وحدة أدارة البحوث مع الأوليات ...

يرتبط قصور الغدة الدرقية بارتفاع دهون الدم والذي يعزى الى انخفاض هرمونات الغدة الدرقية، وتشير الأبحاث الحديثة الى ان هرمون تحفيز الغدة الدرقية (TSH) يؤثر بشكل مستقل في عملية التمثيل الغذائي للدهون. تعد (miRNAs) جزيئات صغيرة احادية السلسلة وغير مشفرة من الحامض النووي RNA والتي تنظم التعبير الجيني ما بعد النسخ. يؤثر خلل تنظيم التعبير الجيني في العديد من جزيئات الحامض النووي miRNAs في المسارات المشاركة في اضطرابات الغدة الدرقية.

هدفت هذه الدراسة الى تحديد مستويات (miRNA_590) في اضطرابات الغدة الدرقية وتقييم حساسية وخصوصية (miRNA)واهميتها التشخيصية. فضلا عن ايجاد العلاقة بين (miRNA_590) وبعض المؤشرات الفسيولوجية مثل هرموني الجريلين والاوبستاتين.

شملت الدراسة الحالية 90 عينة من النساء منها 60 يعانين من اضطرابات الغدة الدرقية فضلا عن 30 عينة من النساء السليمات. تم جمع البيانات الديموغرافية للمريضات من خلال تقنية (الاستبيان) واخضاعهن للتاريخ السريري الكامل والفحص السريري مضافا لها الفحوصات المختبرية كما تم تحديد نوع الاضطراب بناءا على التقدير الكمي لهرمونات الغدة الدرقية. تم قياس تراكيز هرمونات الجريلين والاوبستاتين بوساطة تقنية الامتصاص المناعي المرتبط بالانزيم (ELISA) ومن نوع التنافسية .

تم اجراء التحليل الاحصائي وتقييم كفاءة القيمة التنبؤية باستخدام خاصية تشغيل المستقبل (ROC)

اشارت النتائج الى ارتفاع تركيز مستوى هرمون الجريلين لدى مريضات قصور الغدة الدرقية والذي بلغ (35.26 ng/ml) مقارنة بمجموعة السيطرة(26.92 ng/ml). كما لوحظ ارتفاع تركيزمستوى هرمون الاوبستاتين (10.99 ng/ml) لدى النساء البدينات المصابات بقصور الغدة الدرقية مقارنه بمجموعة السيطرة (7.22 ng/ml).

وفي هذه الدراسة تم قياس عتبة الدورة (CT) للحامض النووي (miRNA_590)عبر تحديد كمي موجود في العينة، اذ أشارت عتبة الدورة المنخفضة الى وفرة اعلى من الحامض النووي (miRNA)المستهدفة .وفي هذه الدراسة تتمتع مجموعة المريضات بقيمة Ct (29.94) اعلى مقارنة مع مجموعة السيطرة(22.14) مما يشير الى مستويات اقل من الحامض النووي (MIRNA_590) لدى مجموعة قصور الغدة الدرقية.

كان التغيير في الطي والذي يشير الى الاختلاف في التعبير الجيني لدى المريضات والسليمات بانه تنظيم تصاعدي واعلى في مجموعة قصور الغدة الدرقية(13.46) مقارنة بمجموعة السيطرة(0.95), وكما أظهرت النتائج المرتفعة للهرمون المحفز للدرقية (TSH)مرتبطة مع الكميات القليلة من الحامض النووي (MIRNA-590) .

تم اجراء تحليل منحنى (ROC) ومساحة تحت المنحنى (Area Under Curve) للتغير في تعبير الحامض النووي (miRNA-590) بين مريضات السمنة المصابات بقصور الغدة الدرقية ومجموعة السيطرة.

واستنتجت الدراسة الى ان التغيير في التعبير الجيني للحامض النووي (MIRNA_590)يوفر قيمة تنبؤيه عالية لتشخيص مرضى قصور الغدة الدرقية وفقا لدراسة منحنى (ROC).

جامعة كربلاء

كلية العلوم

قسم علوم الحياة

قياس مستوى الاديبوكينات والحامض النووي الرايبوزي_590 وبعض المعايير الفسلجية لدى النساء البدينات المصابات بقصور الغدة الدرقية

رسالة

مقدمة الى مجلس كلية العلوم -جامعة كربلاء

وهي جزء من متطلبات نيل شهادة الماجستير في علوم الحياة

من قبل الطالبة

طيبة حيدر عبد الأمير الرفاعي

بكالوريوس علوم/علوم الحياة _2017

بأشراف:

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جاسم حنون هاشم العوادي
دكتوراه انسجة وفسلجة حيوانية
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2025م 1446ه