



University of Kerbala

College of Pharmacy

Department of Pharmacology and Toxicology



**The Impact of *THRA1* gene Polymorphisms on the
Therapeutic Response of Levothyroxine in Female
Patients with Hypothyroidism in Kerbala Province**

A Thesis

**Submitted to the Council of College of Pharmacy / University of
Kerbala as Partial Fulfillment of the Requirements for the Degree of
Master in Pharmacology and Toxicology Department**

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The Impact of *THRA1* gene Polymorphisms on the Therapeutic Response of Levothyroxine in Female Patients with hypothyroidism in Kerbala Province

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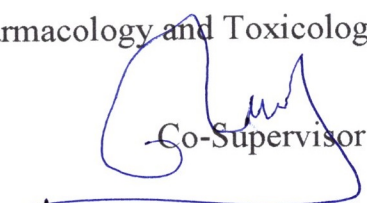


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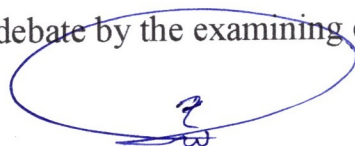
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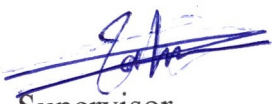
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Dedication

To my family.

To the owner of the fragrant biography, they was the first to have the credit for my attaining higher education, my beloved father and my beloved mother , my God bless their souls.

To my husband and son.

To all my teachers who never hesitated to help me.

To everyone encourage and help me.

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List of Abbreviations

4-AAP	4-Aminoantipyrine
AF1	Activation function
AMI	acute myocardial infarction
AS-PCR	Allele specific Polymerase Chain Reaction
BMI	Body mass index
BP	Blood pressure
bp	Base pair
CHE	Cholestrol ester
CVD	cardiovascular disease
D1	deiodinase
DBD	DNA-binding domain
DM	Diabetes mellitus
ECLIA	electrochemiluminescence immunoassay
FSG	fasting serum glucose
FSI	fasting serum insulin
GK	glocukinase
GOD	glucose oxidase
GPD	glycerol 3 phosphate dehydrodenase
H2O2	Hydrogen peroxide
HDL	High density lipoprotein
HOMA-IR	homeostasis model assessment-insulin resistance
HT	hypertension
kDa	kelodalton
LBD	ligand binding dimerization
LDL	Low density lipoprotein

LT4	Levothyroxine
mIU/L	Mili-international unit per liter
NAFLD	Non-alcoholic fatty liver disease
NAFLD	Non-alcoholic fatty liver disease
NHANES III	The National Health and Nutrition Survey
NIS	sodium/iodide symporter
OATP2B1	Organic Acid Transporting Polypeptide 2B1 transporter
POD	peroxidase
PRL	prolactin
RFLP-PCR	Restriction fragment length polymorphism polymerase chain reaction
rT3	reverse T3
SERCA	sarcoplasmic reticulum-calcium ATPase
SPSS	Statistical Package for the Social Sciences
T3	Triiodothyronine
T4	Thyroxine
Tg	thyroglobulin
TG	Triglyceride
Tmax	to peak plasma concentration
TPO	thyroid peroxidase
TRH	thyrotropin-releasing hormone
TR β	Thyroid receptor beta
TSH	Thyroid stimulating hormone
THRA1	Thyroid hormone receptor alpha 1
VLDL	Very low density lipoprotein

Abstract

Background: Thyroid dysfunctions are among the most prevalent endocrine disorders worldwide, with hypothyroidism being a common manifestation. clinical hypothyroidism is characterized by elevated thyroid-stimulating hormone (TSH) levels while free thyroxine (T4) remains below the normal range. Thyroid hormone receptor alpha 1 (TR α 1) plays a crucial role in thyroid function, and genetic variations may influence treatment responses.

Aim: This study investigates the impact of rs939348 (T>C) and rs12939700 (C>A) single nucleotide polymorphisms (SNPs) in the TR α 1 gene on the therapeutic response to levothyroxine (L-T4) in Iraqi female patients with hypothyroidism in Kerbala province.

Methodology: A case-control study was conducted on 100 Iraqi female patients (≥ 40 years) with primary hypothyroidism undergoing L-T4 therapy, compared with 50 healthy females as controls. Thyroid hormone levels (free and total T4, free and total T3, TSH) were assessed. The rs939348 SNP was analyzed using restriction fragment length polymorphism polymerase chain reaction (RFLP-PCR), while rs12939700 SNP detection was performed using allele specific polymerase chain reaction (AS-PCR).

Results: The results of this study reveal that rs939348 (T>C) SNP, genotype distribution in patients was TT: 33%, CC: 48%, and CT: 19%, compared to TT: 80% and CC, CT: 10% in controls. A significant

association was found between rs939348 and TSH levels ($p < 0.01$) as well as total T3 and free T4 ($p < 0.001$) compared to controls.

For the rs12393700 (C>A) SNP, genotype distribution in patients was CC: 90%, AA: 4%, and CA: 6%, while all controls exhibited the CC genotype (100%). A highly significant association ($p < 0.001$) was observed between rs12393700 and TSH and free T4 levels.

Conclusion: The rs939348 (T>C) SNP, especially the CT and CC genotypes, was significantly associated with changes in thyroid hormone levels, which could contribute to resistance to levothyroxine (L-T4) therapy. Similarly, the rs12393700 (C>A) SNP showed a strong correlation with variations in thyroid hormone levels, indicating its potential role in influencing treatment outcomes. These findings suggest that genetic variations in the *TRα1* gene may affect the efficacy of levothyroxine, underscoring the importance of adopting personalized treatment approaches for managing hypothyroidism.

Chapter One
Introduction
&
Literature Review

1. Introduction

1.1 Thyroid gland

The thyroid gland is a butterfly-shaped structure that sits between the second and third tracheal rings, anterior to the trachea. The synthesis of thyroid hormones is regulated by three main factors: The thyroid follicle, the thyroid gland's functional unit where thyroid hormone is synthesized and released into the bloodstream, comes first, the hypothalamus and the pituitary gland make up the remaining two parts (Branca et al., 2022). Wherein pituitary thyroid-stimulating hormone (TSH) is produced in response to hypothalamic thyrotropin-releasing hormone (TRH), and TSH in turn stimulates the thyroid gland to create thyroxine (TH). Only a minor portion of T3, the functional hormone, is produced by the thyroid; the majority of the hormone is the prohormone (Pirahanchi, Toro and Jialal, 2020). In a negative-feedback system, T3 and T4 control the production of TRH and TSH. There are 2 main types of thyroid hormone: thyroxine (also called T4 because it carries 4 iodine atoms) and triiodothyronine (also called T3 because it carries 3 iodine atoms). Under normal circumstances, about 90% of the thyroid output is in the form of T4 and about 10% is T3 (Groeneweg et al., 2019).

1.2. Synthesis of thyroid hormone

First Step: Iodide Uptake This component is essential to the thyroid gland's operation, as shown by the quantity of iodine in each molecule of thyroid hormone. To make thyroid hormone, thyrocytes must concentrate iodine against a gradient in concentration. The sodium/iodide symporter (NIS) is a crucial component of the active, energy-intensive molecular mechanism that underpins this synthesis(Köhrle, 2023).

Second Step: Iodide Oxidation and Organification

Iodide travels apically upon entering the cytoplasm of the polarized thyrocyte, where it undergoes oxidation and forms a covalent bond with thyroglobulin (Tg). H₂O₂ and thyroid peroxidase (TPO) are needed for this phase. TPO is a 100 kDa heme-containing protein that is a member of the same family as myeloperoxidase, lactoperoxidase, and eosinophil peroxidase in the human peroxidase family (Aghajanova et al., 2011; Pirahanchi and Jialal, 2018). To get the final protein, posttranslational changes such as glycosylation, heme fixing, proteolytic trimming, and dimerization are necessary. TPO oxidizes iodide and donates H₂O₂. The resultant molecule can interact with Tg (Glinioer, 1997; Cignini et al., 2012; Pirahanchi and Jialal, 2018; Pirahanchi, Toro and Jialal, 2020).

Third Step: Secretion of Iodothyronines

Through endocytosis, Tg is internalized by thyrocytes through the apical membrane. Colloid droplets result from the invasion of the apical membrane by pseudopod development (Aghajanova et al., 2011; Saiselet et al., 2012; Pirahanchi, Toro and Jialal, 2020; Pizzimenti et al., 2022).

At least three variables control the metabolism and production of thyroid hormones: Iodine availability, TSH-induced stimulation, and deiodinases activity. The thyroid gland secretes thyroid hormone (T3), which is crucial for metabolism, differentiation, and development. The lack of T3 in early human development results in growth disturbances and severe mental retardation, a disease called cretinism. Later in life, T3 plays an important role in metabolic balance. T3 action is mediated by nuclear T3 receptors (TRs) that can bind T3 with high affinity (van der Spek, Fliers and Boelen, 2017).

1.3. The physiological role of thyroid hormone

Thyroid hormones, primarily thyroxine (T4) and triiodothyronine (T3), are essential for regulating numerous physiological functions, including metabolism, growth, and development. These hormones increase the basal metabolic rate (BMR), stimulate thermogenesis, and regulate the breakdown and synthesis of proteins, carbohydrates, and lipids (Sue and Leung, 2020). Thyroid hormones are vital for normal fetal development, particularly for the formation of the nervous system, and they play a key role in bone growth and cognitive function during childhood (Bernal, Morte and Diez, 2022). They also help regulate the cardiovascular system by increasing heart rate, contractility, and vasodilation, ensuring adequate blood flow to tissues (Danzi and Klein, 2012). In the nervous system, thyroid hormones influence mood, cognition, and neurotransmitter synthesis, and they are crucial for normal brain function. Furthermore, they regulate reproductive health, with normal thyroid function being necessary for menstrual regularity, fertility, and ovulation (Danzi and Klein, 2012; Bernal, Morte and Diez, 2022; Zhu et al., 2022). Thyroid hormones also help manage cholesterol levels by promoting the clearance of low-density lipoprotein (LDL) cholesterol from the bloodstream. Imbalances in thyroid hormone levels can lead to conditions like hypothyroidism, characterized by fatigue, weight gain, and depression, or hyperthyroidism, which can cause weight loss, anxiety, and tachycardia, affecting multiple body systems including the gastrointestinal and musculoskeletal systems (Sue and Leung, 2020).

1.4 Thyroid Dysfunctions

Thyroid dysfunctions are the most prevalent endocrine disorders worldwide, with diabetes mellitus coming in second. The main factors influencing thyroid diseases include changes in the production of thyroid hormones. Thyroid hormone imbalances can result in either hyperthyroidism or hypothyroidism (Biondi, Kahaly and Robertson, 2018).

Thyroid abnormalities are more common depending on age, sex, geography, and iodine intake, among other things. According to the geographic distribution of thyroid disorders, iodine deficiency areas had the highest rates of thyroid disorders; inadequate iodine consumption is linked to symptoms of insufficient thyroid hormone production and a high frequency of hypothyroidism (Odeniyi, Olopade and Fasanmade, 2021).

X chromosome inactivation on the thyroid gland and immune system, which greatly contributes to the female predilection of autoimmune thyroiditis, and female sex hormone imbalances, such as estrogen hormone linked to thyroid problems, which are among the most common endocrine dysfunctions in the world, especially in females (Leng and Razvi, 2019a).

Due to the uncertainty in the economic situation and its effect on the level of living, females are more likely than males to experience thyroid disorders. This is especially true for Iraqi women, who were expected to take on greater home obligations. Women had a higher probability of being antibody positive and running the risk of having their hypothyroidism worsen (Elenkov and Chrousos, 2002).

1.5 Hypothyroidism

Hypothyroidism refers to the common pathological condition of thyroid hormone deficiency. It can have major negative health repercussions and even cause death if left untreated. The definition of hypothyroidism is primarily biochemical due to the wide range in clinical presentation and general lack of symptom specificity. Thyroid-stimulating hormone (TSH) concentrations above the reference range and free thyroxine concentrations below the reference range are indicative of overt or clinical primary hypothyroidism. A common indicator of early thyroid failure, mild or subclinical hypothyroidism is characterized by TSH levels above the reference range and free thyroxine levels within the normal range (Leng and Razvi, 2019a). A common endocrine condition that can be easily identified and treated, hypothyroidism can be lethal in severe situations if left untreated (Glinoer et al., 1994). A sizable section of the world's

population is now affected by the rising prevalence of hypothyroidism. Iodine deficiency continues to be the leading cause of hypothyroidism worldwide (Calsolaro et al., 2019b). Hypothyroidism is categorized as primary (dysfunction of the thyroid gland), secondary (dysfunction of the pituitary), or tertiary (dysfunction of the hypothalamus). Women are up to 8–9 times more often than males to have primary hypothyroidism, and the condition is more common as people age, peaking between the ages of 30 and 50 (Vanderpump, 2011). Though the clinical presentation of hypothyroidism can include a wide range of symptoms that vary with age, sex, and time between onset and diagnosis, the most common symptoms of the condition are dry skin, cold sensitivity, fatigue, muscle cramps, voice changes, constipation, heavy menstrual periods, and weight gain (Vanderpump, 2011; Calsolaro et al., 2019b).

1.5.1 Epidemiology of hypothyroidism

The National Health and Nutrition Survey (NHANES III) confirmed that both TSH level and the presence of antithyroid antibodies are increased with age, and that the prevalence of hypothyroidism is higher in females (5.9%) than in males (2.3%). Hypothyroidism is more common in white people and obese people (BMI > 30) (Vanderpump, 2011). 4.6% of Americans suffered from hypothyroidism (0.3% from overt and 4.3% from subclinical). Total, overt, and subclinical hypothyroidism were more common in women in Europe (6.40%, 0.80%, and 5.86%) than in men (3.37%, 0.30%, and 3.45%, respectively) (Madariaga et al., 2014). Among Arab nations, subclinical hypothyroidism is 11.1% and overt hypothyroidism is 4% in Saudi Arabia, while the prevalence of hypothyroidism in Libya is 6.18%. According to a study done in the Kirkuk province of Iraq, hypothyroidism affects 22.7% of people, whereas 4% of women in Kurdistan are hypothyroid (Ajlouni et al., 2022; Mirahmad et al., 2023).

1.5.2. Pathophysiology

Thyroid gland hormone synthesis is directly stimulated by TSH, which is synthesized and secreted in the anterior pituitary in response to activation of thyrotropin-releasing hormone generated in the brain. People with a healthy hypothalamic-pituitary-thyroid axis have a negative feedback regulatory system that controls the thyroid gland's metabolism. As biosensors of thyroid hormone levels, free-thyroxine (FT4) and free-triiodothyronine (FT3) levels provide feedback to the pituitary gland, which in turn regulates TSH levels (Dogantekin, Dogantekin and Avci, 2011; Gupta and Kumar, 2022).

When thyroid hormone synthesis decreases, there is an increase in TSH secretion. The control system responds slowly, and during non-equilibrium periods—which occur at the onset of hypothyroidism—differences between the amounts of thyroid hormone in plasma and TSH can be detected. TSH measurement is considered the gold standard for diagnosing thyroid disease, specifically overt and subclinical hypothyroidism, for three main reasons. First, there is an inverse log-linear relationship between the TSH and FT4 concentrations. Consequently, small linear drops in FT4 concentration are accompanied by an exponential increase in TSH levels. Second, most people with hypothyroidism in clinical practice have a primary thyroid gland ailment. Thirdly, TSH immunometric tests exhibit greater than 99% sensitivity and specificity (Chai, 2020).

1.5.3 Clinical and biochemical features of hypothyroidism

Clinical signs and symptoms of hypothyroidism can range from nonexistent to potentially fatal, as in the instance of myxedema coma. Originally identified in the late 1900s as the result of severe hypothyroidism that had gone untreated for a long time, myxedema coma is now an uncommon illness. However, early detection is crucial due to the disease's remarkable trajectory, which includes a 40% death rate even in the face of treatment (Hashemipour, Rad and Dalili, 2021).

Myxedema coma leads to an altered mental status, hypothermia, progressive lethargy, and bradycardia and can eventually result in multiple organ dysfunction syndrome and death. Therefore, early initiation of thyroid hormone therapy and other supportive measures is crucial (Mathew et al., 2011).

1.5.4. Signs and symptoms of hypothyroidism

Although the clinical presentation of hypothyroidism in adults can involve a wide range of symptoms that vary with age, sex, and time between onset and diagnosis, the most common symptoms include fatigue, lethargy, cold sensitivity, weight gain, constipation, change in voice, and dry skin (Ambachew et al., 2021; Hashemipour, Rad and Dalili, 2021). Hypothyroidism is diagnosed based on non-specific symptoms, particularly in older patients who exhibit less and fewer traditional signs and symptoms compared to younger people. Since a change in seven or more symptoms in the last year increases the risk of hypothyroidism (likelihood ratio 7–8), an increase in symptom severity may be a good indicator of hypothyroidism (Hashemipour, Rad and Dalili, 2021).

The symptoms and consequences of hypothyroidism in clinical settings (Chiovato, Magri and Carlé, 2019; Gottwald-Hostalek and Schulte, 2022)

- 1 .Gaining weight, 2. Intolerance to cold 3 .Exhaustion 4 .Hoarseness in speech
- 5 .a decline in hearing, taste, or vision 6 .Memory impairment, 7. paresthesia
8. mood disturbance 9 .Indigestion 10.Subfertility and infertility
- 11 .irregular menstruation 12 .Galactorrhea 13 .Weakness in muscles ,
- 14 .spasms in the muscles 15 .Pain in the joints 16 .Bleeding, 17.fatigue,
- 18 .dry skin 19 .Loss of hair 20 .A decline in kidney function.

Although almost all major organs are affected clinically by hypothyroidism, the cardiovascular system has received the greatest research attention. Increased vascular resistance, lower cardiac output, impaired left ventricular function, and alterations in multiple other measures of cardiovascular contractility are the outcomes of hypothyroidism. Patients with hypothyroidism have higher rates of myocardial damage and pericardial effusions than comparable euthyroid controls (Grais and Sowers, 2014; Berta et al., 2019a). In addition, individuals with hypothyroidism frequently exhibit characteristics of the metabolic syndrome, such as dyslipidemia, elevated waist circumference, and hypertension, as well as a higher prevalence of cardiovascular risk factors. Additionally, the levels of homocysteine, low-density lipoprotein, and total cholesterol are raised by hypothyroidism (Khatiwada et al., 2016).

1.5.5. Diagnosis of hypothyroidism

The majority of the symptoms, particularly in moderate cases, are nonspecific and sometimes mistaken for other conditions or the aging process itself, making the diagnosis of hypothyroidism difficult. This is particularly problematic for elderly people, as many symptoms like exhaustion, difficulty focusing, dry skin, and many more are mistakenly believed to be typical aspects of aging. Hypothyroidism, sadness, and the prevalence of anemia are three distinct clinical disorders that are frequent in older adults and share vague symptoms in common. According to the World Health Organization, anemia is defined as having a frequency of more than 10% among community-dwelling individuals 65 years of age and older, and it is often linked to other clinical disorders (Bensenor, Olmos and Lotufo, 2012b; Effraimidis, Watt and Feldt-Rasmussen, 2021). Older adults frequently experience sadness or depressive symptoms, particularly when they are paired

with additional medical conditions known as comorbidities. Differentiating between these three circumstances is critical in this context (Bensenor, Olmos and Lotufo, 2012a). Without TSH and FT4 readings, a diagnosis of hypothyroidism cannot be verified based solely on clinical symptoms. Since thyroid illnesses are more common with age and cannot be ruled out without laboratory testing, routine screening for thyroid diseases is advised by numerous guidelines beyond a specific age (Stockigt, 2000; Yazdaan et al., 2023). On the other hand, a normal or low value suggests central hypothyroidism's level assessment is one of the biochemical tests that is very sensitive and reliable, but it needs to be done in conjunction with other thyroid assays. TSH typically ranges from 0.5 to 5.0 mIU/L, but in older people, the highest limit can reach 7.9 mIU/L; a high TSH value suggests primary hypothyroidism (Sheehan, 2016).

Second, total T4 is used instead of free T4, as some individuals may have anomalies in their proteins or be taking medications that can produce false positive results for free T4. However, while T3 is not sensitive to thyroid hypofunction, low blood T3 values can occur even in euthyroid people due to hunger and numerous medications, as T3 levels stay normal until severe hypothyroidism develops. Moreover, the blood thyroid peroxidase (TPO) antibodies can be measured to look into hypothyroidism linked to autoimmune diseases (Paczkowska et al., 2020a).

1.3.5 Complications of hypothyroidism

If hypothyroidism is not addressed, it can result in several health issues. The medical name for severe hypothyroidism—when the condition has worsened over an extended period of time without receiving treatment—is myxedema. Because it is extremely unlikely that you would not identify the symptoms and seek treatment, myxedema is a very rare condition. This type of hypothyroidism poses a serious risk to life. Myxedema has the potential to eventually cause a person's metabolism to stall to the point of coma. See a doctor right away if you suffer from myxedema symptoms like severe exhaustion or cold intolerance. Recognizing the signs of hypothyroidism and getting the right medical attention are essential to avoiding the disorder's consequences. With the correct care, hypothyroidism can be controlled and need not interfere with daily activities (Khan et al., 2017; Ono et al., 2017; Ito et al., 2022).

Antiperoxidase antibodies in the serum are a sign of autoimmune (lymphocytic) thyroiditis, which is typically the cause of subclinical hypothyroidism (normal serum T4, elevated TSH) and may be linked to coronary artery disease. For instance, in a

postmortem examination, lymphocytic thyroiditis was found in the tissues of 20% of men and 50% of women who died from a fatal myocardial infarction, but not in the tissues of 10% of men and women who died from other causes (Duntas, 2015; Paparo et al., 2022). The prevalence of cardiovascular disease (CVD) was found to be higher in hypothyroid people with circulating TSH levels > 10 mIU/L, indicating that TH dysfunction is a major risk factor for lipid metabolic diseases. Since the development of atherosclerotic plaque, which in turn promotes atherosclerotic-related CVDs, is directly linked to the progression of elevated circulating LDL into the sub-endothelium, the focus of research has recently shifted to understanding circulating lipid catabolism in hypothyroidism and the underlying mechanisms of hypothyroidism-induced hyperlipidemia (Su et al., 2022). The kidneys' capacity to excrete water is typically reduced by hypothyroidism. Consequently, serum creatinine levels may be abnormally high or blood sodium levels may be abnormally low. Thyroid hormone replacement therapy can resolve these issues. However, healing from these renal problems may take longer if extremely low hormone levels continue (Naguib and Elkemary, 2023). Symptoms of hypothyroidism include nerve damage and muscle weakness. Carpal tunnel syndrome may also be more common in people who have untreated hypothyroidism (Azhary et al., 2010; Kotwal et al., 2016). Thyroid hormones have a significant impact on pregnancy and reproduction. Many reproductive diseases, including irregular menstruation, infertility, and aberrant sexual development, are linked to thyroid dysfunction. Insufficient thyroid function is linked to elevated TRH production, which prompts the pituitary to release PRL and TSH. Due to its detrimental effects on ovarian function and GnRH pulsatility, hyperprolactinemia lowers reproductive potential (Stagnaro-Green, Dong and Stephenson, 2020; Hatting et al., 2023). Women with hypothyroidism have lower fertility and an increased risk of different obstetric illnesses when they do conceive. These consist of placental abruption, postpartum hemorrhage, abortion, and pregnancy-induced hypertension (Stagnaro-Green, Dong and Stephenson, 2020).

1.6. Levothyroxine

When the body lacks the natural T4 hormone, levothyroxine, a synthetic version that is identical to the natural one both physiologically and biochemically, is given. For acquired primary (thyroidal), secondary (pituitary), and tertiary (hypothalamic) hypothyroidism, oral levothyroxine medication is therefore recommended. In addition, it is used as a supplement to surgery and radioiodine therapy to treat euthyroid goiters, such as thyroid nodules, subacute or chronic lymphocytic thyroiditis, multinodular goiter, and thyroid

cancer patients who have had thyroidectomies (Colucci et al., 2013). Some of common levothyroxine **side effects** include weight lose,tachycardia, tremors, sweating,heat intolerance, diarrhea and menstrual irregularities.

1.6.1 The Optimal dose determination

Levothyroxine Replacement Therapy for Primary Hypothyroidism: Most individuals with this condition respond well to levothyroxine treatment, which involves taking 1.6 µg/kg of ideal body weight on a daily average (Ramírez Stieben et al., 2022). The requirements are significantly higher for children (>2 /µg/kg body weight and age dependent) and newborns (10 to 15 µg/kg body weight. Age-related reductions in thyroid hormone metabolism led to a decrease in levothyroxine needs. Additionally, there is a favorable correlation between the ideal replacement levothyroxine dosage and the degree of serum TSH rise at diagnosis. Normalizing serum TSH is a plausible objective in the management of these individuals due to the sensitivity of the current TSH assays and the very narrow normal range for serum TSH. The serum TSH levels should range from 0.5 to 3.0 mU/L with the right treatment. When treating hypothyroid adults under 65 years of age, we start them on a complete replacement dose of around 1.6 µg/kg body weight (or ideal body weight in situations of substantial obesity). For most women, the dosage is between 75 and 100 /xg/d, while for most men, it is between 100 and 150 µg/d (Efthymidis, Watt and Feldt-Rasmussen, 2021). After two months of medication, serum TSH levels should be rechecked since they gradually decline. It takes longer for the serum TSH level to return to normal the higher it was initially. The daily levothyroxine dose can be increased by 25 /µg if the serum TSH concentration is not normal by 4 months and the serum free T4 index has increased, showing compliance. Six to eight weeks later, testing on thyroid function are performed. Once hypothyroidism is treated, there is a possibility that the metabolic clearance of T4 would increase. Therefore, it is important to evaluate the serum TSH value again six months after normalization to make sure it has stable (Antonelli et al., 2021a). When a patient with primary hypothyroidism is on levothyroxine, a serum TSH levels below normal typically denotes overtreatment. If this happens, the dosage needs to be gradually lowered, and three months later, the serum TSH levels needs to be assessed. Once the right dosage has been determined, medication can be monitored with an annual TSH assay. As a measure of free thyroid hormone concentrations, free thyroid hormone indices account for individual differences in circulating thyroid hormone-binding proteins. Serum free T4 index values in patients on levothyroxine medication that is appropriate typically fall within the upper half of the

normal range (Duntas and Jonklaas, 2019a). However, unless the blood TSH concentration is elevated and concerns about bioavailability or compliance develop, a free T4 index measurement is typically not necessary for periodic monitoring of patients on a stable levothyroxine dosage. Because there is a greater chance of side effects from levothyroxine therapy, hypothyroid patients with cardiac disease or those who are elderly (65 years of age and above) may not have identified heart disease should be treated with greater caution. Thyroid hormone increases the heart's contractility and pace, which raises the oxygen consumption of the heart. The lowered systemic vascular resistance (afterload) and end diastolic volume (preload) result from the enhanced myocardial contractility, which lowers oxygen consumption (Colucci et al., 2013; Duntas and Jonklaas, 2019a; Antonelli et al., 2021a; Ramírez Stieben et al., 2022). These conflicting effects on oxygen consumption need to balance each other out, theoretically. In a study of people with hypothyroidism, 2% (average age, 71 years) experienced angina that started suddenly while using levothyroxine. In those who already had angina, symptoms decreased or vanished in 38%, remained unchanged in 45%, and got worse in 16% of cases. Nonetheless, the 1-year cardiovascular death rate was just 3% overall, which is lower than the total mortality rate recorded for all patients suffering from coronary artery disease during that time (Omidi et al., 2019). Levothyroxine therapy should therefore be started at 25 µg/d for hypothyroid patients with heart disease and for older people without overt heart disease. This dosage should be increased by 25 µg every eight weeks until the serum TSH level returns to normal. Levothyroxine medication should be discontinued in patients who initially develop angina while waiting for the heart condition to be assessed. The medication should be stopped or the dose lowered, and the cardiac condition should be reassessed, in the event that the current anginal symptoms get worse (Duntas and Jonklaas, 2019a). Angioplasty and cardiac and noncardiac surgery can be performed on untreated hypothyroid patients without increasing mortality. Patients with severe hypothyroidism may also be more susceptible to developing postoperative myxedema coma. While up to 20% of patients with Hashimoto thyroiditis may recover on their own, therapy for primary hypothyroidism is typically lifelong. It is unclear if women using levothyroxine replacement therapy are more susceptible to decreasing bone mineral density, even in cases when the replacement dose is properly prescribed and closely monitored (Ladenson et al., 1984; Malhotra and Bhadada, 2022; Amouzeshi et al., 2023). These findings point to a patient who either stopped taking the drug or took it inconsistently before returning to therapy in the days leading up to the test. Following

levothyroxine intake, serum T4 concentration rises more quickly than serum TSH concentration falls. By switching to a new brand of preparation, it may be possible to explore the possibility of lower bioavailability if the serum TSH concentration rises in a compliant patient without any clinical issues (Duntas and Jonklaas, 2019a).

1.6.2. Mechanism of action of Levothyroxine

As a prohormone that deiodinases enzymes convert to the active hormone triiodothyronine (T3), levothyroxine is the L-form of the endogenous thyroid hormone thyroxine (T4). Through its binding to thyroid receptors (TR) and subsequent activation or repression of particular genes, T3 regulates differentiation and metabolic rate. Members of the nuclear receptor superfamily include thyroid hormone receptors (TRs). The thyroid receptor comes in two isoforms: TR α and TR β . Whereas TR β is found in the brain, retina, inner ear, kidney, liver, and lung, TR α is expressed in the brain, heart, and skeletal muscle (Bianco and da Conceição, 2018). The complex will undergo conformational changes following T3's attachment to the thyroid receptor, and it will then bind to the DNA response element as a heterodimer in a certain orientation. Binding of the complex to DNA causes either expression or repression of specific genes, also in the absence of any ligand, TR can bind to DNA but this binding represses or silences basal target gene transcription, T3 can cause an increase in the left ventricular contractility because it stimulates the transcription of myosin heavy chain α and inhibits the expression of myosin heavy chain β (Yen et al., 1992).

1.6.3 Levothyroxine pharmacokinetic

Generally speaking, the gut absorbs between 70 and 80 percent of an oral dose of LT4. This absorption may include the LT4 molecule being transported via the Organic Acid Transporting Polypeptide 2B1 (OATP2B1) transporter. While one study found no significant changes in LT4 absorption between hypothyroidism-positive and hypothyroidism-negative people, another investigation showed that subjects with hypothyroidism or hyperthyroidism had increased LT4 bioavailability when compared to euthyroid controls. It has been demonstrated that after radiolabeled LT4 was administered, the jejunum and ileum absorbed roughly half of an oral dose of the hormone (Braun et al., 2013; Meyer Zu Schwabedissen et al., 2018). Compared to younger adults, older subjects (>70 years) showed a slight reduction in LT4 absorption. This age effect on LT4 absorption would be taken into account in ordinary clinical practice by titrating the LT4 dose in an old patient to an appropriate age-specific reference range. In contrast

to T₄, T₃ is almost absorbed (100%) from the gut after an oral dosage (Lipp, 2021). According to reports, individuals with primary hypothyroidism had a 3-hour time to peak plasma concentration (T_{max}) of LT₄, whereas euthyroid controls had a 2-hour T_{max}. The labeling requirement to take LT₄ tablets on an empty stomach, such as 30 minutes before breakfast or 3 hours after the evening meal, is due to the fact that concurrent food intake decreases the bioavailability of LT₄. Three peripheral deiodinases mediate the conversion of LT₄ to T₃ and subsequent deactivation, which is the primary metabolic pathway and the route most pertinent to its physiological activities (P. et al., 2013; Kumar et al., 2017). To put it briefly, deiodinase D1 can mediate the conversion of T₄ to T₃, increasing the tissue-specific availability of T₃. As a result, LT₄ can be seen as primarily serving as a prodrug to distribute T₃ to peripheral tissues. When it comes to producing T₃, D2 is more crucial than D1, but D1 is particularly crucial for eliminating reverse T₃ (rT₃), an inactive thyroid hormone metabolite, from the body by converting it into another inactive metabolite, deiodinated thyroid hormone. Thyroid hormone breakdown is the primary activity of deiodinase D3. The peripheral conversion of LT₄ to T₃ may be inhibited by polymorphisms of deiodinases, which has been suggested as an explanation for the partial resolution of hypothyroidism symptoms in certain individuals following exogenous LT₄ treatment. In individuals with hypothyroidism, the average elimination half-life of LT₄ following oral administration is 7.5 days, which is similar with once-daily treatment. In euthyroid patients, a somewhat shorter elimination half-life (average 6.2 days) was observed. Interestingly, T₃ has a substantially shorter elimination half-life (1–1.4 days, on average) which could make it more difficult to administer LT₄–T₃ combination therapy in the future (Bianco and da Conceição, 2018).

1.6.4. Factors that may reduce pharmacokinetics of levothyroxine

1. Diurnal Variation

Taking LT₄ at night (for example, three hours after dinner) as opposed to in the morning slightly but significantly raised blood levels of LT₄ and decreased blood levels of TSH. As a result, shifting the regular LT₄ administration time from the morning to the evening has been suggested. This is especially because a number of secondary variables (such as creatinine, lipids, BMI, heart rate, and quality of life) did not vary between the morning and bedtime administration periods. Nonetheless, taking LT₄ in the morning is still generally advised (but at least half an hour before having tea, coffee, or food) (Bolk et al., 2007).

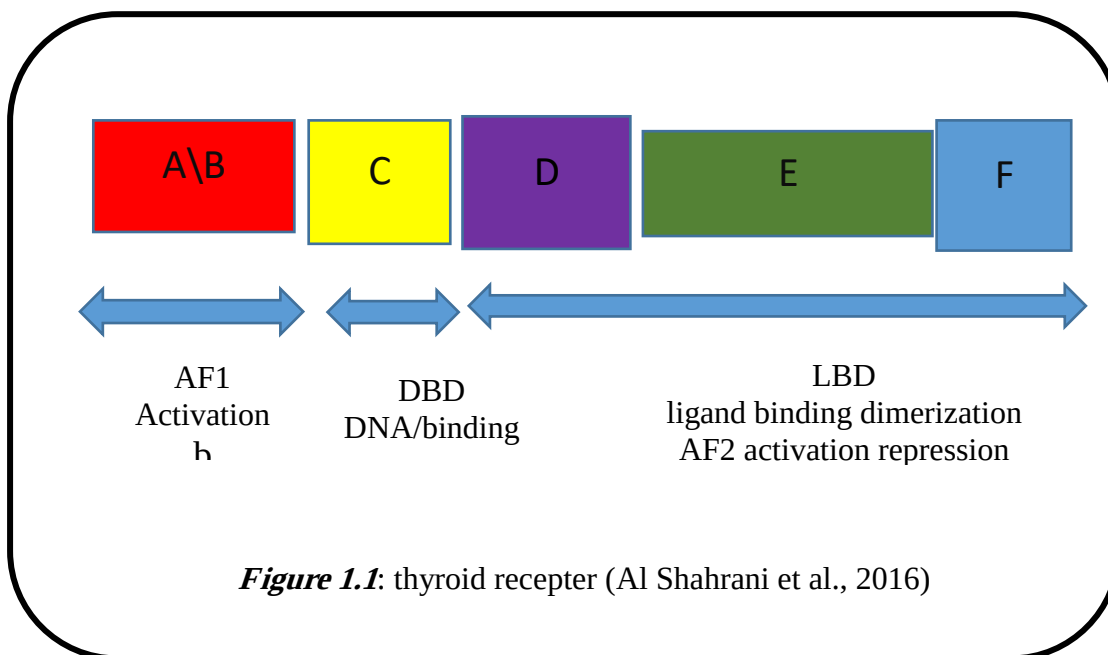
2. Malabsorption of, and Suboptimal Adherence to, Levothyroxine

The absorption of LT4 into the bloodstream can be impeded by a variety of factors, such as pre-existing intestinal disorders (such as celiac disease, previous gut resection, or some types of bariatric surgery), concurrent intakes of certain supplements containing metal ions (such as calcium, iron, or antacids), medications (such as sevelamer, laxatives, or proton pump inhibitors), or soy protein. As pH drops, LT4 becomes more soluble. Proton pump inhibitors raise the pH and decrease the stomach's acidity, which may result in a 40% decrease in LT4's bioavailability. In contrast, co-administration of ascorbic acid (vitamin C) lowers the pH of the stomach and enhances LT4 absorption. Even in the presence of high dosages of exogenous LT4, malabsorption of LT4 can lead to lower-than-expected blood levels of LT4 and higher than expected levels of TSH. Patients with established LT4 malabsorption may be able to benefit from formulations other than the standard tablet. When there is suspicion of suboptimal adherence to LT4 medication, the LT4 absorption test, which measures thyroid hormone levels following an LT4 dose under supervision, can be helpful in differentiating between cases of LT4 non-adherence and actual LT4 malabsorption. For patients with severe intestinal LT4 malabsorption, intramuscular LT4 treatment has been suggested, however this strategy is still in the research stages right now (McMillan et al., 2016; Skelin et al., 2017; Liu et al., 2023).

1.7. Thyroid hormone receptors

TRs are members of the nuclear receptor superfamily, which also comprises "orphan receptors" lacking known ligands and receptors for retinoids, vitamin D, fatty acids, and prostaglandins. Two distinct genes, TRa and TRb, which are found on different chromosomes (17 and 3, respectively, in humans) encode TR. Multiple TR isoforms are produced by alternative splicing from each gene: TRa1, TRa2, and TRa3 from the TRa gene and TRb1 and TRb2 from the TRb gene (Aranda and Pascual, 2001; Rehman et al., 2023). TRs have modular architectures with six regions (A–F) and three functional domains, similar to other nuclear receptors Figure1.1: The thyroid receptor's structure; Three functional domains are The thyroid receptor's structure; Three functional domains are found in each of the six sections (A–F) that make up nuclear receptors, including TR. LBD, ligand-binding domain (D, E, and B areas); AF1, activation function 1 (A and B regions); DBD, DNA-binding domain (C region); a The central nervous system, bone, heart, skeletal muscle, and gastrointestinal tract are the main sites of expression for TRα1 expression, but several tissues express the non-T3-binding isoform TRα2, such as the

brain and testis. The THRB locus generates two distinct isoforms, TR β 1 and TR β 2, which differ in their amino-terminal regions. In the liver, kidney, and thyroid, TR β 1 is regarded as the predominant isoform. Additionally, TR β 1 is mostly expressed in the pituitary, brain, and inner ear, where it mediates functions that overlap with those of TR β 2. The expression pattern of TR β 2 is more limited, regulating neurosensory (hearing, vision) development as well as the HPT axis (Jin and Li, 2010).



1.7.1. Thyroid Receptor Alpha 1

TRalpha1 is a well-characterized TR found in the heart that has been extensively studied in animal. Interestingly, TRalpha1, which is abundantly expressed, is in an unliganded state throughout fetal life due to low levels of T3. This acts as an apo receptor, inhibiting the development of the adult gene program and permitting the proliferation of cardiomyocytes and a rise in cardiac mass. Following birth, a spike in T3 causes TRalpha1 to become liganded. This, in turn, functions as a homoreceptor, causing the production of adult genes that support the development and maturity of the heart. The observation that TRalpha1 functions as a "molecular switch" throughout the formation of the heart is intriguing, as its classification as an apo- or holoreceptor regulates the proliferation and differentiation of cardiomyocytes (Pilhatsch et al., 2010; Elliott et al., 2018). Moreover, a recent study in a mouse heart found that a dominant negative TRalpha1—which is unable to bind T3 and develop into a holoreceptor—prevented

myocardial cells from completing differentiation and instead allowed their proliferation, which in turn allowed the myocardium to regenerate following an acute myocardial infarction (AMI). Importantly, the prenatal profile of the TRalpha1-T3 axis recurs in adulthood to stressful stimuli and during disease states, such as HF. The heart may be trying insufficiently to recreate its developmental pathway and allow cardiomyocytes to multiply and ultimately regenerate, as evidenced by this recapitulation of fetal-like signaling characteristics (Mantzouratou et al., 2023a). Cell hypertrophy without reductive mitosis is the outcome of the embryonic profile of the TRalpha1-T3 axis during stress in organs with relatively limited regeneration capability, like the heart. Although this hypertrophy can temporarily offset the malfunction, it becomes maladaptive and ultimately fails when combined with the adoption of a low energy profile. Therefore, following stress, unliganded TRalpha1 travels to the nucleus to cause dedifferentiation and reactivation of fetal genes, as well as proliferation in cardiomyocytes (Pantos and Mourouzis, 2018). This process is known as "low T3 syndrome." The primary tissue-level regulators of T3 concentration are TH-activating or TH-inactivating enzymes known as deiodinases. Iodothyronine deiodinases of types 1, 2, and 3 show distinct tissue distributions and catalytic characteristics. T3 availability both locally in wounded tissue and systemically is dependent on deiodinase type 3 (D3), which has a strong propensity for inactivating T3. It's interesting to note that after myocardial damage, D3 is strongly expressed in the heart. Furthermore, it has been demonstrated that the adrenergic system's activation regulates the shuttling of TRalpha1 to the nucleus. This system is hypothesized to be involved in both cardiac remodeling and fetal gene reactivation. A decrease in the ratio of sarcoplasmic reticulum-calcium ATPase (SERCA) to phospholamban (PLN), an increase in the beta myosin heavy chain (the predominantly fetal type of myosin, which is slower and less energy-consuming than the adult alpha type of myosin), and the "energy shift" of the stressed myocardium to the use of glucose as an energy substrate instead of fatty acids (like in fetal life) are landmarks of the fetal-like shift in the myocardium. The organ's structure and function continue to deteriorate as a result of this transcriptional shift, which in the short term enables the cardiomyocytes to recover to a less energy-demanding state by having a lower maximum shortening velocity (Vale et al., 2019; Alhayali and Al-Jawadi, 2022; Mantzouratou et al., 2023b).

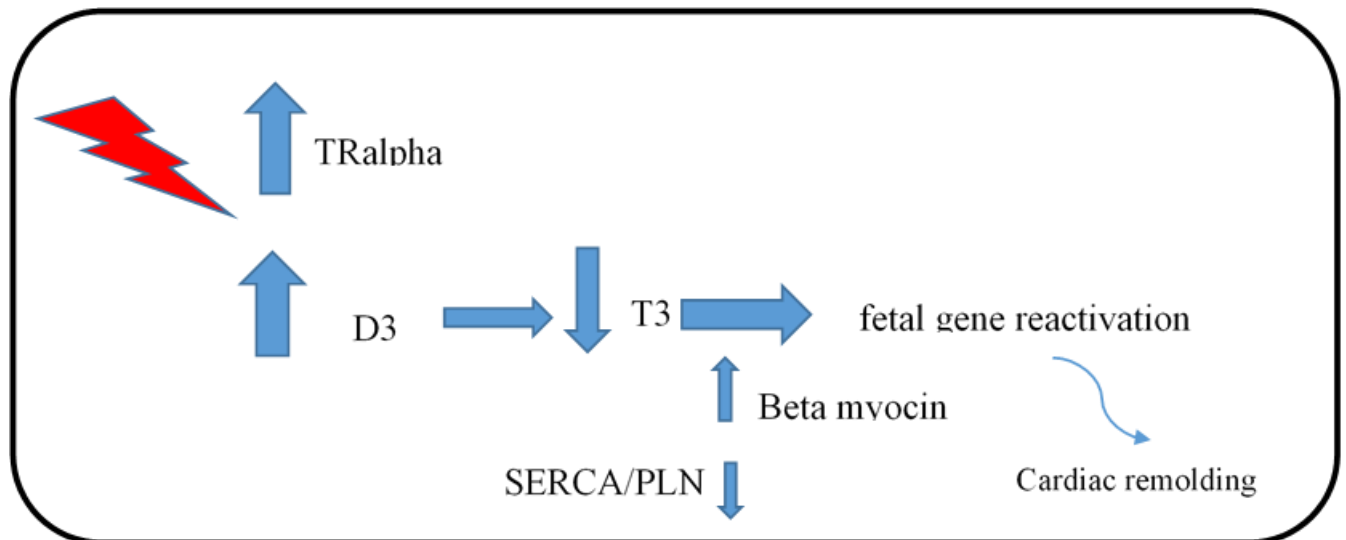


Figure 1.2: THRA1 Following stress, D3 inactivates T3, whereas TRalpha1 rises in the nucleus. Consequently, fetal gene reactivation and cardiac remodeling are caused by TRalpha1 acting as an aporeceptor. (Mantzouratou et al., 2023)

1.7.2. Thyroid Receptor Alpha 1 Gene Polymorphisms Effect

The human TRalpha1 gene encodes the thyroid receptor alpha 1 and is found on chromosome 17. On chromosome 17q11.2, the human THRA gene is arranged into 10 exons that are spaced along 27 kbp of genomic DNA. The location of the human THRA's introns Despite their different lengths, is well preserved when compared to the chicken gene. Two exons encode the 5' untranslated region and the N terminal A/B domain. It's interesting to note that three exons combine to form the hormone binding domain, while one exon encodes each of the putative zinc fingers of the receptor DNA-binding domain. Two distinct messenger RNAs are produced by alternative splicing of the gene's final two exons. Furthermore, ear-1, a member of the nuclear receptor superfamily, is another gene. has the opposite transcriptional direction when it links with the 3' region of THRA (Boumaza et al., 2019; Krieger et al., 2019; Furman et al., 2021).

The *THRA* gene results in the generation of two distinct receptor types, each with a unique C-terminal region. One receptor, called *THRA1*, has 410 amino acids and binds to T3, activating target genes like the growth hormone gene to start transcription. The other receptor, called *THRA2*, is 490 amino acids long and functions as a major negative modulator of TRs without binding T3. Suppression of transactivation mediated by either *THRA1* or *THRB* gene products is how *THRA2* exerts dominant negative control (Krieger et al., 2019). While mutations in one allele cause dwarfism and aberrant lipid metabolism,

as well as a distinct phenotype from TRb mutations, mutations in the other allele result in mortality soon after delivery. The location and kind of mutation have an impact on the phenotype caused by TRa1 mutations. These variations in the final phenotype may derive from diverse ways that TRa1 interacts with different corepressors, repressing distinct target genes in different ways. The ultimate binding of T3 to the TR in the cell nucleus can be influenced by a variety of variables, therefore circulating (measurable) amounts of thyroid hormones may not accurately reflect the levels in certain tissues. For organs like the brain, which have systems in place to protect local tissue from changes in circulating levels, this may be particularly crucial (Wu and Koenig, 2000; Bernal and Morte, 2013; Anyetei-Anum, Roggero and Allison, 2018).

SNPs, or single nucleotide polymorphisms, are minute differences in the genomes of members of the same species. These variations enhance individual variability and, occasionally, may alter a person's susceptibility to illness or how they react to drugs or other substances. Serum thyroid values have a considerable degree of interindividual variance, despite a minor intraindividual variation. A recent study found that age and environmental factors, including cigarette smoking and iodine intake, account for 35% of the variation in blood thyroid parameters, with hereditary factors accounting for 65% of the variation. More recently, it was discovered that the concentration of free T4 (FT4) and free T3 (FT3) is less affected by genetic regulation, whereas TSH is largely determined by it. The first intron after the translation start site contains a frequent mutation in the general population called rs939348 C/T. in the that was discovered to be connected to higher incidence of hypertension and systolic blood pressure (Syvänen, 2001; Goumidi et al., 2011a; Wibowo, Hastuti and Susanti, 2021a).

THR genotyping has been shown in another study to be useful in predicting atherogenic dyslipidemia in hypothyroid individuals. The levels of FT4 concentrations in THRA rs-939348 differed significantly. It has been demonstrated that factors other than genetic differences are related to the dosage of L-T4. For instance, a high dose of L-T4 was linked to increased levels of FT4, a reduced body weight, TSH, and BMI. These findings are consistent with what was previously known about the use of L-T4 in hypothyroid individuals. The 3' untranslated SNPs' second region of THRA intron 9 were genotyped: SNP (A/G) (rs12939700). Accordingly, Fernandez-Real et al. was associated with obesity in a group considered at high cardiovascular risk (Al-Azzam et al., 2014a; S.I. et al., 2014a; ElRawi et al., 2019).

Aim of the Study

1. To investigate the presence of genetic polymorphisms in the *THRA1* gene among Iraqi female patients diagnosed with primary hypothyroidism.
2. To evaluate whether these polymorphisms may contribute to Levothyroxine resistance in the affected patients.

Chapter Two

Patients, Materials, & Methods

2.1. Patients

This study was performed from September 2023 to July 2024. A total of 150 female were enrolled in this case-control study when they visit a private clinic to get medication and advice about their cases. All the patients were already diagnosed with primary hypothyroidism. The patients were receiving levothyroxine for at least 4 months.

2.1.1. Patient Criteria

A. Inclusion Criteria

Female patients with hypothyroidism who are 40 years of age or older and who start receiving levothyroxine for thyroid hormone replacement therapy four months or more.

B. Exclusion Criteria

1. patients whose course of treatment is shorter than four months.
- 2 .The patient, who is under 40 years old.
- 3 .The patients were given any medicine that affects the expression or activity of TRA1 or interacts with levothyroxine .
4. The patient had a thyroidectomy.

2.1.2 Scientific and Ethical Approval

The Scientific and Ethical Committee at the College of Pharmacy at University of Kerbala discussed and approved the proposal of this research.

All subjects completed a written consent form outlining the goal of the research and were asked to complete a specially created questionnaire before being officially included in the study.

2.1.3 Study Design

This is a case-control study that included 100 female patients from Iraq who had been diagnosed with hypothyroidism. Patients who have fasted for the whole night have had blood samples drawn for biochemical, hormonal, and genetic analyses.

2.2 Materials

2.2.1 Instruments and equipment

Chapter Two..... Patients, Materials, and Methods

The instruments that used in the current study are illustrated in Table 2-1.

Table 2-1 Instruments and the manufacturing companies

Equipment	Company	Country
Cobas e 411	Roch	Germany
Centrifuge	Hettich	Germany
Digital camera	Canon	England
Distillatory	GEL	Germany
Hood	Lab tech	Korea
Incubator	Binder	Germany
Micropipettes	Slammed	Japan
Nano pac 500 power suppliers for electrophoresis	Cleaver	UK
PCR machine	veriti	Singapore
Sensitive balance	AND	Taiwan
UV-trans illuminator	Syngene	England
Vortex mixer	Human twist	Germany

1.2.2. Chemicals, Kits, and Suppliers

Chemicals and kits used in this study are listed in the Table 2-2 accompanied by producing companies and countries.

Table 2-2 chemicals and kits and their producing company.

Categories	Chemicals and Kits	Company	Country
Chemicals	Agarose	Bio Basic	Canada
	Ethanol	SDI	Iraq
	Ethidium Bromide	Intron	Korea
	Nuclease free water	Bioneer	Korea
	TBE buffer	Bioneer	Korea
Biochemical kits	Fasting serum glucose kit	Mindary	China
Hormonal kits	FT3 kit	Snibe Dignostic	China
	FT4 kit	Snibe Dignostic	China
	Insulin kit	Mindary	China
	TSH kit	Snibe Dignostic	China
	TT3 kit	Snibe Dignostic	China
	TT4 kit	Snibe Dignostic	China
Lipid profile kits	Cholesterol kit	Mindary	China
	Triglyceride kit	Mindary	China
	HDL kit	Mindary	China

	LDL kit	Mindary	China
	VLDL kit	Mindary	China
Kits of the genetic investigation	DNA Extraction Kit	gsync	Taiwan
	PCR Master mix Kit	biolabs	korea
	DNA ladder Marker (100 bp)	Genome	korea
	Primers for detection of TRA1 gene snp	oligo	korea
	MseI enzyme	biolabs	korea

2.3 Methods

2.3.1 Sample collection

Following an overnight fast and vein puncture, patients' 5 ml of blood were drawn; these samples were separated into two portions, the first of which, 2 ml, was stored in an EDTA tube for the extraction of DNA, and the remainder of which Second part: 3 ml were placed in a gel tube for the purpose of extracting sera, which was utilized to evaluate the other biochemical test and thyroid function tests.

2.3.2 Biochemical and Hormonal Assay Methods

2.3.2.1 Estimation of Thyroid Function

2.3.2.1.A Estimation of Serum Thyroid Stimulating Hormone

An immunoassay was used to quantitatively quantify TSH in vitro. The electrochemiluminescence immunoassay, or "ECLIA," is intended to be used with the Cobas e immunoassay analyzer. It creates a sandwich complex using two different monoclonal antibodies that are particularly targeted against thyroid-stimulating hormone. The surface of the electrode attracts the microparticles by magnetic attraction. When a voltage is given to the electrode, chemiluminescent emission is produced, which is measured by a photomultiplier.

2.3.2.1.B Estimation of Serum Total Thyroxine

The method used to determine serum total T4 is a competitive chemiluminescence immunoassay. Prior to the sample being incubated at 37 degrees, the serum sample's thyroxine competes with the T4 antigen that has been immobilized on the magnetic

microbeads for a small number of binding sites, which results in the formation of immune complexes. When the chemiluminescent reaction starts, a photomultiplier detects the light signal, which is inversely proportional to the concentration of T4 in the sample.

2.3.2.1.C Estimation of Serum Free Thyroxine

As previously mentioned in total T4, the test for serum-free T4 determination is a competitive chemiluminescence immunoassay.

2.3.2.1.D Estimation of Serum Total Triiodothyronine

As previously mentioned in total T4, the test for serum-total T3 determination is a competitive chemiluminescence immunoassay.

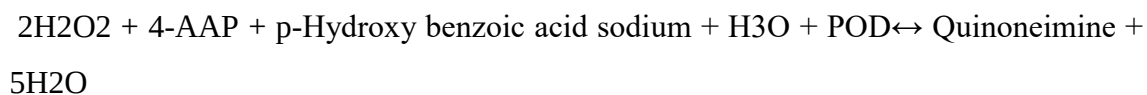
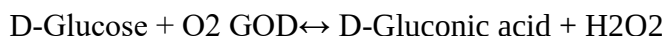
2.3.2.1.E Estimation of Serum Free Triiodothyronine

As previously mentioned in total T4, the test for serum-free T3 determination is a competitive chemiluminescence immunoassay.

2.3.2.2 Determination of Glycemic Indices

2.3.2.2.A Estimation of Fasting Serum Glucose Level

Glucose is oxidized by glucose oxidase (GOD) to produce H₂O₂, which oxidizes 4-Aminoantipyrine (4-AAP) with sodium p-hydroxybenzoic acid to make quinonimine dye in the presence of peroxidase (POD). The rise in absorbency is closely correlated with the amount of glucose present.



2.3.2.2.B Estimation of Fasting Serum Insulin Level

Using an ELISA kit, the fasting serum insulin level was measured. The sandwich principle is the foundation of this kit. An aliquot of patient samples is first incubated in the coat well with an enzyme conjugate, which is a monoclonal anti-insulin antibody coupled with biotin. This is the basic idea of the two-step incubation procedure. During the second incubation phase, the streptavidin-peroxidase enzyme complex binds the

biotin anti-insulin antibody. The quantity of bound peroxidase complex is inversely associated with the amount of insulin present in the sample. The color produced is proportionate to the quantity of insulin in the patient sample when the right insulin standards are used. Spectrophotometric analysis was used to determine the absorbance at 450 nm. μ IU/ml was used to express the results.

2.3.2.2.C Estimation of Insulin Resistance

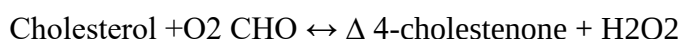
Developed by Matthews in 1985 (84) the homeostasis model assessment-insulin resistance (HOMA-IR) is a non-invasive, quick, inexpensive, and accurate method of estimating insulin resistance. It is computed from fasting serum insulin and fasting serum glucose using the following formula:

$$\text{HOMA} = \text{FSI } (\mu\text{IU/ml}) * \text{FSG (mg/dl)} / 405 (\text{Sengupta et al., 2018})$$

2.3.2.3 determination of lipid profile

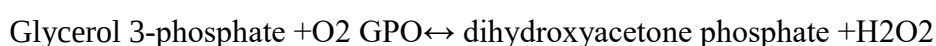
2.3.2.3.A. Estimation of cholesterol

By catalysis of CHE and CHO, cholesterol ester is catalyzed to yield H_2O_2 which oxidate 4-aminoantipyrine with phenol to form a colored dye of quinonimine. The absorbency increase is directly proportional to the concentration of cholesterol.



2.3.2.3.B. Estimation of triglycerides

Through a sequence of enzymatic catalysis steps by lipase ,GK,and GPD, triglycerides is catalyzed to yield H_2O_2 ,which oxidize 4-aminoantipyrinel to yield a colored dye of quinonimine. The absorbency increase is directly proportional to the concentration of triglycerides.



2.3.2.3. C. .Estimation of high density lipoprotein (HDL)

The system monitor the change in absorbance at 600 nm. This change in absorbance is directly proportional to the concentration of cholesterol in the sample and is used by the system to calculate and express the HDL-c concentration.

1. HDL CHE+CHO $\leftrightarrow$ cholestenone +H₂O₂



2. LDL, VLDL, chylomicrons CHE+CHO $\leftrightarrow$ cholestenone +H₂O₂



2.3.2.3.D. Estimation of low-density lipoprotein (LDL)

As previously mentioned in HDL estimation.

2.3.2.3.E. Estimation of very low-density lipoprotein (VLDL)

As previously mentioned in HDL estimation.

2.3.3 Measurement of Body Mass Index

The computation of an individual's Body Mass Index (BMI) is dependent on their height and weight. The BMI, which is measured in kilograms per square meters, is computed by dividing the body weight by the square of the body height .the mass in kilograms and the height in meters, expressed as a meter (kg/m²).

$$\text{BMI} = \text{Weight} / (\text{Height})^2$$

A BMI of 18.5-24.9 indicates normal weight, a BMI of 25–30 indicates overweight, and a BMI of more than 30 indicates obesity.(Golden et al., 2012)

2.3.4 Genetic analysis

2.3.4.1 Extraction of genomic DNA from a blood sample

Genomic DNA was extracted from the whole blood using gsyncTM genomic DNA extraction Mini kit.

Step 1-Sample Preparation

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1. Up to 200µl blood was transferred to a 1.5ml microcentrifuge tube. If the sample volume is less than 200µl, add the appropriate volume of PBS.
2. Proteinase K (20µl) was added to the sample and mix by pipetting, then incubated for 5 minutes at 60°C.

Step 2 –Cell Lysis

3. GSB Buffer (200µl) was added to the sample and mixed by the vortex.
4. The mixture was incubated in a 60°C water bath for 5 minutes to lyse the sample, inverting the tube every 2 minutes.
5. The required elution buffer (for Step 5 DNA elution) was preheated in a 60°C water bath.

Step 3 – Binding

6. Ethanol (96-100%) with volume 200µl was added to the sample and vortexed for 10 seconds. (Pipetting if there is any precipitate.)
7. A GS column was placed in a 2ml collection tube. The sample mixture (including any precipitate) was transferred carefully to GS column, centrifuged for 5 minutes at speed (14,000 - 16,000 x g) for 1 minute To remove contaminations and the 2 ml collection tube was discarded. The GS column was placed in a new 2ml collection tube.

Step 4 – Washing

8. GS column was washed with 400µl W1 Buffer. Then was centrifuged for 1minute at speed (14,000- 16,000 x g) and the flow through was discarded.
9. The GS column was placed back in the 2ml Collection tube. The GS column was washed with 600µl wash buffer (ethanol added), then was centrifuged for 1 minute at full speed (14,000 - 16,000 x g) and the flow-through was discarded.
10. The GS column was placed back in the 2ml collection tube. Then was centrifuged for an additional 3 minutes at full speed (14,000 -16,000 x g) to dry the column.

Step 5 – Elution

11. The dry GS column was transferred in a new 1.5ml microcentrifuge tube.

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12. A volume of 100µl of preheated elution buffer was added to the membrane center of the GS Column.

13. The GS column was left stranded at least 3 minutes to allow elution buffer to be completely absorbed.

14. It was centrifuged for 1 minute at full speed (14,000 - 16,000 x g) to elute the purified DNA.

15. The DNA was stored at -20°C.

2.3.3.2 Restriction fragment length polymorphism polymerase chain reaction (RFLP – PCR)

1. DNA extraction extract DNA from sample.

2. PCR amplification use PCR to amplify aspecific DNA region that contaian aknown restriction site.

3. restriction enzyme (Msel enzyme) treat the PCR product with arestriction enzyme that cut at aspecific sequence, if amutation is present ,it may eliminate anew restriction site. The restriction enzyme Msel digestion was done in 1µl containing 10 units of enzyme Msel with 5µl of *10 buffer and 1µg of PCR product and incubated at 37 °C for 30 min.

4. gel electrophoresis run the digested DNA fragments on agarose gel,smaller fragment move faster.

5. visualization stain the gel with ehidium bromide and view under UV light,analyze the band to detect differences between samples.

2.3.3.2.A. Restriction digestion conditions

1. enzyme selection choose an enzyme that recognize a polymorphic site and make sure the enzyme cut only one allele.

2. reaction PCR Product mix with restriction enzyme and buffer

3. incubation at temperature typically 37 °C for 30min .

2.3.3.2.B Primer preparation

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Polymerase Chain Reaction (PCR) was performed using a specific primer to amplify the desired DNA fragments of the TRA1 gene. Two SNPs were investigated, the first is rs939348; T>C SNP and second was rs12939700; C/A SNP. Restriction fragment length polymorphism -PCR (RFLP-PCR) technique was used to detect rs939348; T>C SNP and Amplification Refractory Mutation System Polymerase Chain Reaction (ARMS-PCR) was used to amplify the DNA fragment encompasses rs12939700; C>A SNP. Primer-BLAST software was used to design the primers, which were then bought as a lyophilized product from oligo, Korea. Each primer was dissolved in specific volumes of nuclease-free water to obtain a stock solution with a concentration of 100 pmol/μl after that, a diluted work solution was made by adding 90μl of nuclease-free water to 10 μl of each stock solution to obtain 10 pmol/μl. This work solution was kept at -20°C until use.

Table 2-3: Primers sequences to detect rs939348 T/C SNP and rs12939700 C>A SNP

Primers		Sequence	Prod uct size (bp)	Referance
Primers sequences of 939348 T > C	F	CCTGTGTCTCCCAGCTTAGC	190	(R.Agus,etal., 2021) Msel enzyme
	R	CCACCAGACTCACAGCCTCT		
Primers sequences 12939700 C > A	F of allele C	AGCTGGGGGCTGAGGGAGACC	355	Primer blast Desgine by dr.Haider Alkaraawi
	F of allele A	AGCTGGGGGCTGAGGGAGACA		
	R	TAGGCCAAGGAAGTGGGTGGGGG		
F: Forward primer, R; Reverse primer				

2.3.3.2.C.Optimization of polymerase chain reaction conditions

The PCR reaction was optimized following a series of tests to determine the ideal annealing temperature and the ideal number of amplification cycles.

2.3.3.2.D. Running the polymerase chain reaction

The PCR reaction was performed by mixing the PCR component with DNA and employing the optimum PCR program as shown in Tables 2-4 and Table 2-5 respectively.

Table 2-4: PCR reaction mixture to amplify a DNA fragment encompasses rs939348 T > C SNP

Component	Volume (µl)
Forward primer	2
Reverse primer	2
DNA template	4
Nuclease free water	4.5
Master mix	12.5
Total volume	25

Table 2-5: PCR reaction mixture to amplify a DNA fragment encompasses rs12939700 C > A SNP

Component	Volume (µl)
Forward primer	2
Reverse primer	2
DNA template	4
Nuclease free water	7
Master mix	10
Total volume	25

Table 2-6: PCR program to detect rs939348 T > C SNP

Steps	Temperatures/c	Time	Cycle
Denaturation	95	3 minutes	1
Initial denaturation	95	35 sec.	30
Annealing	62	45 sec.	
Extension	72	55 sec.	
Final extension	72	5minuts	1

Table 2-7: PCR program to amplify a DNA fragment encompasses rs12939700 C > A SNP

Steps	Temperatures/c	Time	Cycle
denaturation	95	3 minutes	1
Initial denaturation	95	35 sec.	32
annealing	66.6	45 sec.	
extension	72	55 sec.	
Final extension	72	5 minuts	1

2.3.3.3 Agarose Gel Electrophoresis

1. Agarose gel was prepared by dissolving 1.5g of agarose powder in 100ml of 1x TBE buffer (PH 8).

2. Heating the solution on a hot plate until boiling.
3. While waiting for cooling then 4 µl of ethidium bromide was added to the solution.
4. The comb was fixed at the end of the tray for making wells used for PCR product loading.
5. The agarose was gently poured into the tray and waited to solidify at room temperature for 30 min.
6. The comb was gently removed from the tray.
7. The tray was fixed in an electrophoresis chamber and filled with a TBE buffer.
8. PCR products were loaded into the wells directly.
9. The electrophoresis apparatus's voltage was set to ensure an electrical field of 5 v for each cm of the distance between the cathode and anode.
10. At the end of the run which is about 90 min, ultraviolet transilluminator was used at 320-336 nm for band detection.
11. The gel was photographed using a digital camera.

2.3.3.4 Sequencing and sequence alignment

Sequencing for the amplified products that were obtained to detect rs12939700 C > A SNP was done by Microgen company, Korea using the forward primer. The National Center for Biotechnology Information's (NCBI) website's Basic local alignment search tool (BLAST) and MEGA7 were both used for homology searches to produce the alignment results. The results were compared with reference sequences of the TRA1 gene obtained from the gene bank as controls.

2.4 Statistical Analysis

The collected data of the present study were entered from patients' sheets and analyzed through the Statistical Package for the Social Sciences (SPSS version 26). The data were presented as frequencies and percentages or mean and standard deviation in appropriate tables and graphs. The distribution of alleles and genotypes in accordance with Hardy-Weinberg equilibrium was examined using the goodness of fit test, followed by a Chi-square analysis. The P value of 0.05 or less was regarded as statistically significant.



Chapter Three



Results

3. Results

3.1. Demographic Analyses of The Study Participants:

The demographic and clinical analyses of the study participants all of whom were females including 50 healthy and 100 patients with hypothyroidism undergoing L-thyroxine treatment was presented in the **(Table3.1)**. The data showed significant differences between the two groups, the mean age of the healthy control group 51.40 ± 8.776 comparing to the patients 52.76 ± 9.358 with a P value 0.3932, indicating no statistically differences in the age. Highly significant differences were noticed in the term of weight/Kg, healthy females had a mean of 71.66 ± 9.983 compared to 84.88 ± 15.96 in hypothyroid patients with a P value of <0.0001 . More likely the BMI/ kg/m² was dramatically higher in the patients (33.26 ± 5.929) than in healthy individuals 28.38 ± 3.600 also with a P value <0.0001 . HT prevalence was higher in the patients with 66% (n=66) compared to 6% (n=3) in the healthy group, resulting in a P value of 0.0272. Parallely, the healthy women had 94%(n=47) without hypertension, whereas 34%(n=34) of patients were without hypertension. Obviously, blood pressure measurement was further certified these findings: the mean SBP was 124.6 ± 7.549 in healthy people compared to 133.8 ± 15.80 in the hypothyroid females with a P value of <0.0001 . In addition, healthy females showed the mean DBP 81.00 ± 5.425 while the hypothyroid patients showed 84.61 ± 7.184 with a P value of 0.0021, indicating significant differences in both SBP and DBP. DM was showed in 37% of hypothyroid patients compared to 16% of healthy individuals, with a P value of 0.048. conversely, 84% of healthy females and 63% of the patients did not have DM. The duration of L-thyroxine treatment among patients was varied as follow: 42% of the patients had been on the treatment for 1-5 years, 44% of the patients had been on the treatment for 6-10 years, 8% for 11-15 years, 3% for 16-20 years, and another 3% for 21-25 years. Regarding the dosage of L-thyroxine treatment among patients also varied between 25mcg-250mcg with specific patient percentage as shown in the Table1. To sum up, the data demonstrated a significant difference between healthy and hypothyroid patients in body weight, BMI, HT, BP, and DM prevalence with patients exhibited higher values in all these parameters.

Table 3.1: Demographic analyses of the study participants

Parameters	Healthy, n=50	Patients, n=100	P value
Age/year, mean $\pm$ SD	51.40 ± 8.776	52.76 ± 9.358	0.3932

40-49, (n)%	(22), 44	(35), 35	
50-59, (n)%	(16), 32	(38), 38	
60-69, (n)%	(12), 24	(22), 22	
70-79, (n)%	-	(4), 4	
80-89, (n)%	-	(1), 1	
Wight/Kg, mean±SD	71.66 ± 9.983	84.88 ± 15.96	<0.0001
BMI/(kg/m²), mean±SD	28.38 ± 3.600	33.26 ± 5.929	<0.0001
Underweight, (n)%	-	-	0.424
Normal, (n)%	(5), 10	(5), 5	
Overweight, (n)%	(30), 60	(27), 27	
Obese, (n)%	(15), 30	(68), 68	
HT			
Yes, (n)%	(3), 6%	(66), 66%	0.0272
No, (n)%	(47), 94%	(34), 34%	
SBP, mean±SD	124.6±7.549	133.8±15.80	0.0001
DBP, mean±SD	81.00±5.425	84.61±7.184	0.0021
DM			
Yes, (n)%	(8), 16	(37), 37	0.048
No, (n)%	(42), 84	(63), 63	
Duration of L-thyroxin treatment/Years, (n)%			
1-5	-	(42), 42%	-
6-10	-	(44), 44%	-
11-15	-	(8), 8%	-
16-20	-	(3), 3%	-
21-25	-	(3), 3%	-
Dose of L-thyroxin mcg, (n)%			
25	-	(3), 3%	

50	-	(15), 15%	
75	-	(12), 12%	
100	-	(56), 56%	
125	-	(4), 4%	
150	-	(6), 6%	
200	-	(2), 2%	
225	-	(1), 1%	
250	-	(1), 1%	

Data is presented as mean±SD or percentages. P<0.05 is considered significant. SBP refers to Systolic blood pressure, DBP refers to Diastolic blood pressure, BMI refers to body mass index, DM refers to diabetes mellitus.

3.2. Analyses of Plasma Thyroid Hormones and Metabolic Markers in Patients and Healthy Control Groups:

The plasma thyroid hormone levels and metabolic biomarkers in female patients with hypothyroidism and healthy individuals were presented in the **(Table3.2)**. Interestingly, the data revealed that the plasma thyroid stimulating hormone (TSH) levels were dramatically high in the hypothyroid patients (4.300 ± 4.708) $\mu\text{U/ml}$ compared to the healthy people (2.130 ± 0.8125) $\mu\text{U/ml}$ with a P value of <0.0015 and a 95% CI 0.8421 to 3.497. Free triiodothyronine (FT3) plasma levels were also higher significantly in patients (5.108 ± 0.979 pmol/L) group compared to the healthy control group (5.450 ± 0.997 pmol/L) with a P value of 0.0483. Conversely, FT4 levels were statistically significant lower in the patient plasma (13.38 ± 2.283 pmol/L) compared to the healthy individuals (14.60 ± 1.804 pmol/L) with a P value of <0.001 and 95% CI (-1.280 to 0.3973). however, we did not notice statistically significant differences in Total Triiodothyronine (TT3) and Total thyroxine (TT4) plasma levels between the groups as shown in the Table3.2.

Metabolic biomarkers analyses indicated that the hypothyroid patients had higher insulin plasma levels with a P value of <0.0001. Similarly, hypothyroid patients presented higher fasting plasma glucose (FPG) (119.4 ± 41.22 mg/dl) compared to the healthy control females (99.13 ± 12.97 mg/dl) with a P value of 0.004 and a 95% CI equal to (5.639 to 29.96). furthermore, the hemostatic model assessment for insulin resistance (HOMA-IR) was dramatically elevated in Hypothyroid patient (5.166 ± 3.186) compared to healthy

individuals (2.120 ± 0.981) with a P value of <0.0001 . Hence, the Hypothyroid patient showed a significant alteration in the thyroid function and metabolic hemostatic compared to the healthy control group.

Table 3.2: Plasma Thyroid Hormone and Metabolic Markers In Patients And Control Groups.

Parameters	means $\pm$ SD		P value	95% CI
	Control N=50	Patients N=100		
TSH, μ U/ml	2.130 ± 0.8125	4.300 ± 4.708	<0.0015	0.8421 to 3.497
TT3, ng/ml	1.726 ± 0.4442	1.772 ± 1.753	0.8546	-0.234 to -0.0214
FT3, pmol/L	5.108 ± 0.979	5.450 ± 0.997	0.0483	0.05292 to 0.6709
TT4, nmol/L	141.8 ± 14.56	138.3 ± 24.71	0.3480	-11.06 to 3.921
FT4, pmol/L	14.60 ± 1.804	13.38 ± 2.283	<0.001	-1.280 to 0.3973
Insulin, μ U/ml	8.552 ± 3.411	16.90 ± 9.827	<0.0001	5.012 to 10.74
FPG, mg/dl	99.13 ± 12.97	119.4 ± 41.22	<0.0044	5.639 to 29.96
HOMA-IR	2.120 ± 0.981	5.166 ± 3.186	<0.0001	2.133 to 3.959
*TSH=thyroid stimulating hormone, TT3=total thyroid hormone, FT3=free thyroid hormone, TT4=total thyroid hormone, FT4=free thyroid hormone, FPG=fasting plasma glucose. Data presented as mean $\pm$ SD, P value $\leq$ 0.05 considered significant.				

3.3. Analyses of blood lipid profile in hypothyroid patients and healthy control group:

Blood lipid profile of hypothyroid patients was compared to healthy individuals as presented in the (Table3.3). Interestingly, patients exhibited significantly higher levels of cholesterol (188.8 ± 39.51 mg/dl) compared to the healthy control (160.3 ± 29.45 mg/dl) with a P value of 0.0001. More likely, Triglycerides (TG) levels were significantly elevated in the blood of hypothyroid patients compared to the healthy people with a P value of 0.002. Unlike cholesterol and TG, there were not statistically significant differences observed in high density lipoprotein (HDL) cholesterol between the two groups with a $P=0.257$, nor in low density lipoprotein (LDL-CHO) cholesterol with a $P=0.145$. Surprisingly, the patients had significantly lower levels of very low-density lipoprotein (VLDL) cholesterol (28.15 ± 13.136 mg/dl) compared to the healthy

individuals (33.81 ± 11.799 mg/dl) with a P value of 0.011. These findings highlighted the significant differences in cholesterol and TG levels between hypothyroidism and controls, indicating the potential influence of thyroid function on lipid metabolism specifically cholesterol and triglycerides.

Table 3.3: Plasma blood lipid profiles in patients and control groups.

Parameters mg/dl	means $\pm$ SD		P value
	Healthy N=50	Patients N=100	
Cholesterol	160.3 $\pm$ 29.45	188.8 $\pm$ 39.51	0.0001
TG	111.22 $\pm$ 34.797	141.91 $\pm$ 63.952	0.002
HDL	48.14 $\pm$ 12.884	50.46 $\pm$ 11.213	0.257
LDL	101.91 $\pm$ 24.445	110.08 $\pm$ 35.414	0.145
vLDL	33.81 $\pm$ 11.799	28.15 $\pm$ 13.136	0.011
*Data presented as mean $\pm$ SD, P value $\leq$ 0.05 considered significant. TG=triglyceride, HDL=high density lipoprotein, LDL=low density lipoprotein, VLDL=very low-density lipoprotein.			

3.4. Duration and distribution of L-thyroxine treatment among patients with hypothyroidism

Table3.4 represents the distribution of hypothyroid patients based on the L-thyroxine treatment in years and the percentage within each category. Noticeably, the data reveals that 42% of the patients have been on the treatment for 1-5 years, followed by 44% have been on the treatment for 6-10 years. However, a smaller proportion of patients have been undergoing treatment for longer time distributed as follow: 8% for 11-15 years, and 3% each for 16-20 years and 21-25 years. Then we measured plasma thyroxine hormones levels based on the L-thyroxine treatment in years as presented in the (**Table3.5**). Clearly the data showed consistent values for various parameters over time. TSH means level remained relatively stable. Similarly, there were no significant changes observed in the levels of TT3, FT3, TT4 and FT4 over the observed periods, which indicating stability in hormone regulation despite varying treatment durations.

Table 3.4: Represents duration of L-thyroxin treatment, mcg /year

Duration of treatment (years)	Patients percentage
1-5	42
6-10	44
11-15	8
16-20	3
21-25	3

Table 3.5: Represents plasma hormone levels according to the duration of L-thyroxine treatment (mcg/year)

Duration of treatment (years)	TSH Mean $\pm$ SD	TT3 Mean $\pm$ SD	FT3 Mean $\pm$ SD	TT4 Mean $\pm$ SD	FT4 Mean $\pm$ SD	P value
1-5	5.817 $\pm$ 5.912	1.634 $\pm$ 0.2191	5.475 $\pm$ 0.8589	134 $\pm$ 26.45	13.01 $\pm$ 2.487	0.556
6-10	3.647 $\pm$ 3.546	1.573 $\pm$ 0.2129	1.573 $\pm$ 0.2129	138.7 $\pm$ 23.06	13.61 $\pm$ 2.151	0.654
11-15	1.913 $\pm$ 1.140	1.544 $\pm$ 0.1111	5.621 $\pm$ 0.9615	144.5 $\pm$ 26.55	14.07 $\pm$ 1.796	0.587
16-20	1.08 $\pm$ 1.195	7.54 $\pm$ 9.929	5.44 $\pm$ 0.8073	146.5 $\pm$ 11.48	12.86 $\pm$ 1.985	0.564
21-25	2.223 $\pm$ 1.348	1.477 $\pm$ 0.125	5.293 $\pm$ 0.7422	165.8 $\pm$ 9.362	14.01 $\pm$ 3.081	0.7654
The data was presented as Mean $\pm$ SD						

3.5. Impact of L-thyroxine treatment on the plasma thyroid hormone levels in hypothyroid patients:

Next, we studied the impact of L-thyroxine treatment on plasma thyroid levels in hypothyroid patients, comparing those treated for 5 years or less with those who treated for more than five years as shown in the (Table3.6). Interestingly, the mean TSH level significantly reduced in patients treated for more than 5 years ($3.202 \pm 3.230 \mu\text{U/ml}$) compared to those who treated for 5 years or less ($4.774 \pm 3.395 \mu\text{U/ml}$) with a P value 0.022, which may indicate that longer treatment duration may improve the response to the treatment allowing the thyroid gland to be function more normally. Unlike TSH levels, no significant changes were observed between the two groups in the mean levels of TT3 ($1.634 \pm 0.2191\text{ng/ml}$ compared to $1.873 \pm 2.298\text{ng/ml}$, $P=0.5042$), FT3 ($5.475\pm0.8589\text{pmol/L}$ compared to $5.431\pm1.094\text{pmol/L}$, $P=0.882$), TT4 ($134.0 \pm 26.45\text{nmol/L}$ compared to $141.3 \pm 23.13\text{nmol/L}$ with a $P=0.1460$), and FT4 ($13.01 \pm$

2.487pmol/L vs 13.66 ± 2.103 pmol/L, $P=0.1627$), suggesting that these thyroid hormones remain stable regardless of the duration of L-thyroxine treatment.

Table 3.6: Effect the duration of L-thyroxine on the plasma thyroid levels in hypothyroid patients

Parameters	Duration of L-thyroxine treatment		P value
	≤5 Years	>5 Years	
TSH, μ U/ml	4.774 ± 3.395	3.202 ± 3.230	0.0225
TT3, ng/ml	1.634 ± 0.2191	1.873 ± 2.298	0.5042
FT3, pmol/L	5.475 ± 0.8589	5.431 ± 1.094	0.882
TT4, nmol/L	134.0 ± 26.45	141.3 ± 23.13	0.1460
FT4, pmol/L	13.01 ± 2.487	13.66 ± 2.103	0.1627
*TSH=thyroid stimulating hormone, TT3=total thyroid hormone, FT3=free thyroid hormone, TT4=total thyroid hormone, FT4=free thyroid hormone. Data presented as mean $\pm$ SD, P value $\leq$ 0.05 considered significant.			

3.6. Impact the duration of L-thyroxine treatment on the clinical biomarkers in hypothyroid patients

We were interested on the studying the effect of L-thyroxine duration on various clinical biomarkers in Hypothyroid patient as shown in the **(Table3.7)**. Patients who treated for more than 5 years showed slightly higher Insulin levels (17.41 ± 11.21 μ U/ml) compared to those who treated for 5 years or less (16.20 ± 7.583 μ U/ml), though these differences were not statistically significant ($P=0.5461$). FPG was significant lower in the patients who treated for more than five years (107.9 ± 24.38 mg/dl) compared to those who treated for five years or less (127.2 ± 53.43 mg/dl with a P value=0.0176). interestingly, the HOMA-IR showed no significant changes between the two groups (5.150 ± 2.867 vs 5.178 ± 3.424 , $P=0.966$). total cholesterol was slightly lower in the long-term treatment group (181.3 ± 39.81 mg/dl compared to the short treatment group 189.9 ± 45.52 , $P=0.3171$). more than five years L-thyroxine treatment significantly lowered TG plasma levels ($P=0.0441$) compared to those who treated for 5 years or less. HDL-cholesterol levels were similar between the two groups (50.59 ± 12.38 vs 50.37 ± 10.40 , $P=0.9221$). LDL-cholesterol levels were significantly lower in the long-term treatment group compared to the short-term group (102.8 ± 35.10 vs 116.6 ± 32.10 , $P=0.0499$). lastly, L-thyroxine long term treatment did not change vLDL-cholesterol levels compared to short term treatment (29.75 ± 14.59 vs 26.99 ± 11.97 , $P=0.3022$). Therefore,

these data suggesting that longer duration of L-thyroxine may improve glucose metabolism and blood lipid profile in the females with hypothyroidism.

Table 3.7: Effect the duration of L-thyroxine on the biochemical markers in hypothyroid patients.

Biomarkers	Duration of L-thyroxine treatment		P value
	≤5 Years	>5 Years	
Insulin, $\mu\text{U/ml}$	16.20 $\pm$ 7.583	17.41 $\pm$ 11.21	0.5461
FPG, mg/dl	127.2 $\pm$ 53.43	107.9 $\pm$ 24.38	0.0176
HOMA-IR	5.150 $\pm$ 2.867	5.178 $\pm$ 3.424	0.9662
CHO mg/dl	189.9 $\pm$ 45.52	181.3 $\pm$ 39.81	0.3171
TG mg/dl	154.3 $\pm$ 72.82	129.3 $\pm$ 48.70	0.0441
HDL mg/dl	50.59 $\pm$ 12.38	50.37 $\pm$ 10.40	0.9221
LDL mg/dl	116.6 $\pm$ 32.10	102.8 $\pm$ 35.10	0.0499
vLDL mg/dl	29.75 $\pm$ 14.59	26.99 $\pm$ 11.97	0.3022
*Data presented as mean $\pm$ SD, P value $\leq$ 0.05 considered significant. TG=triglyceride, HDL=high density lipoprotein, LDL=low density lipoprotein, VLDL=very low-density lipoprotein.			

3.7. Study age related variations in thyroid hormones and metabolic biomarkers in hypothyroidism and healthy controls:

To examine whether thyroid function and metabolic parameters are intricately linked and can be impacted by age, we measured different variations including: thyroid hormones, glucose metabolism and insulin resistance across different age groups as presented in the (Table3.8). Significant differences were observed across several parameters. Not surprisingly, TSH levels were consistently higher in hypothyroid patients across all age groups compared to the healthy control females, with the most pronounced differences noted in the 50-59 age category (5.233 $\pm$ 5.795 $\mu\text{U/ml}$ in patients vs 2.041 $\pm$ 0.759 in healthy people, P <0.01). for the age categories 70-79 and 80-89, comparisons between patients and healthy females were not possible due to the absence of healthy participants in these age groups in our study. Interestingly, we did not find significant age-related changes in the levels of TT3 nor in the levels of TT4 as presented in the Table3.8. We noticed that FT4 significantly reduced based on age groups, as we see in the Table8 and Figure3D age category 40-49 years FT4 levels were 12.92 $\pm$ 1.752 in patients vs 14.086 $\pm$ 2.164 in healthy individuals with a P<0.01. Also, age group 50-59years FT4 levels were 13.192 $\pm$ 2.49nmol/L in Hypothyroid patient vs

14.714±1.857nmol/L in healthy control people with a P<0.01 as shown in the **Table3.8**. FT3 was elevated in hypothyroid patients, particularly in the 60-69 age group (5.646±0.961pmol/L vs 4.696±0.905pmol/L with a P<0.01. Insulin levels were significantly elevated in Hypothyroid patient compared to the healthy control participants across the age groups 40-49years and 50-59years, with a P value less than 0.001. correspondingly, FPG and HOMA-IR were markedly higher in Hypothyroid patient compared to the healthy control groups across all age categories with a P value less than 0.01, as shown in the **Table3.8**. Hence these findings suggest that hypothyroidism is associated with significant disruptions in both thyroid function and metabolic regulation, with variations becoming more notable with advancing age.

Table 3.8: Age related variations in thyroid hormone and metabolic parameters in hypothyroid females compared to healthy individuals.

Parameters	Age category/ Years mean±SD							
	40-49		50-59		60-69		70-79	80-89
	Healthy N=22	Patients N=35	Healthy N=16	Patients N=38	Healthy N=12	Patients N=22	Patients N=4	Patients N=1
TSH, μ U/ml	1.956±0.73	4.821±5.258	2.041±0.759	5.233±5.795	2.429±0.886	3.883±2.071	3.153±2.541	5.27±0.00
P value	0.01		0.01		0.01		-	
TT3, ng/ml	1.708±0.49	2.082±2.9476	1.666±0.314	1.626±0.201	1.83±0.512	1.613±0.29	1.4425±0.21	1.31±0.00
P value	0.9401		>0.999		0.9983		-	
FT3, pmol/L	4.761±0.72	5.281±0.982	5.431±0.949	5.51±0.832	4.696±0.905	5.646±0.961	6.542±1.228	6.21±0.00
P value	0.0437		.9996		0.0139		-	
TT4, nmol/L	142.881±14.774	137.243±23.774	142.868±14.119	138.029±24.629	138.475±15.489	143.353±27.074	120.857±22.978	139.4±0.00
P value	0.994		0.897		0.995		-	
FT4, pmol/L	14.086±2.164	12.92±1.752	14.714±1.857	13.192±2.49	13.907±1.396	13.831±2.203	12.225±2.044	14.61±0.00
P value	0.0342		0.0327		0.987		-	
Insulin, μ U/ml	8.3±2.55	16.742±7.93	7.901±3.387	17.49±11.3	10.006±3.84	16.309±10.7	18.92±10.034	19.99±0.
P value	0.0042		0.0026		0.3835			

FPG, mg/dl	98±12.066	115.744±38. 357	101.687± 9.06	130.657± 53.887	95.583±8. 511	109.45 4±21.9 45	105.25 ±17.65 1	101±0.0
P value	0.0460		0.0382		0.0443		-	
HOMA- IR	2.14±0.94	4.677±2.555	1.925±0. 569	5.535±3. 492	2.127±1.4 51	5.133± 3.47	7.975± 2.99	5±0.00
P value	0.0075		0.0001		0.0319		-	
*TSH=thyroid stimulating hormone, TT3=total thyroid hormone, FT3=free thyroid hormone, TT4=total thyroid hormone, FT4=free thyroid hormone. Data presented as mean±SD, P value≤0.05 considered significant.								

3.8. Study age related variations in blood lipid profiles among hypothyroidism and healthy individuals:

In examining age-related variations in blood lipid profiles between Hypothyroid patient and healthy people, several notable emerged across different age categories as presented in the (Table3.9). For the age categories 70-79 and 80-89, comparisons between patients and healthy females were not possible due to the absence of healthy participants in these age groups in our study. Total cholesterol levels were consistently elevated in Hypothyroid patient across different age groups compared to the healthy females. In the age 40-49 (187.657 ± 29.344 mg/dl in patients group compared to 154.772 ± 23.382 mg/dl in healthy control with a P value less than 0.05), at age 50-59 (191.361 ± 41.631 mg/dl in hypothyroidism group vs 155.133 ± 22.918 mg/dl in the healthy individuals with a $P < 0.05$), at age 60-69 (198.8 ± 58.988 mg/dl in the patients compared to 154.555 ± 21.772 mg/dl in the healthy control with a P-value less than 0.01). More likely, TG levels were significantly elevated in Hypothyroid patient compared to healthy individuals in the age groups 40-49 and 50-59, but not in the 60-69 age group. HDL showed variability between the two groups but consistently remained not significant across all age groups . although LDL levels showed no statistically differences across all age groups, they were generally higher

in Hypothyroid patient compared to the healthy individuals . vLDL levels showed mixed patterns but tended to be lower in the patients across all the age groups, in particular those aged 70-79 and 80-89. However, these findings shed light on the complex interplay between hypothyroidism and lipid metabolism across different stages of adulthood, emphasizing potential implications for cardiovascular healthcare in this population.

Table 3.9: Age related variations in blood lipid profiles in females with hypothyroidism compared to healthy individuals.

Parameters	Age category/ Years mean±SD							
	40-49		50-59		60-69		70-79	80-89
	Healthy N=22	Patients N=35	Healthy N=16	Patients N=38	Healthy N=12	Patients N=22	Patients N=4	Patients N=1
CHO	154.772±2 3.382	187.657 ±29.344	155.133 ±22.918	191.36 1±41.6 31	154.555 ±21.772	198.8± 58.988	176.25 ±49.22 9	191±0.0 0
P value	0.0178		0.0226		0.0383		-	
TG	96.5±30.1 64	148.187 5±51.83	113±21. 095	169.13 8±69.4 45	131.5±4 4.056	145.22 7±69.6 37	126.5± 48.617	94±0.00
P value	0.0112		0.0141		0.9820		-	
HDL	46.272±13 .302	50.571± 9.55	53.125± 8.845	48.710 ±11.17 7	44.916± 15.506	53.059 ±13.02 8	46.25± 10.242	73±0.00
P value	0.7520		0.7993		0.3768		-	
LDL	94.209±26 .663	109.742 8±26.35 5	100.856 ±17.694	110.69 4±34.2 73	117.441 ±22.234	106.10 4±49.6 41	131.9± 31.354	99.2±0.0 0
P value	0.4856		0.9085		0.9230		-	
vLDL	33.5 ± 12.12	28.26 ± 11.37	30.6625 ± 12.47	29.418 ± 15.06	38.58 ± 9.40	26.42 ± 13.22	27.05 ± 11.60	18.8 ± 0
P value	0.6635		0.9995		0.0937		-	
*Data presented as mean±SD, P value≤0.05 considered significant. TG=triglyceride, HDL=high density lipoprotein, LDL=low density lipoprotein, VLDL=very low-density lipoprotein.								

3.9. Genotyping of *THRA1* rs939348 T>C gene:

In the **Figure3.1**, the RFLP-PCR yielded a clear 190 bp and 142 bp amplicons, which were comparison with a 100-1500 bp DNA ladder. Lane M refers to the DNA ladder for size reference. Lanes 1-12 refer to the individuals (10-23) represent TT genotype (wild alleles), lanes (24 and 25) refer to the mutant genotype (CC, homozygote), and (34 and 42) indicate the mutant genotypes (CT/TC, heterozygote). The electrophoresis gel was conducted at 45V.

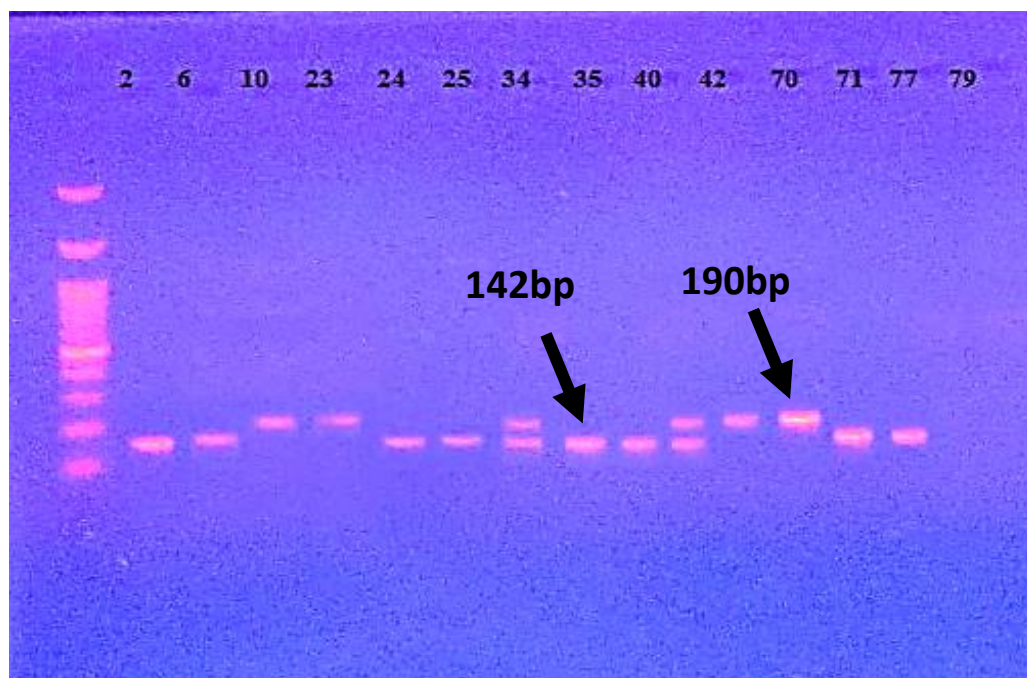


Figure 3.1: Detection of *THRA1* rs939348 T>C genetic polymorphism by using PCR-RFLP. Lane M refers to the DNA ladder for size reference. Lanes 1-12 refer to the individuals, (10-23) represent TT genotype (wild alleles), lanes (24 and 25) refer to the mutant genotype (CC, homozygote), and (34 and 42) indicate the mutant genotypes (CT/TC, heterozygous). The electrophoresis gel was conducted at 45V.

3.10 Examine the association of *THRA1* rs939348 T>C genotypes with hypothyroid risk: a comparative study:

Next, we were interested in understanding gene polymorphism in particular *THRA1* rs939348 T>C gene variations in the context of hypothyroidism. Studying the rs939348 T>C polymorphism may help to understand the genetic basis of hypothyroidism and identify individuals who might be at higher risk for developing the disease. Our findings revealed that the frequency distribution of the *THRA1* rs939348 T>C genotypes had a significant difference between healthy females and Hypothyroid patient as presented in the (**Table3.10**). The TT genotype (wild alleles) was significantly more common in

healthy control (80%) compared to patients (33%), suggesting a promising protective effect $P<0.001$. Conversely, the CC genotype (mutant homozygote alleles) was more prevalent in patients (48%) than in healthy individuals (10%), indicating a strong correlation with increased risk of hypothyroid $P<0.001$. The CT genotype (mutant heterozygote alleles) showed a higher frequency in patients (19%) than in healthy people (10%), but this difference was not statistically significant $P=0.199$, indicating it might not be a major risk factor for the disease. The pairwise comparison of allele frequencies for the *THRA1* rs939348 gene variations among hypothyroid patients, using Chi-square (χ^2) tests, to calculate the differences as shown in the Table3.10. The comparisons revealed a substantial statistical difference between wild genotypes (TT) and mutant genotypes (CT and CC) with a $P<0.001$. These data indicated that *THRA* rs939348 T>C genotypes are significantly different from one another among hypothyroidism patients, underscoring the potential genetic influence of the *THRA* rs939348 T>C gene variations on the disease.

Table 3.10: Frequency distribution of *THRA1* rs939348 T>C in healthy individuals and Hypothyroid patient

Genotype, (n)%	Healthy, n=50	Patients, n=100	P value
Wild (TT)	(40), 80	(33), 33	<0.001
Mutant heterozygous (CT)	(5), 10	(19), 19	0.199
Mutant homozygous (CC)	(5), 10	(48), 48	<0.001
Chi-square was used to find the differences between healthy and patients genotypes. Data was presented as number and frequencies.			

Table 3.11: Pairwise comparison of *THRA1* rs939348 T>C allele frequencies in hypothyroid patient

Pairwise comparisons	Chi-Square (χ^2)	p-value
TT vs. CT	83.12	<0.001
TT vs. CC	343.09	<0.001
CT vs. CC	496.75	<0.001

Table3.12 present the data of pairwise comparisons of alleles frequencies for the *THRA1* rs939348 T>C polymorphism among healthy individuals, using chi-square (χ^2) tests. The TT (wild type) and CT (mutant heterozygous) genotypes yielded a chi-square value of 54.44 with a p-value of <0.0001, indicating a highly significant difference. Similarly, the TT vs. CC (mutant homozygous) comparison also showed a chi-square value of 54.44 with a p-value of <0.0001, demonstrating another significant difference. However, the

comparison between the CT and CC genotypes resulted in a chi-square value of 0 with a p-value of 1, indicating no significant difference between these two genotypes. These results indicating that although the wild alleles (TT) significantly differ in frequency from both mutant genotypes (CT and CC) in healthy people, the frequencies of the mutant genotypes do not differ significantly from each other.

Table 3.12: Pairwise comparison of *THRA1*rs939348 T>C allele frequencies in healthy subjects.

Pairwise Comparison	Chi-Square (χ^2)	p-value
TT vs. CT	54.44	<0.0001
TT vs. CC	54.44	<0.0001
CT vs. CC	0	1

3.11. Analyses of *THRA1* rs939348 T>C genotype frequencies in hypothyroid patient and healthy individuals using Hardy-Weinberg equilibrium:

The analyses of the *THRA1* rs939348 T>C genotype frequencies in Hypothyroid patient using Hardy Weinberg equilibrium. The calculation involved of 100 sample size and comparing observed frequencies and those expected under Hardy-Weinberg equilibrium. The statistical analyses were assessed using Fischer exact test. The observed genotype frequencies were 42.5% for the TT allele and 57.5% for the C allele. The expected frequencies under Hardy-Weinberg equation were 18.1% for the T allele, 33.1% for the C allele, and 48.9% for the TC/CT genotype. We found significant deviation from Hardy-Weinberg expectation with a P-values of 0.0002 for the TT genotype, 0.0001 for the CT genotype, and 0.001 for the CC genotype, all below the significant threshold of 0.05, indicating a statistically relevant difference between the observed and expected genotype frequencies in the patient population.

Healthy control group of 50 individuals showed that the observed genotype frequencies were 85% for the allele TT and 15% for the allele C as presented in the (Table3.14). Under Hard-Weinberg equilibrium calculations, the T allele showed 72.25%, 2.25% for the allele C and 25.5% for the TC/CT genotype. Interestingly Fischer exact test results showed notable differences between Hardy-Weinberg analyses with a P value of 0.0002 for the TT genotype. 0.001 for the CC genotype, and 0.001 for the CT/TC genotype. This suggests meaningful differences between the observed and expected genotype in the healthy control population.

Table 3.13: Analyses of THRA rs939348 T>C genotype frequencies: Hardy-Weinberg and Fischer exact test calculations in hypothyroid patients.

Genotype/observed % n=100		Hardy–Weinberg equilibrium/ expected %			Fischer exact test	P value
T	C	T	C	TC/CT	TT/observed vs TT/expected	0.0002
0.425	0.575	0.180625	0.330625	0.48875	CT/observed vs CT/expected	0.0001
					CC/observed vs CC/expected	0.001
P <0.05 considered significant, data was showed as percentage.						

Table 3.14: Analyses of THRA rs939348 T>C genotype frequencies: Hardy-Weinberg and Fischer exact test calculations in healthy control people.

Genotype/observed % n=50		Hardy–Weinberg equilibrium/ expected %			Fischer exact test	P value
T	C	T	C	TC/CT	TT/observed vs TT/expected	0.0002
0.85	0.15	0.7225	0.0225	0.255	CT/observed vs CT/expected	0.0001
					CC/observed vs CC/expected	0.0001
P <0.05 considered significant, data was showed as percentage.						

3.12. Genotyping of *THRA1* rs12393700 C>A gene:

In the Figure3.2, the AS-PCR yielded a clear 355 base pair (bp) amplicon, which was comparison with a 100-1500 bp DNA ladder. Lane M refers to the DNA ladder for size reference. Lanes # refer to the individual's identification numbers. The samples were loaded in the two wells, for example (2-2) as shown in the figure8. If the bands appear in both wells, the sample is considered as wild-type (CC), indicating two copies of the normal allele. If the band appear in only one well the sample counted as mutant (CA, heterozygous). If no bands appear in the either well, the sample counted as mutant (AA, homozygous), indicating two copies of mutant allele. The electrophoresis gel was conducted at 45V.

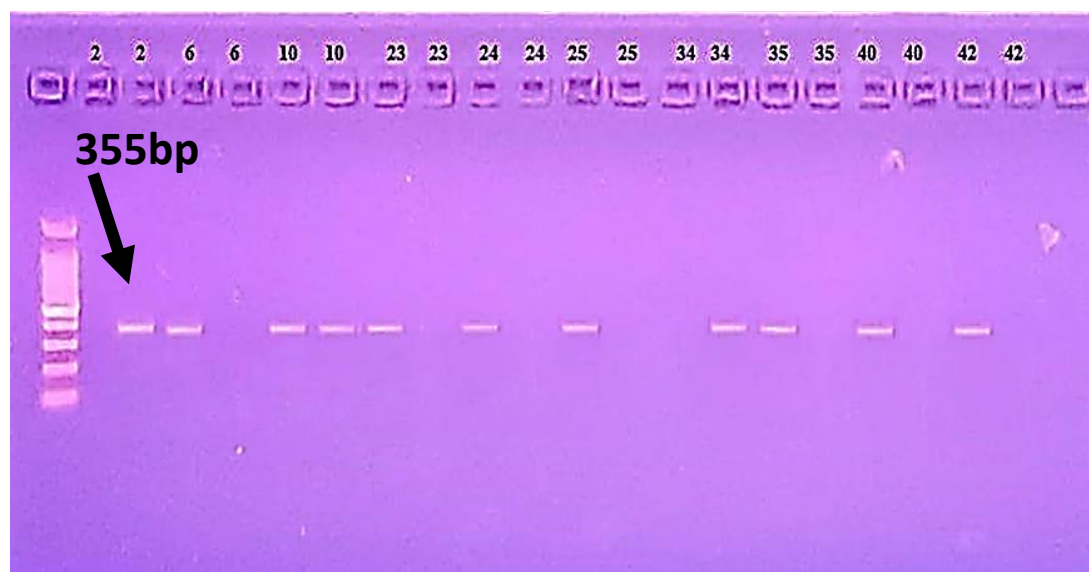


Figure 3.2: Detection of *THRA1* rs12393700 C>A gene polymorphism by using AS-PCR. Lane M refers to the DNA ladder for size reference. Lanes numbers refer to the individual's identification number. (10-10) represent CC genotype (wild alleles), lanes (34-34) refer to the mutant genotype (AA, homozygote), and (42-42) indicate the mutant genotypes (CA/AC, heterozygous). The electrophoresis gel was conducted at 45V.

3.13. Investigating the association of *THRA1* rs12393700 C>A genotypes with hypothyroidism risk: a comparative study:

Investigating the frequency distribution of the *THRA1* rs12393700 C>A polymorphism in healthy and Hypothyroid patient helps in understanding genetic susceptibility to hypothyroidism by unmasking any significant correlation between gene variations and the disease development. The results revealed that the frequency distribution of the *THRA1* rs12393700 C>A genotypes had a significant difference between healthy group and hypothyroid patient as presented in the (Table3.15). All the healthy samples (sample size=50, 100%) exhibited the wild type CC genotype. 90% of the Hypothyroid patient exhibited the wild type CC genotype, 6% were mutant heterozygous (CA), and 4% were mutant homozygous (AA). The differences in genotype frequencies between healthy individuals and Hypothyroid patient were statistically significant, with p-values of less than 0.001 for all comparisons (CC, CA, and AA genotypes). These results, derived from a Chi-square test, indicate a notable disparity in the distribution of the *THRA1* rs12393700 C>A genotypes between the two groups.

The pairwise comparison of allele frequencies for the *THRA1* rs12393700 C>A gene variations among hypothyroid patients, using Chi-square (χ^2) tests, to calculate the differences as shown in the **Table3.16**. The comparisons revealed a substantial statistical difference between wild genotypes (CC) and mutant genotypes (CA and AA) with a $P < 0.001$. However, the comparison between mutant homozygous (AA) and mutant heterozygous (CA) yielded χ^2 value of 0.25 with a P-value 0.123 indicating no notable differences between these two genotypes. These data indicated that there was a substantial difference between wild and both mutant genotypes, but not between the two mutant genotypes in hypothyroid patients.

Table 3.15: Frequency distribution of THRA rs12393700 C>A in healthy individuals and Hypothyroid patient

Genotype, (n)%	Healthy, n=50	Patients, n=100	P value
Wild (CC),	(50), 100	(90), 90	<0.001
Mutant heterozygous (CA)	0	(6), 6	<0.001
Mutant homozygous (AA)	0	(4), 4	<0.001
Chi-square was used to find the differences between healthy and patients genotypes. Data was presented as number and frequencies.			

Table 3.16: Pairwise comparison of THRA rs12393700 C>A allele frequencies in Hypothyroid patient

Pairwise comparisons	Chi-Square (χ^2)	p-value
CC vs. CA	63.64	<0.001
CC vs. AA	68.18	<0.001
CA vs. AA	0.25	0.123

3.14. Analyses of *THRA1* rs12393700 C>A genotype frequencies in hypothyroid patient and healthy individuals using Hardy-Weinberg equilibrium:

The genotype frequencies of the *THRA* rs12393700 C>A were presented in the (Table3.17). The data involved observed values from 100 patients with hypothyroidism, and expected values under Hardy-Weinberg equilibrium (HWE). The observed frequencies were 90% for CC. 4% for CA/AC, and 6% for AA. According to HWE the

expected values were 88.36% for CC, 11.28% for CA/AC, and 0.36% for AA. Significant statistical differences were noticed between observed and expected frequencies for CA/AC with a $P=0.0412$ and AA with a $P=0.0012$, suggesting deviations from Hardy-Weinberg expectation for these genotypes, while CC genotype did not show significant deviations from HWE expectation. Surprisingly, the analyses of *THRA1* rs12393700 C>A genotype frequencies in 50 healthy participants revealed 100% CC and 0% for both CA/AC and AA as shown the **Table3.18**. These frequencies are exactly as expected under Hardy-Weinberg equilibrium. This perfect alignment indicates that the genotype distribution in this healthy population follows Hardy-Weinberg law precisely, with no deviations observed.

Table 3.17: Analyses of *THRA1* rs12393700 C>A genotype frequencies: Hard-Weinberg and Fischer exact test calculations in patients with hypothyroidism.

Genotype/observed % n=100			Hardy–Weinberg equilibrium/ expected %			Fischer exact test	P value
CC	CA/AC	AA	CC	CA/AC	AA	CC/observed vs CC/expected	0.6302
0.90	0.04	0.06	0.8836	0.1128	0.0036	CA/observed vs CA/expected	0.0412
						AA/observed vs AA/expected	0.0012
P <0.05 considered significant, data was showed as percentage.							

Table 3.18: Analyses of *THRA1* rs12393700 C>A genotype frequencies: Hard-Weinberg and Fischer exact test calculations in healthy individuals.

Genotype/observed % n=50			Hardy–Weinberg equilibrium/ expected %		
CC	CA/AC	AA	CC	CA/AC	AA
1	0	0	1	0	0

3.15 Demographic and clinical characteristics of participants with *THRA1* rs939348 T>C genotype: a comparative study between hypothyroid patient and healthy individuals.

According to the *THRA1* rs939348 T>C genotype, the demographic parameters among participants revealed notable differences between healthy control group (n=50) and Hypothyroid patient (n=100) as presented in the **Table3.19**. The mean age was slightly higher in patients with all genotypes (TT: 51.82±8.897, TC: 52.68±8.159, CC: 53.44±10.2) compared to healthy participants (TT: 52.25±8.602, TC: 51.8±10.45, CC: 44.2±6.38) as presented in the **Table3.19**. Age distribution revealed a higher percentage of older individuals among patients with CC genotype.

BMI and mean body weight were statistically higher in the patients with all the genotypes compared to the healthy individuals. However, within each group either healthy individuals or patients, no statistical differences were observed based on the genotypes see **Table3.19**. The distribution of BMI showed statistically significant indifferences in the prevalence of individuals grouped as normal weight (TT: 10%, TC: 0%, CC: 3%) and overweight (TT: 46%, TC: 6%, CC: 8%) between patients and healthy participants as described in the Table20. These findings underscore the clinical relevance of the *THRA1* rs939348 T>C genotype in predisposing individuals to hypertension, diabetes, and varying BMI profiles, highlighting the importance of targeted interventions based on genetic profiles.

Chi-square calculation showed that the prevalence of HT was significantly higher in patients (TT: 20%, TC: 14%, CC: 32%) than in healthy participants (TT: 6%, TC: 0%, CC: 0%) as described in the Table20. SBP was significantly elevated in the Hypothyroid patient with TT genotype compared to the healthy individuals with TT genotype whereas no statistically differences were noticed in the other genotypes. DBP in the other hand was constant and did not show significant differences between the two groups as described in the **Table3.20**. Intriguingly, DM was more common in the patients compared to the healthy participants ($\chi^2=11.67$, 5, $P<0.0396$) see **Table3.20**. Duration and dose of L-thyroxin treatment were recorded only for patients, with the majority requiring higher doses over longer periods. These results suggesting that *THRA1* rs939348 T>C gene variations may be associated with various demographic and clinical parameters, underscore the need for personalized management strategies in Hypothyroid patient with these genotypes.

Table 3.19: Differences among demographic analyses in the study's participants with *THRA1* rs939348 T>C genotype

Parameter	Healthy TT	Healthy TC	Healthy CC	Patient TT	Patient TC	Patient CC
Age (years)	52.25±8.60	51.8±10.45	44.2±6.38 ^(d)	51.82±8.90	52.68±8.16	53.44±10.2

Weight (kg)	72.1±10.37	71.6±9.56	68.2±7.95	82.48±16.97 ^(e)	86.42±13.51 ^(e)	85.92±16.26 ^(e)
BMI (kg/m²)	28.32±3.65	30.47±3.54	26.82±2.78 ^(f)	32.48±5.82 ^(f)	33.62±5.16 ^(f)	33.64±6.34 ^(f)
SBP (mmHg)	124.3±7.98	124.8±7.16	126.2±4.66	134.2±17.47 ^(g)	134.5±12.99 ^(g)	134.4±16.01 ^(g)
DBP (mmHg)	80.98±5.86	81.6±2.61	79.2±2.49	85.15±6.20 ^(h)	83.16±6.64 ^(h)	85.38±7.79 ^(h)
HT Yes (%)	7.5	0.0	0.0	60.6 ^(a)	73.7 ^(a)	66.7 ^(a)
DM Yes (%)	17.5	20.0	0.0	30.3	21.1	47.9 ^(c)
Normal BMI (%)	14.7	0.0	14.3	6.5	0.0	6.5
Overweight BMI (%)	67.6	60.0	42.9	35.5	26.3	17.4
Obese BMI (%)	17.6	40.0	14.3	64.5 ^(b)	73.7 ^(b)	80.4 ^(b)
^(a) : Significantly higher Hypertension in patient groups (p < 0.00000001) ^(b) : Significantly higher Obesity in patient groups (p = 0.0019) ^(c) : Significantly higher Diabetes Mellitus in Patient CC group (p = 0.0197) ^(d) : Significantly lower Age in Healthy CC group ^(e) : Significantly higher Weight in Patient groups compared to Healthy ^(f) : Significantly higher BMI in Patient groups compared to Healthy ^(g) : Significantly higher SBP in Patient groups compared to Healthy ^(h) : Significantly higher DBP in Patient groups compared to Healthy Data was presented as mean±SD						

Table 3.20: Differences among clinical parameters in the study's participants with THRA rs939348 T>C genotype

Parameters	Healthy, n=50			Patients, n=100				
HT, (n)%	TT	TC	C C	TT	TC	CC	Chi-square, df	P value
Yes	(3), 6	-	-	(20), 20	(14), 14	(32), 32	$\chi^2=49.34$, 5	<0.0001
No	(37), 74	(5), 10	(5), 10	(13), 13	(5), 5	(16), 16		
DM, (n)%	TT	TC	C C	TT	TC	CC	Chi-square, df	P value
Yes	(7), 14	(1), 2	(0), 0	(10), 10	(4), 4	(23), 23	$\chi^2=11.67$, 5	0.0396
No	(33), 66	(4), 8	(5), 10	(23), 23	(15), 15	(25), 25		

BMI/(kg/m ²), (n), %	TT	TC	C C	TT	TC	CC	Chi- square, df	P value
Normal	(5), 10	-	(1), 2	(2), 2	-	(3), 3	$\chi^2=6$	0.0419
Overweight	(23), 46	(3), 6	(3), 6	(11), 11	(5), 5	(8), 8		
Obese	(12), 24	(2), 4	(1), 2	(20), 20	(14), 14	(37), 37		

3.16 Demographic and clinical characteristics of participants with *THRA1* rs12393700 C>A genotype: a comparative study between Hypothyroid patient and healthy individuals.

In this experiment we examined the distribution of the demographic and clinical parameters in the participants based on the *THRA1* rs12393700 C>A genotypes; the analyses involved two groups of Hypothyroid patients (n=100) and healthy control (n=50). It is worth to be notice that healthy females exhibited only the wild type CC genotype and did not show any of the other genotypes. Consequently, the analysis was narrowed to comparing patients with various genotypes exclusively to the CC genotype in the healthy group as described in the **Table3.21**, **Table3.22**. The mean age was consistent across groups, with patients with mutant type CA genotype showed slightly higher mean age compared to the other groups see **Table3.21**. The age distribution showed that the majority of both patients and healthy people were withing 40-49 age range, with patients having slightly higher proportion in the age range 50-49 years.

The mean body weight remained constant within the patients group regardless the genotype distribution as shown in the Table. However, patients with wild-type CC genotype showed a significantly higher mean body weight (85.16±16.17 Kg) compared to the healthy controls with wild-type genotype (71.66±9.983 Kg, P-value <0.0001). Similarly, Patients with wild-type CC genotype were significantly more likely to fall within the range of obesity (33.3±6.015) compared to the healthy females (28.38±3.6, P<0.0001) whereas the mean of the BMI remained constant within patient groups. Furthermore, a significant prevalence of obesity was found among patients (CC: 61%, $\chi^2=37.29$, P=0.04) compared to healthy individuals, where 12% had a normal BMI, 58% were overweight, and 30% were obese see **Table3.22**.

Hypothyroid patient in particular those with wild-CC genotype significantly suffered from HT disease ($\chi^2=48.72$, P=0.001) compared to the healthy individuals as presented in the **Table3.21** and **Table3.22**. DM did not show a statistically significant difference

between patients and healthy individuals ($\chi^2=7.9$, $P=0.162$), though a higher percentage of patients with the CC genotype (32%) had DM compared to healthy individuals (16%) see **Table3.22**. Both SBP and DBP were elevated in patients, particularly in those with wild-CC genotype (SBP: 134.1 ± 16.3 mmHg, DBP: 84.58 ± 7.333 mmHg) compared to healthy individuals (SBP: 124.6 ± 7.549 mmHg, DBP: 81 ± 5.425 mmHg). Regarding L-thyroxin treatment, the majority of patients had been on treatment for 1-10 years, with CC genotype patients showing the highest percentage (1-5 years: 38%, 6-10 years: 39%). Most patients required a 100-mcg dose of L-thyroxin, with the highest percentage observed in CC genotype patients (49%).

Table 3.21: Differences among demographic analyses in the study's participants with THRA rs12393700 C>A genotype

Parameter	Healthy CC	Healthy CA	Healthy AA	Patient CC	Patient CA	Patient AA
Age (years)	51.4 $\pm$ 8.776	—	—	52.52 $\pm$ 9.31	57 $\pm$ 10.24 ^(c)	51.75 $\pm$ 10.24
Weight (kg)	71.66 $\pm$ 9.983	—	—	85.16 $\pm$ 16.17	86.83 $\pm$ 12.8	75.75 $\pm$ 16.01
BMI (kg/m ²)	28.38 $\pm$ 3.6	—	—	33.3 $\pm$ 6.015	35.2 $\pm$ 4.754 ^(d)	29.23 $\pm$ 4.45
SBP (mmHg)	124.6 $\pm$ 7.549	—	—	134.1 $\pm$ 16.3	132.2 $\pm$ 8.954	129.8 $\pm$ 13.82
DBP (mmHg)	81 $\pm$ 5.425	—	—	84.58 $\pm$ 7.333	85.67 $\pm$ 5.888	83.75 $\pm$ 6.85
HT Yes (%)	6	—	—	58 ^(a)	5	3
DM Yes (%)	16	—	—	32	3	2
Normal BMI (%)	12	—	—	4	0	1
Overweight BMI (%)	58	—	—	25	1	3
Obese BMI (%)	30	—	—	61 ^(b)	5	0
^(a) HT significantly higher in-Patient CC vs Healthy CC. ^(b) Obesity significantly higher in-Patient CC vs Healthy CC. ^(c) Age significantly higher in-Patient CA vs other groups. ^(d) BMI significantly higher in-Patient CA vs Healthy CC and Patient AA. Data was presented as mean $\pm$ SD						

Table 3.22: Differences among demographic analyses in the study's participants with THRA rs12393700 C>A genotype

Parameters	Healthy, n=50			Patients, n=100				
HT, (n)%	CC	CA	AA	CC	CA	AA	Chi-square, df	P value
Yes	3	-	-	58	5	3	$\chi^2=48.72$	0.001
No	47	-	-	32	1	1		
DM, (n)%	CC	CA	AA	CC	CA	AA	Chi-square, df	P value
Yes	8	-	-	32	3	2	$\chi^2=7.9$	0.162
No	42	-	-	58	3	2		
BMI/(kg/m ²), (n), %	CC	CA	AA	CC	CA	AA	Chi-square, df	P value
Normal	(6), 12	-	-	(4), 4	-	(1), 1	$\chi^2=37.29$	0.04
Overweight	(29), 58	-	-	(25), 25	(1), 1	(3), 3		
Obese	(15), 30	-	-	(61), 61	(5), 5	-		

3.17Analyses of plasma thyroid hormones in patients and healthy individuals with *THRA1* rs939348 T>C genotypes.

This study aimed to investigate the idea of genetic variations at the *THRA1* rs939348 T>C locus influences plasma thyroid hormone levels and metabolic markers in both healthy individuals and hypothyroid patients. **Table3.23** show comparisons of plasma thyroid hormones between the two groups of Hypothyroid patients (n=100) and healthy control (n=50). Interestingly, patients with CC-mutant type showed two folds higher plasma TSH levels (4.74 ± 3.636) compared to the healthy people with CC genotype (2.132 ± 0.9426 , $P < 0.01$) as detailed in the **Table3.23**. However, TSH levels were relatively consistent across all the other genotypes between healthy and hypothyroidism patients. Total T3 levels were relatively consistent across genotypes, while comparing patients to controls, there were significantly differences in FT3 levels across different genotypes as explained in the **Table3.23**. However, within each group (patients or controls), FT3 levels showed no astatically differences, which may indicate the effect of gene variations on the FT3 levels is crucial in the context of comparing patients to controls but not in the same group. We did not notice statistically important differences between the two groups in the level of TT4 as shown **Table3.23**. Not surprisingly, healthy people with TT, TC and CC variations presented higher levels of FT4 compared to hypothyroid patients, as detailed in the **Table3.23**. This is consistent with the hypothesis that genetic

variations significantly impact thyroid hormone levels in the context of comparing patients to controls, rather than within each group (patients or controls). Hence the results revealed significant variations in thyroid hormone levels between patients and healthy control groups, indicating the influence of *THRA1* rs939348 T>C polymorphism on thyroid physiological functions.

Table 3.23: Plasma thyroid hormones in patients and healthy people with *THRA1* rs939348 T>C genotypes.

Parameter	Healthy TT	Healthy TC	Healthy CC	Patient TT	Patient TC	Patient CC
TSH (μU/ml)	2.228±0.801 ^a	1.352±0.302 ^b	2.132±0.942 ^a	4.144±4.471 ^c	5.031±7.428 ^c	4.74±3.636 ^c
TT3 (ng/ml)	1.744±0.429	1.752±0.608	1.558±0.464	1.648±0.195	1.651±0.259	1.906±2.527
FT3 (pmol/L)	5.233±1.011 ^a	4.544±0.591 ^b	4.672±0.808 ^b	5.582±1.011 ^a	5.214±1.019 ^b	5.452±0.984 ^{ab}
TT4 (nmol/L)	140.5±14.97	146.9±15.61	147.3±9.10	142.5±27.04	137.8±24.67	135.5±23.12
FT4 (pmol/L)	14.51±1.601 ^a	15.79±1.678 ^b	13.41±2.75 ^c	13.52±2.478 ^a	13.95±1.597 ^{ab}	13.07±2.361 ^c
Superscript letters (a, b, c) indicate statistically significant differences between groups within the same row. Values sharing the same letter are not significantly different from each other. Values with different letters are significantly different (p < 0.05). Data presented as mean±SD, TSH=thyroid stimulating hormone, TT3=total thyroid hormone, FT3=free thyroid hormone, TT4=total thyroid hormone, FT4=free thyroid hormone.						

3.18 Analyses of glucose hemostasis in patients and healthy individuals with *THRA1* rs939348 T>C genotypes:

Glucose hemostasis profiles of individuals with *THRA1* rs939348 T>C gene variations revealed notable differences between healthy people and patients. Hypothyroid patient showed more than two folds increased in insulin levels for both wild and mutant genotypes (TT 16.13 ± 9.238 μU/ml, for the CT 17.50 ± 8.410 μU/ml, for the CC 17.39 ± 10.76) compared to healthy individuals (for the TT 8.905 ± 3.541 μU/ml, 6.294 ± 1.701 μU/ml, 8.149 ± 3.189 μU/ml), with a P-value<0.01 see **Table3.24**. Although insulin level was significantly higher in patients compared to healthy controls, it was remained relatively constant within both patient and control groups, as shown in the **Table3.24**. This indicates that *THRA1* rs939348 T>C gene polymorphism significantly impact

insulin stability in Hypothyroid patient compared to healthy controls but does not cause notable differences within the same groups.

FPG is a critical parameter used to study glucose hemostasis because it provides a baseline measurement of blood glucose levels without the influence of recent food intake, reflecting the ability of body to blood glucose through endogenous insulin production and response to glucose regulation mechanisms. Interestingly, our results showed elevated of FPG in Hypothyroid patient with wild type TT genotype and mutant type CC genotype ($P<0.01$) compared to healthy individuals as shown in the **Table3.24**. However, no notable differences were observed within both patient and healthy control groups as shown in the **Table3.24**.

HOMA-IR was considerably higher in patients across all genotypes compared to healthy people, specifically in the TT genotype (5.024 ± 2.672 compared to 2.060 ± 0.8242 in healthy people with a $P\text{-value}<0.0001$) and in the CC genotype (5.227 ± 3.280 compared to 1.743 ± 0.9396 with a $P\text{-value}<0.01$) as shown in the **Table3.24**.

Table 3.24: Glucose profile in patients and healthy individuals with *THRA1* rs939348 T>C genotypes.

Parameter	Healthy TT	Healthy TC	Healthy CC	Patient TT	Patient TC	Patient CC
Insulin ($\mu\text{U/ml}$)	8.91 ± 3.54 ^a	6.29 ± 1.70 ^b	8.15 ± 3.19 ^a	16.13 ± 9.24 ^c	17.50 ± 8.41 ^c	17.39 ± 10.76 ^c
FPG (mg/dl)	101.0 ± 13.22 ^a	92.60 ± 6.88 ^b	93.29 ± 12.80 ^b	125.5 ± 58.40 ^c	114.5 ± 38.90 ^c	117.1 ± 25.22 ^c
HOMA-IR	2.06 ± 0.82 ^a	2.55 ± 1.75 ^{ab}	1.74 ± 0.94 ^a	5.02 ± 2.67 ^c	5.26 ± 3.87 ^c	5.23 ± 3.28 ^c
*Data presented as mean $\pm$ SD, P value $\leq$ 0.05 considered significant. FPG=fasting plasma glucose, Bold superscript letters (^a , ^b , ^c) indicate statistically significant differences ($p \leq 0.05$). Same letters mean no significant difference. Different letters indicate statistically significant difference between groups						

3.19 Analyses of blood lipid profiles in patients and healthy individuals with *THRA1* rs939348 T>C genotypes:

Patients with wild type TT genotype showed significant higher blood cholesterol level (187.9 ± 37.23 mg/dl) compared to healthy individuals (157.5 ± 23.86 mg/dl, $P\text{-value}<0.001$) as detailed in the **Table3.25**. Cholesterol levels were relatively similar between patients and healthy controls in the other genotypes as shown in the **Table3.25**. TG blood levels were significantly higher in Hypothyroid patient with TT and CC

genotypes compared to the healthy females with a P value<0.01 as detailed in the **Table3.25**. Healthy people with mutant CC genotype showed the lowest level of TG compare to other gene variations as shown in the **Table3.25**, while TG levels remained constant across the patient groups. In addition, HDL, LDL and vLDL levels were consistent and showed no significant differences between patients and healthy individuals, and they remained constant within both groups as well.

Table 3.25: Blood lipid profile in patients and healthy individuals with *THRA1* rs939348 T>C genotypes.

Parameter	Healthy TT	Healthy TC	Healthy CC	Patient TT	Patient TC	Patient CC
Cholesterol	157.5±23.86 ^a	183.0±63.13 ^b	159.6±19.90 ^a	187.9±37.23 ^b	188.1±44.14 ^b	189.6±39.88 ^b
TG	110.0±29.37 ^a	141.6±60.03 ^b	78.86±34.05 ^c	139.5±70.93 ^b	144.7±61.36 ^b	142.5±61.15 ^b
HDL	49.28±12.39 ^a	48.80±15.75 ^a	38.40±12.52 ^b	49.27±9.15 ^a	53.37±12.27 ^a	50.13±12.07 ^a
LDL	99.5±22.42 ^a	122.8±28.54 ^b	100.4±31.62 ^a	104.9±31.09 ^a	111.2±33.73 ^{ab}	113.2±38.97 ^b
VLDL	34.34±11.90 ^a	34.58±9.70 ^a	34.04±9.94 ^a	25.99±9.79 ^b	28.04±11.90 ^b	28.32±12.35 ^b
*Data presented as mean±SD, P value≤0.05 considered significant. TG=triglyceride, HDL=high density lipoprotein, LDL=low density lipoprotein, VLDL=very low-density lipoprotein. Bold superscript letters (^a, ^b, ^c) indicate statistically significant differences ($p \leq 0.05$). Same letters mean no significant difference. Different letters indicate statistically significant difference between groups						

3.20 Investigate of duration L-Thyroxine treatment on plasma thyroid hormones in hypothyroid patient with *THRA1* rs939348 T>C genotypes:

To investigate whether the *THRA1* rs939348 T>C genotypes polymorphism influences the response to L-thyroxine treatment in patient with hypothyroidism. **Table3.26** describes the duration of L-thyroxine treatment in patients with different *THRA1* rs939348 T>C genotypes (TT, TC, CC) as percentages. For patients treated for equal five years or less, 12% of the patients had wild genotype (TT), 9% of the patients with heterozygous mutant (TC) and 21% had the homozygous mutant genotype (CC) as detailed in the **Table3.26**. For patients who treated for more than 5years, 21% had the wild genotype (TT), 10% of the patients with the mutant genotype (heterozygous) and 27% had the homozygous mutant genotype (CC). statistically, no statistical differences were found in duration of treatment among the genotypes (P-value of 0.1496), and there

were no statistical differences in genotype distribution between the two treatment durations (P-value 0.0721). Therefore, it is worthwhile to study the correlation between the duration of the treatment, the genotypes, and TSH levels to gain a better understanding of their interplay and influence on treatment outcomes.

To examine whether the duration of L-thyroxine treatment is affected by thyroid hormone receptor alpha polymorphism, which may influence the interaction with thyroid hormones and consequently the metabolic and biological response to L-thyroxine, we investigated the duration of L-thyroxine treatment in Hypothyroid patient with *THRA1* rs939348 T>C genotypes. Interestingly, for patients treated for five years or less, TSH levels were highest in the TC genotype (7.47 ± 9.65 μ U/ml) compared to patients with the same genotype treated for more than five years (2.16 ± 1.83 μ U/ml, with a $P < 0.01$) as detailed in **Table3.27**. In contrast, TSH levels was slightly higher but not statistically significant for patients with CC and TT genotypes treated for ≤ 5 compared to those who treated for > 5 years as detailed in **Table3.27**.

TT3 levels were relatively similar across genotypes for both treatment durations, with a notable increase in the CC genotype for > 5 years (2.166 ± 3.369 ng/ml) as presented in the Figure14B. FT3 levels remained fairly consistent across all genotypes and treatment durations. FT3, TT4 and FT4 plasma levels were relatively similar across all genotypes for ≤ 5 compared to patients treated for > 5 years as explained in the **Table3.27**. These results indicate that prolonged L-thyroxine treatment did not lead to improve thyroid hormone levels, especially in the TT and TC mutant genotypes, suggesting that alpha thyroid gene variations may strongly correlated to the effectiveness of the treatment in Hypothyroid patient and should be considered in their care and management.

Table 3.26: Duration of L-thyroxine treatment in patients with THRA rs939348 T>C genotypes.

Time/years	Patients%, N=100			P value	
	TT	TC	CC	Row Factor	0.1496
≤5 years	12	9	21		
>5 years	21	10	27	Column Factor	0.0721
*Data presented as percentage, P value≤0.05 considered significant.					

Table 3.27: Effect the duration of L-thyroxine on plasma thyroid hormones in Hypothyroid patient with THRA rs939348 T>C genotypes.

Parameter	≤5 Years			>5 Years		
Genotype	TT	TC	CC	TT	TC	CC
TSH (μU/ml)	5.53 ± 5.11 ^a	7.47 ± 9.65 ^a	5.37 ± 4.23 ^a	3.35 ± 3.98 ^a	2.16 ± 1.83 ^a	3.47 ± 3.00 ^a
TT3 (ng/ml)	1.714 ± 0.222 ^a	1.674 ± 0.251 ^a	1.570 ± 0.192 ^a	1.610 ± 0.172 ^a	1.629 ± 0.273 ^a	2.166 ± 3.369 ^b
FT3 (pmol/L)	5.735 ± 0.782 ^a	5.241 ± 1.102 ^a	5.426 ± 0.788 ^a	5.632 ± 1.160 ^a	5.189 ± 0.997 ^a	5.471 ± 1.128 ^a
TT4 (nmol/L)	137.38 ± 33.76 ^a	135.68 ± 29.83 ^a	131.39 ± 38.68 ^a	145.50 ± 22.70 ^a	139.65 ± 20.46 ^a	138.68 ± 24.64 ^a
FT4 (pmol/L)	12.54 ± 2.38 ^a	14.64 ± 1.920 ^b	12.57 ± 2.55 ^a	14.07 ± 2.40 ^a	13.33 ± 0.96 ^a	13.45 ± 2.17 ^a
TSH = Thyroid-Stimulating Hormone TT3 / FT3 = Total / Free Triiodothyronine TT4 / FT4 = Total / Free Thyroxine Data are presented as mean ± standard deviation (SD) Bold superscript letters (^a , ^b) indicate significant differences at p ≤ 0.05 within each row and duration group. Values sharing the same letter are not significantly different; different letters indicate a statistically significant difference.						

3.21 Impact of L-thyroxine treatment duration on clinical parameters in hypothyroid patient with *THRA1* rs939348 T>C genotypes:

Thyroid hormones play a significant regulation role on various clinical parameters. Thyroid hormones in particular T4 and T3 play a crucial role for glucose hemostasis and lipid profiles. Hypothyroidism often leads to increase insulin resistance, elevated FPG, and alter blood lipid profiles. Therefore, we aimed to study the impact of L-thyroxine treatment duration on clinical biomarkers in Hypothyroid patient with *THRA1* rs939348

T>C gene polymorphism to assess whether genetic variations influence treatment outcomes. Interestingly, our study did not reveal notable differences in glucose hemostasis parameters-including insulin, FPG and HOMA-IR between patients treated with L-thyroxine for 5 years or less and those treated for more than 5 years across all genotypes as detailed in the **Table3.28**. These data did not show a clear pattern of effect of L-thyroxine treatment on the clinical parameters across different genotypes. This may suggest either a lack of response to the treatment or thyroid alpha receptor gene (THRA) variations, in particular rs939348 T>C genotypes influence responses to treatment in ways that not evident in these patients.

Table 3.28: Effect the duration of L-thyroxine on clinical parameters in Hypothyroid patient with THRA rs939348 T>C genotypes.

Parameter	≤5 Years			>5 Years		
Genotype	TT	TC	CC	TT	TC	CC
Insulin (μU/ml)	14.60 ± 7.68 ^a	17.14 ± 11.37 ^a	16.70 ± 5.61 ^a	17.01 ± 10.09 ^a	16.82 ± 5.99 ^a	14.29 ± 5.76 ^a
FPG (mg/dl)	140.25 ± 80.42 ^a	122.67 ± 51.17 ^{ab}	121.76 ± 33.24 ^b	117.05 ± 41.16 ^a	107.10 ± 23.91 ^a	113.18 ± 17.44 ^a
HOMA-IR	4.50 ± 1.67 ^a	6.14 ± 5.05 ^a	5.10 ± 2.10 ^a	5.32 ± 3.11 ^a	4.46 ± 2.41 ^a	4.01 ± 1.70 ^a
CHO (mg/dl)	182.75 ± 53.92 ^a	198.89 ± 53.00 ^a	190.19 ± 38.20 ^a	182.62 ± 34.25 ^a	178.40 ± 34.34 ^a	181.37 ± 46.47 ^a
TG (mg/dl)	149.83 ± 104.62 ^a	166.89 ± 63.69 ^a	151.38 ± 56.00 ^a	130.58 ± 43.47 ^a	124.70 ± 54.66 ^a	135.52 ± 65.07 ^a
HDL (mg/dl)	50.08 ± 11.30 ^a	51.89 ± 14.44 ^a	50.33 ± 12.63 ^a	48.81 ± 7.94 ^a	54.70 ± 10.56 ^a	49.98 ± 11.86 ^a
LDL (mg/dl)	106.18 ± 32.35 ^a	124.54 ± 30.09 ^a	119.06 ± 32.79 ^a	104.10 ± 31.13 ^a	99.14 ± 33.63 ^a	108.73 ± 43.24 ^a
VLDL (mg/dl)	30.48 ± 21.72 ^a	30.71 ± 12.85 ^a	28.93 ± 10.47 ^a	26.53 ± 9.44 ^a	25.64 ± 11.08 ^a	27.85 ± 14.19 ^a
FPG = Fasting Plasma Glucose CHO = Cholesterol TG = Triglycerides HDL / LDL / VLDL = High / Low / Very Low-Density Lipoproteins Data are presented as mean ± SD Bold superscript letters (^a , ^b) indicate statistically significant differences at p ≤ 0.05 within each row and duration group Values with the same superscript are not significantly different; different letters indicate a significant difference						

3.22 Measuring the impact of *THRA1* rs939348 mutation on the TSH levels in hypothyroid patient and healthy people:

In this experiment, we were interested to measure TSH levels in broader categories of wild genotypes vs mutant genotypes instead of the specific genotypes (TT, TC, CC) to increase the power of the statistical calculations by increase the sample size for each

group. This would help the researchers to understand the impact of the mutation that providing a basis for more detailed studies if significant differences were found. Interestingly, as detailed in the (Table3.29) Hypothyroid patient with wild genotypes showed no significant differences in TSH plasma levels ($4.441 \pm 4.471 \mu\text{U/ml}$) compared to the healthy individuals with wild genotypes ($2.228 \pm 0.801 \mu\text{U/ml}$, $P > 0.05$). However, patients with mutant genotypes exhibited fourfold higher levels of plasma TSH compared to the healthy individuals with mutant genotypes with ($P < 0.01$). Additionally, Patients with mutant genotypes showed significantly higher levels of TSH ($P < 0.01$) compared to healthy controls with wild genotypes. These results indicate that the *THRA1* rs939348 T>C mutation has a strong impact on TSH levels in hypothyroidism patients. Hypothyroid patient with mutant genotypes (TC or CC) exhibited significant higher TSH levels compared to the healthy participants with the same genotypes, suggesting a potential genetic influence of the mutant allele on the severity of the hypothyroidism and responses to L-thyroxine. In contrast, there is no clinically relevant differences between patients with wild genotypes and healthy individuals with the same genotype, indicating that the wild type does not strongly influence TSH levels in the context of hypothyroidism.

Table 3.29: Plasma TSH levels in wild type and mutant *THRA1* rs939348 T>C genotypes among hypothyroid patient and healthy people.

TSH, $\mu\text{U/ml}$ mean $\pm$ SD			
Wild/Patients	Wild/Healthy	Mutant/Patients	Mutant/Healthy
4.441 $\pm$ 4.471	2.228 $\pm$ 0.801	4.490 $\pm$ 4.4882	1.568 $\pm$ 0.6799
*Data presented as mean $\pm$ SD, P value $\leq$ 0.05 considered significant.			

3.23 Examine the impact of *THRA1* rs939348 T>C on the treatment responsiveness in patients with hypothyroidism:

The results of the previous experiment heightened responsiveness among patients with wild genotype (TT). Therefore, in the next experiment we measured TSH levels in wild type genotype based on the duration of L-thyroxine treatment aiming to examine whether genotype influence the efficacy of treatment by regulating TSH levels. Table3.30 presented plasma TSH levels in Hypothyroid patient and healthy participants according to the *THRA1* rs939348 mutant and wild genotypes, categorized by the duration of L-thyroxine treatment. Not surprisingly, for patient with wild type genotypes, TSH levels

were consistent and no significant changes were noticed between patients who treated for 5 years or less and patients who treated for more than 5 years compared to compared to healthy individuals (2.132 ± 0.9425 $\mu\text{U/ml}$ across both duration groups). In contrast, patients with mutant genotypes exhibited higher TSH levels regardless of treatment duration: 6.262 ± 7.300 $\mu\text{U/ml}$ (≤ 5 years) and 3.124 ± 3.513 $\mu\text{U/ml}$ (> 5 years), compared to healthy individuals (2.130 ± 0.808 $\mu\text{U/ml}$ in both duration groups). These findings strongly indicate that *THRA1* rs939348 T>C mutation may contribute to elevated TSH levels in Hypothyroid patient and influence their response to the therapy.

Table 3.30: TSH plasma levels in Hypothyroid patient with *THRA1* rs939348 T>C genotypes compared to healthy individuals considering the duration of L-thyroxin treatment

L-thyroxin duration/Year	TSH, $\mu\text{U/ml}$ mean $\pm$ SD			
	Wild/Patients	Wild/Healthy	Mutant/Patients	Mutant/Healthy
≤ 5 years	5.369 ± 4.233	2.132 ± 0.9425	6.262 ± 7.300	2.130 ± 0.808
> 5 years	3.469 ± 3.003	2.132 ± 0.9425	3.124 ± 3.513	2.130 ± 0.808
*Data presented as mean $\pm$ SD, P value ≤ 0.05 considered significant.				

3.24 Examine the effect of L-thyroxine dose variations on TSH plasma levels in Hypothyroid patient with *THRA1* rs939348 T>C genotypes compared to healthy individuals:

This experiment was conducted based on our observations that patients with wild alleles responded effectively to the therapy. We aimed to examine whether this responsiveness varies according to different doses of L-thyroxine. Consistently, patients with *THRA1* rs939348 T>C wild genotype exhibited consistent and stable TSH plasma levels compared to the healthy individuals with the same genotypes, ranging from 4.253 ± 2.336 $\mu\text{U/ml}$ to 3.390 ± 1.854 $\mu\text{U/ml}$, with no data available for dosages below 50mcg or ≥ 200 mcg as detailed in the **Table3.31**. In contrast, patients with mutant genotypes (TC or CC) showed significant elevated in the levels of TSH compared to the healthy participants with the same genotypes. These results indicate that genotype specific response to L-thyroxine treatment among Hypothyroid patient underscoring the importance of gene polymorphism testing in tailoring therapeutic strategies.

Table 3.31: The effect of L-thyroxine on TSH plasma levels in Hypothyroid patient with THRA rs939348 T>C genotypes compared to healthy individuals.

L-thyroxine Dose (mcg)	Wild / Patients	Wild / Healthy	Mutant / Patients	Mutant / Healthy
25	–	2.23 ± 0.80 ^a	4.51 ± 1.79 ^b	1.65 ± 0.70 ^c
50	4.25 ± 2.34 ^b	2.23 ± 0.80 ^a	7.34 ± 7.79 ^b	1.65 ± 0.70 ^c
75	3.14 ± 2.26 ^b	2.23 ± 0.80 ^a	3.64 ± 2.50 ^b	1.65 ± 0.70 ^c
100	2.92 ± 2.04 ^b	2.23 ± 0.80 ^a	3.16 ± 1.85 ^b	1.65 ± 0.70 ^c
125	1.69 ± 0.23 ^c	2.23 ± 0.80 ^a	4.07 ± 3.57 ^b	1.65 ± 0.70 ^c
150	3.39 ± 1.85 ^b	2.23 ± 0.80 ^a	4.28 ± 3.90 ^b	1.65 ± 0.70 ^c
≥200	–	2.23 ± 0.80 ^a	12.03 ± 6.40 ^d	1.65 ± 0.70 ^c

TSH = Thyroid Stimulating Hormone (μU/ml)
 Data are presented as mean ± SD
 Bold superscript letters (^a, ^b, ^c, ^d) indicate statistically significant differences ($p \leq 0.05$) within each row
 Same letter = no significant difference
 Different letters = significant difference between values

3.25 Analyses of plasma thyroid hormones in patients and healthy individuals with *THRA1* rs12393700 C>A genotypes.

We aimed to investigate the idea of genetic variations at the *THRA1* rs12393700 C>A locus influences plasma thyroid hormone levels and metabolic markers in both healthy individuals and hypothyroid patients. **Table3.32** present plasma thyroid hormone levels in Hypothyroid patient and healthy individuals with wild-type genotype (CC) and mutant genotypes (CA and AA) of the THRA rs12393700 C>A polymorphism. Notably all healthy individuals were reported with CC genotypes; thus, the comparison between healthy and patients was solely based on the CC genotypes. Patients with wild genotype (CC) showed two folds higher TSH plasma levels ($4.39 \pm 4.88 \mu\text{U/ml}$) compared to healthy people with the same type (2.13 ± 0.81 , $P < 0.001$). No significant differences were noticed between healthy individuals and Hypothyroid patient with the CC genotypes in the mean levels of TT3, FT3 and TT4 as detailed in the **Table3.32**. In contrast, healthy people with CC genotype showed a significant increase in the mean levels of FT4 ($14.42 \pm 2.033 \text{ pmol/L}$) compared to the patients with the same genotype ($13.17 \pm 2.204 \text{ pmol/L}$, $P < 0.001$).

Table 3.32: Plasma thyroid hormones and metabolic markers in patients and healthy people with *THRA1* rs12393700 C>A genotypes.

Parameters	Healthy (N=50)			Patients (N=100)		
	CC	CA	AA	CC	CA	AA
TSH (μU/ml)	2.13 ± 0.81	0.00 ± 0.00	0.00 ± 0.00	4.39 ± 4.88 ^a	2.87 ± 2.52 ^b	4.33 ± 3.23 ^a

TT3 (ng/ml)	1.726 ± 0.44	0.00 ± 0.00	0.00 ± 0.00	1.597 ± 0.216 ^a	1.652 ± 0.22 ^{ab}	1.548 ± 0.11 ^b
FT3 (pmol/L)	5.108 ± 0.97	0.00 ± 0.00	0.00 ± 0.00	5.424 ± 0.96 ^b	5.85 ± 1.327 ^a	5.435 ± 1.489 ^b
TT4 (nmol/L)	141.8 ± 14.56	0.00 ± 0.00	0.00 ± 0.00	139 ± 25.28 ^a	134.2 ± 15.02 ^{ab}	128.2 ± 25.11 ^b
FT4 (pmol/L)	14.42 ± 2.033	0.00 ± 0.00	0.00 ± 0.00	13.17 ± 2.204 ^b	15.09 ± 2.139 ^a	14.63 ± 1.547 ^{ab}
Superscript letters (e.g., ^a , ^b) indicate statistical significance between genotypes within the patient group only. Values sharing the same letter are not significantly different. Values with different letters are significantly different. Healthy CA and AA groups were excluded from statistical comparison due to missing data (0.00 ± 0.00).						

3.26 Metabolic profiles associated with *THRA1* rs12393700 C>A genotypes in patients and healthy individuals:

The association of glucose metabolic parameters and lipid metabolism markers with *THRA1* rs12393700 C>A polymorphism were compared between healthy people (n=50) and hypothyroid patient (n=100) as described in the **Table3.33**. Since healthy individuals reported with wild-type CC genotype, the comparison between the two groups was narrowed based on the CC genotype. Patients with wild genotype showed dramatically higher levels of plasma insulin (16.59±10.19 µU/ml) compared to the healthy participants with the same genotype (9.023±4.019 µU/ml, P<0.0001), see **Table3.33**. Similarly, fasting plasma glucose and HOMA-IR were notably elevated (P<0.01) in Hypothyroid patient with wild-type CC genotype compared to healthy controls, see **Table3.33**.

Patients with wild type CC genotype showed significant higher blood cholesterol level (183.8±43.61 mg/dl) compared to healthy individuals (160.3±29.45 mg/dl, P-value<0.001) as detailed in the **Table3.33**. TG blood levels were significantly higher in Hypothyroid patient with CC genotypes compared to the healthy females with a P-value<0.01 as detailed in the **Table3.33**. Healthy people with wild CC genotype showed the lowest level of HDL and LDL, but was not statistically significant compare to the patients with the same genotype. Intrinsically, healthy group reported higher mean levels of vLDL (34.21±11.24mg/dl) compared to Hypothyroid patient (28.19±13.46mg/dl, P<0.01) as shown in the **Table3.33**.

Table 3.33: Metabolic profiles in patients and healthy individuals with *THRA1*rs12393700 C>A genotypes.

Parameters	Healthy (N=50)			Patients (N=100)		
	CC	CA	AA	CC	CA	AA

Insulin (μ U/ml)	9.023 $\pm$ 4.019	0.00 $\pm$ 0.00	0.00 $\pm$ 0.00	16.59 $\pm$ 10.19 ^b	20.36 $\pm$ 4.158 ^a	18.63 $\pm$ 6.873 ^{ab}
FPG (mg/dl)	99.7 $\pm$ 12.86	0.00 $\pm$ 0.00	0.00 $\pm$ 0.00	119 $\pm$ 43.35 ^a	94.85 $\pm$ 39.6 ^b	116.8 $\pm$ 10.97 ^a
HOMA-IR	2.12 $\pm$ 0.9815	0.00 $\pm$ 0.00	0.00 $\pm$ 0.00	5.101 $\pm$ 3.287 ^b	6.483 $\pm$ 2.268 ^a	4.65 $\pm$ 1.439 ^b
Cholesterol	160.3 $\pm$ 29.45	0.00 $\pm$ 0.00	0.00 $\pm$ 0.00	183.8 $\pm$ 43.61 ^b	204.5 $\pm$ 25.42 ^a	182 $\pm$ 26.92 ^b
TG	111.2 $\pm$ 34.8	0.00 $\pm$ 0.00	0.00 $\pm$ 0.00	141 $\pm$ 64.68 ^b	136.7 $\pm$ 66.28 ^b	169.8 $\pm$ 49.12 ^a
HDL	48.14 $\pm$ 12.88	0.00 $\pm$ 0.00	0.00 $\pm$ 0.00	50.56 $\pm$ 11.51 ^b	45.67 $\pm$ 7.146 ^c	55.5 $\pm$ 7.55 ^a
LDL	101.9 $\pm$ 24.45	0.00 $\pm$ 0.00	0.00 $\pm$ 0.00	108.4 $\pm$ 36.24 ^b	134.7 $\pm$ 25.28 ^a	112.1 $\pm$ 7.405 ^b
VLDL	34.21 $\pm$ 11.24	0.00 $\pm$ 0.00	0.00 $\pm$ 0.00	28.19 $\pm$ 13.46 ^{ab}	27.38 $\pm$ 12.97 ^b	28.4 $\pm$ 6.194 ^{ab}
Bold superscript letters indicate statistical groupings: Same letter = not significantly different. Different letters = significantly different. Comparisons are only made among the patients (CC, CA, AA). Healthy CA and AA groups were excluded from analysis due to lack of data (0.00 $\pm$ 0.00). Abbreviations: FPG = fasting plasma glucose, TG = triglyceride, HDL = high-density lipoprotein, LDL = low-density lipoprotein, VLDL = very low-density lipoprotein.						

3.27 Investigate of duration L-Thyroxine treatment on plasma thyroid hormones in hypothyroid patient with *THRA1* rs12393700 C>A polymorphism:

To investigate whether the *THRA* rs12393700 C>A genotypes polymorphism influences the response to L-thyroxine treatment in hypothyroid patients. **Table3.34** describes the duration of L-thyroxine treatment in patients with different *THRA* rs12393700 C>A genotypes (CC, CA, AA) as percentages. For patients treated for equal five years or less, 38% of the patients had wild genotype (CC), 2% of the patients with heterozygous mutant (CA) and 2% had the homozygous mutant genotype (AA) as detailed in the Table33. For patients who treated for more than 5 years, 52% had the wild genotype (CC), 4% of the patients with the mutant genotype (heterozygous) and 2% had the homozygous mutant genotype (AA). Statistically, there was no significant differences noticed in the treatment duration across genotypes. In contrast, the column factor (genotype distribution) reported a P value Of 0.0232, indicating a significant difference in the distribution of genotypes among patients with hypothyroidism based on treatment duration. Therefore, it is worthwhile to study the correlation between the duration of the treatment, the genotypes, and TSH levels to gain a better understanding of their interplay

and influence on treatment outcomes. To examine whether the duration of L-thyroxine treatment is affected by thyroid hormone receptor alpha polymorphism, which may influence the interaction with thyroid hormones and consequently the metabolic and biological response to L-thyroxine, we investigated the duration of L-thyroxine treatment in hypothyroid patient with *THRA1* rs12393700 C>A genotypes. Interestingly, for patients treated for five years or less, TSH levels were highest in the CC genotype (5.92 ± 6.16 μ U/ml) compared to patients with the same genotype treated for more than five years (3.28 ± 3.32 μ U/ml, with a $P < 0.01$) as detailed in **Table3.34**. Unlikely, TSH levels was slightly higher but not statistically significant for patients with CA and AA genotypes treated for ≤ 5 compared to those who treated for > 5 years as detailed in **Table3.34**.

TT3 levels were relatively similar across genotypes for both treatment durations, with a notable increase in the CC genotype for > 5 years (1.91 ± 2.43 ng/ml) as presented **Table3.34**. FT3 levels remained fairly consistent across all genotypes and treatment durations. TT4 and FT4 plasma levels were relatively similar across all genotypes for ≤ 5 compared to patients treated for > 5 years as explained in the **Table3.35**. These results indicate that

prolonged L-thyroxine treatment had led to improve TSH plasma levels, especially in the wild-type CC genotypes, suggesting that *THRA* rs12393700 C>A variations may not correlate with the effectiveness of the treatment in patients with hypothyroidism.

Table 3.34: Duration of L-thyroxin treatment in patients with *THRA1* rs12393700 C>A genotypes.

Time/years	Patients%, N=100			P value	
	CC	CA	AA	Row Factor	0.3468
≤5 years	38	2	2	Column Factor	0.0232
>5 years	52	4	2		
*Data presented as percentage, P value≤0.05 considered significant.					

Table 3.35: Effect duration of L-thyroxine treatment on plasma thyroid hormones in Hypothyroid patient with *THRA1* rs12393700 C>A genotypes.

Parameters	≤5 Years (mean ± SD)			>5 Years (mean ± SD)		
	CC	CA	AA	CC	CA	AA
TSH (μU/ml)	5.92 ± 6.16 ^a	3.78 ± 2.15 ^b	5.85 ± 4.21 ^a	3.28 ± 3.32 ^{ab}	2.41 ± 2.86 ^b	2.82 ± 2.10 ^b
TT3 (ng/ml)	1.62 ± 0.22 ^b	1.89 ± 0.26 ^a	1.64 ± 0.03 ^b	1.91 ± 2.43 ^{ab}	1.54 ± 0.08 ^b	1.46 ± 0.04 ^b
FT3 (pmol/L)	5.44 ± 0.87 ^b	5.42 ± 1.15 ^b	6.16 ± 0.33 ^a	5.41 ± 1.03 ^b	6.07 ± 1.52 ^a	4.72 ± 2.11 ^b
TT4 (nmol/L)	134.96 ± 26.77 ^{ab}	143.50 ± 21.35 ^a	106.70 ± 0.28 ^b	141.90 ± 23.98 ^{ab}	129.50 ± 11.70 ^b	149.70 ± 6.51 ^a
FT4 (pmol/L)	12.63 ± 2.30 ^b	17.15 ± 0.92 ^a	15.96 ± 0.36 ^a	13.64 ± 2.18 ^b	14.06 ± 1.76 ^{ab}	14.30 ± 1.41 ^{ab}
Within each treatment group (≤5 years or >5 years): Values sharing the same superscript letter (e.g., ^a) are not significantly different. Values with different letters are significantly different ($p \leq 0.05$). Comparisons are only made within each treatment duration to avoid confounding between duration effects and genotypes. Abbreviations: TSH = Thyroid-stimulating hormone TT3/TT4 = Total triiodothyronine/thyroxine FT3/FT4 = Free triiodothyronine/thyroxine						

3.28 Impact of L-thyroxine therapy on glucose hemostasis and blood lipid profiles in Hypothyroid patient with *THRA1* rs12393700 C>A genotypes:

The impact of the duration L-thyroxin treatment on various clinical parameters was investigated in Hypothyroid patient with *THRA* rs12393700 C>A polymorphism as detailed in the **Table3.36**. The duration of L-thyroxin therapy was divided into ≤5years and >5years grouped by genotypes CC, CA and AA. For glucose hemostasis parameters (insulin, FPG and HOMA-IR), no notable changes were observed between patients who treated for five or less compared to those who treated for more than 5years across all genotypes as presented in the **Table3.36**. Similarly, blood lipid profiles including CHOL, TG, HDL, LDL and vLDL were constant with minimal changes between the two treatment durations across all the genotypes ($P>0.05$). It is worth to mention that blood cholesterol generally decreased with longer treatment as shown in the **Table3.36**. Hence, the data may indicate that the prolonged treatment did not significantly change or improve the clinical parameters, suggesting that the response to the treatment does not correlate

with the duration of the treatment. This lack of correlation might be related to other factors, such as gene polymorphism.

Table 3.36: Effect the duration of L-thyroxine therapy on clinical parameters in Hypothyroid patient with *THRA1* rs12393700 C>A genotypes.

Parameter	≤5 Years (mean ± SD)			>5 Years (mean ± SD)		
	CC	CA	AA	CC	CA	AA
Insulin (μU/ml)	15.86 ± 7.60 ^b	23.70 ± 2.97 ^a	15.01 ± 9.31 ^b	17.12 ± 11.77 ^b	18.69 ± 3.84 ^b	22.25 ± 1.63 ^a
FPG (mg/dl)	128.42 ± 55.90 ^a	117.00 ± 26.87 ^{ab}	115.00 ± 11.31 ^b	112.19 ± 29.98 ^{ab}	83.77 ± 43.38 ^b	118.50 ± 14.80 ^a
HOMA-IR	5.11 ± 2.93 ^{ab}	6.75 ± 2.89 ^a	4.15 ± 2.19 ^b	5.08 ± 3.56 ^{ab}	6.35 ± 2.38 ^a	5.15 ± 0.64 ^b
CHO (mg/dl)	191.08 ± 47.27 ^{ab}	188.50 ± 2.12 ^a	169.50 ± 37.48 ^b	178.40 ± 40.36 ^b	212.50 ± 28.63 ^a	194.50 ± 12.02 ^{ab}
TG (mg/dl)	154.84 ± 73.60 ^{ab}	97.50 ± 60.10 ^b	200.00 ± 52.33 ^a	130.92 ± 55.90 ^b	156.25 ± 67.71 ^{ab}	139.50 ± 28.99 ^b
HDL (mg/dl)	50.60 ± 12.63 ^b	42.00 ± 4.24 ^a	59.00 ± 8.49 ^a	50.53 ± 10.74 ^b	47.50 ± 8.10 ^b	52.00 ± 7.07 ^a
LDL (mg/dl)	115.89 ± 33.27 ^{ab}	136.50 ± 19.09 ^a	109.20 ± 1.70 ^b	102.85 ± 37.63 ^b	133.75 ± 30.67 ^a	115.00 ± 11.31 ^{ab}
VLDL (mg/dl)	30.48 ± 14.96 ^a	19.65 ± 10.54 ^b	25.95 ± 9.40 ^{ab}	26.52 ± 12.13 ^b	31.25 ± 13.54 ^a	30.85 ± 1.63 ^a
Values with different bold letters (e.g., ^a , ^b) in the same row and treatment group are significantly different ($p \leq 0.05$). Same letters indicate no significant difference. Comparisons are made within the same treatment duration group only (≤5 years or >5 years). FPG = Fasting Plasma Glucose, CHO = Cholesterol, TG = Triglycerides, HDL/LDL/VLDL = High-/Low-/Very Low-Density Lipoprotein						

3.29 Impact of *THRA1* rs12393700 C>A gene polymorphism on TSH levels and L-thyroxine response in patients with hypothyroidism:

The rational for conduiting this experiment is to investigate the influence of *THRA* rs12393700 C>A gene polymorphism on TSH levels in broader categories of wild genotypes vs mutant genotypes instead of the specific genotypes (CC, CA, AA) to increase the power of the statistical calculations by increase the sample size for each group. This would help the researchers to understand the impact of genetic variations on thyroid function and dysfunction and whether duration treatment correlate with *THRA* rs12393700 C>A polymorphism that providing a basis for more detailed studies if

significant differences were found. All healthy people were reported with wild-type (CC) genotype; therefore, the comparison was made between wild-type patients, mutant patients and wild-type healthy individuals. Interestingly, as detailed in the (Table3.37) hypothyroid patient with wild genotypes showed significant elevation in TSH plasma levels ($4.394 \pm 4.878 \mu\text{U/ml}$) compared to the healthy individuals with wild genotypes ($2.130 \pm 0.812 \mu\text{U/ml}$, $P < 0.005$). Additionally, patients with mutant genotypes exhibited no significant differences in plasma TSH levels compared to the healthy individuals with wild genotypes, and other patients with mutant genotype as presented in the Figure21. These results indicate that the *THRA* rs12393700 C>A mutation has a significant impact on TSH levels in hypothyroid patients. Patients with mutant genotypes (CA or AA) exhibited nearly normal TSH levels compared to the healthy participants, suggesting a potential genetic influence of the mutant allele on the severity of the hypothyroidism and responses to L-thyroxine. In contrast, there are strong clinically relevant differences between patients with wild genotypes and healthy individuals with the same genotype, indicating that the wild type strongly influences TSH levels in the context of hypothyroidism.

Table 3.37: plasma TSH levels in wild type and mutant *THRA* rs12393700 C>A genotypes among Hypothyroid patient and healthy people.

TSH, $\mu\text{U/ml}$ mean $\pm$ SD			
Wild/Patients	Wild/Healthy	Mutant/Patients	Mutant/Healthy
4.394 $\pm$ 4.878	2.130 $\pm$ 0.812	4.052 $\pm$ 2.316	0.000 $\pm$ 0.000
*Data presented as mean $\pm$ SD, P value $\leq$ 0.05 considered significant.			

3.30 Genetic influence of *THRA1* rs12393700 C>A genotypes on the levels of TSH in Hypothyroid patient undergoing L-thyroxine therapy:

Next, to understand how genetic variations specifically *THRA* rs12393700 C>A polymorphism influence thyroid function as measured by TSH levels. TSH is important indicator of thyroid physiological functions with higher levels of TSH indicating of hypothyroidisms. By categorized patients based on genotype and duration of L-thyroxine treatment (a standard therapy for hypothyroidism), scientists can unmask whether certain gene variations affect TSH regulation differently compared to healthy individuals.

Table3.38 presents TSH plasma levels defined by *THRA* rs12393700 C>A genotypes compared to healthy individuals based on the duration of L-thyroxine therapy. For patients with the wild-type genotype, those using L-thyroxin for ≤ 5 years showed a mean TSH level of 5.92 ± 6.16 μ U/ml, significantly higher than the healthy control group at 2.13 ± 0.81 μ U/ml. In patients using L-thyroxin for >5 years, the TSH levels decreased to 4.01 ± 3.37 μ U/ml, still higher than in healthy individuals. For patients with the mutant genotype (C>A), TSH levels were 4.82 ± 2.98 μ U/ml in those using L-thyroxin ≤ 5 years and 2.54 ± 2.41 μ U/ml in those using it >5 years. Notably, the mutant genotype group lacked data for healthy controls. The beauty our data that TSH plasma levels reduced significantly showing a two-fold decrease compared to those treated for more than 5years with no significant difference observed compared to healthy individuals.

Table 3.38: TSH plasma levels in Hypothyroid patient with *THRA1* rs12393700 C>A genotypes compared to healthy individuals considering the duration of L-thyroxin treatment

L-thyroxin duration/Year	TSH, μ U/ml mean $\pm$ SD			
	Wild/Patients	Wild/Healthy	Mutant/Patients	Mutant/Healthy
≤ 5 years	5.92 $\pm$ 6.16	2.13 $\pm$ 0.81	4.82 $\pm$ 2.98	-
>5 years	4.01 $\pm$ 3.37	2.13 $\pm$ 0.81	2.54 $\pm$ 2.41	-
*Data presented as mean $\pm$ SD, P value $\leq$ 0.05 considered significant.				

3.31 Impact of different doses of L-thyroxin therapy on TSH levels in Hypothyroid patient with *THRA1* rs12393700 C>A genotypes:

This experiment was conducted based on the hypothesis that gene variations may impact the effectiveness of the treatment response. We aimed to examine whether this responsiveness varies according to different doses of L-thyroxine as explained in the **Table3.39**. Consistently, patients with *THRA* rs12393700 C>A wild-type genotype, TSH levels showed significant and constant increase with different doses of L-thyroxine. The highest dose (≥ 200 mcg) resulting in a threefold elevation in TSH levels (12.03 ± 6.398 μ U/ml, $P < 0.0001$) compared to the lowest dosage, indicating a potential overcompensation or resistance. Mutant patients show a distinct pattern, with an initial high TSH level at 50 mcg (8.2 ± 0.7041 μ U/ml), which then normalizes at higher doses (2.815 ± 2.1 μ U/ml at 75 mcg and 2.866 ± 2.296 μ U/ml at 100 mcg). These results indicate

a more responsive adjustment in TSH levels for mutant patients with moderated doses of L-thyroxine.

Table 3.39: the effect of L-thyroxine on TSH plasma levels in Hypothyroid patient with *THRA1* rs12393700 C>A genotypes compared to healthy individuals.

L-thyroxine (mcg)	Wild/Patients	Wild/Healthy	Mutant/Patients	Mutant/Healthy
25	4.51 ± 1.79 ^a	2.13 ± 0.81 ^b	—	—
50	6.16 ± 7.93 ^a	2.13 ± 0.81 ^b	8.20 ± 0.70 ^a	—
75	3.50 ± 2.41 ^a	2.13 ± 0.81 ^b	2.82 ± 2.10 ^{ab}	—
100	3.63 ± 3.84 ^a	2.13 ± 0.81 ^b	2.87 ± 2.30 ^{ab}	—
125	4.13 ± 3.73 ^a	2.13 ± 0.81 ^b	—	—
150	3.66 ± 3.21 ^a	2.13 ± 0.81 ^b	—	—
≥200	12.03 ± 6.40 ^a	2.13 ± 0.81 ^b	—	—
Values with different bold letters (^a , ^b) in the same row are significantly different ($p \leq 0.05$). Same letters mean no significant difference. Comparisons were made between patients and healthy individuals for the same genotype and dose group.				

3.32 Impact of *THRA1* rs939348 T>C genotype mutation on L-thyroxine response based on TSH levels in patients with hypothyroidism:

The hypothesis of this study is to investigate whether gene variations in the thyroid hormone receptor gene influence the response to treatment in hypothyroidism, potentially leading to either effectively response or non-response to therapy. Accordingly, hypothyroid patient (n=100) was segmented based on *THRA1* rs939348 T>C genotypes and categorized as responders and non-responders depending on the plasma TSH levels, normalized to the levels observed in the healthy individuals ranged (0.5-5μU/ml). Among responders, 33% were wild genotype and 20% were mutant, while among non-responders, 11% were wild and 36% were mutant. Statistically, non-responder patient proportions with mutant genotype were significantly higher than responder patients with wild genotype as detailed in the **Table3.40**, indicating a strong association between the *THRA* rs939348 T>C polymorphism and the response to the therapy. Interestingly, TSH levels were close to normal ($2.019 \pm 1.09\mu\text{U/ml}$) in responder patients with the wild-type genotype compared to the healthy controls, while TSH dramatically elevated in non-responder patients with the wild-type genotype $P < 0.0001$ compared to responder patients with the same genotype. Among patients with mutant genotypes (TC or CC), responders

had TSH levels ($2.243 \pm 1.220\mu\text{U/ml}$) higher than healthy individuals ($1.398 \pm 0.562\mu\text{U/ml}$, with a $P < 0.02$) whereas non-responder patients with mutant genotype showed a six-fold elevation in TSH levels compared to the responders or healthy individuals as presented in the **Table 3.41**. These findings suggest that the responsiveness to L-thyroxine treatment significantly impacts TSH levels in wild-type hypothyroid patient with less effective treatment in patients with mutant genotypes.

Table 3.40: Response of Hypothyroid patient to L-thyroxine based on THRA rs939348 T>C genotypes.

Response to L-thyroxin	Patients %			
	Wild	Mutant	Chi-square (χ^2) statistic	p-value
Responders	33	20	13.730	0.000211
Non-responders	11	36		
*Data was presented as percentage and the statistical values was calculated by Chi-square				

Table 3.41: Plasma TSH levels in Hypothyroid patient undergoing L-thyroxine based on THRA1 rs939348 T>C genotypes, categorized by responders and no responders.

TSH, $\mu\text{U/ml}$ mean $\pm$ SD					
Wild patients-Responders	Wild patients-Non responders	Wild Healthy	Mutant patients-Responders	Mutant patients-Non responders	Mutant Healthy
2.019 ± 1.09	8.394 ± 5.628	2.228 ± 0.801	2.243 ± 1.220	6.662 ± 5.889	1.398 ± 0.562
*Data presented as mean $\pm$ SD, P value ≤ 0.05 considered significant.					

3.33 Impact of *THRA1* rs12393700 C>A genotype mutation on L-thyroxine response based on TSH levels in hypothyroid patients:

Based on TSH plasma levels normalized to the TSH levels of healthy individuals, which range from 0.5 to 5 μ U/ml, we categorized Hypothyroid patient into responders, with TSH levels within the normal range, and non-responders, with TSH levels greater than 5 μ U/ml. Furthermore, the patients were segmented based on *THRA1* rs12393700 C>A gene variations into patients with wild-type genotype and patients with mutant genotypes (CA or AA). The data revealed that 55% of wild genotype patients and 6% of mutant genotype patients being responders compared to 35% of wild-type patients and 6% of mutant genotype patients were non-responders. However, according to Chi-square calculation, which yielded a P-value of 0.9455, indicating no significant differences in treatment response between wild and mutant genotypes sees **Table3.42**. Surely, TSH levels were close to normal ($1.911 \pm 0.9545\mu\text{U/ml}$) in responder patients with the wild-type genotype compared to the healthy controls, while TSH dramatically elevated in non-responder patients with the wild-type genotype $P < 0.0001$ compared to responder patients with the same genotype. Among the mutant genotypes; mutant non-responder mutant patients showed a sixfold elevation in TSH levels ($6.218 \pm 1.949 \mu\text{U/ml}$) compared to the responder mutant patients with the same genotypes (1.609 ± 1.078 , $P < 0.0001$) as detailed in the **Table3.43**. It is worth to be noticed that healthy individuals did not report mutant genotypes; therefore, we do not have data to compare mutant responders or non-responders with healthy controls. Instead, we compared the mutant patients for both responders and non-responders, to healthy wild genotype individuals. Surprisingly, no significant differences were noticed in the TSH levels in mutant patients for both responders and non-responders compared to the healthy individuals with the same genotypes, indicating that mutation in the SNP rs12393700 C>A does not seem to influence the efficacy of L-thyroxine therapy in hypothyroid patients. In contrast, the responsiveness to L-thyroxine treatment significantly impacts TSH levels in Hypothyroid patient with *THRA* rs12393700 C>A wild-type.

Table 3.42: Plasma TSH levels in Hypothyroid patient undergoing L-thyroxine based on *THRA1* rs12393700 C>A genotypes, categorized by responders and no responders

Response to L-thyroxin	Patients %			
	Wild	Mutant	Chi-square (χ^2) statistic	p-value
Responders	55	6	0.004670	0.9455
Non-responders	35	4		
*Data was presented as percentage and the statistical values was calculated by Chi-square				

Table 3.43: response of Hypothyroid patient to L-thyroxine based on *THRA1* rs12393700 C>A genotypes

TSH, μ U/ml mean $\pm$ SD				
Wild patients- Responders	Wild patients-Non responders	Wild Healthy	Mutant patients- Responders	Mutant patients-Non responders
1.911 $\pm$ 0.9545	8.297 $\pm$ 5.933	2.13 $\pm$ 0.8125	1.609 $\pm$ 1.078	6.218 $\pm$ 1.949
*Data presented as mean $\pm$ SD, P value $\leq$ 0.05 considered significant.				

3.34 Investigate BMI variations among Hypothyroid patient by *THRA1* rs939348 T>C polymorphism and treatment response:

BMI is a significant parameter for assessing the response to hypothyroidism treatment. Normal BMI measurements often correlate with a positive response to L-thyroxine treatment, indicating adequate thyroid hormone replacement therapy. By Investigating how gene variations impact BMI and treatment outcomes, scientists can potentially identify genetic markers that predict treatment response and help enhance the specificity and precision therapies description for individual patients.

Table3.44 present the data of 100 Hypothyroid patient and 50 healthy individuals. Patients were categorized based on *THRA1* rs939348 T>C genotypes into wild-type genotype and mutant genotypes and further segmented into responders and non-responders according to TSH levels normalized to those of healthy individuals. For patients with the wild genotype, responders had a mean of BMI 31.569 $\pm$ 2.402 Kg/m², while non-responders had a mean of BMI 31.047 $\pm$ 6.664 Kg/m²; both groups showed no significant differences compared to the healthy controls with a mean of BMI 28.317 $\pm$

3.651 Kg/m². In contrast, among patients with mutant genotypes, for both responder and non-responder patients reported significantly increased in BMI levels ($P < 0.001$) compared to the healthy individuals with the same genotypes. Additionally, there was no statistically significant difference in BMI between responder and non-responder groups among mutant patients. These data suggest that patients with the wild-type THRA rs939348 genotype had BMI levels similar to healthy controls, indicating a promising treatment response. While mutant patients exhibited significantly higher BMI levels regardless of treatment response status.

Table 3.44: Body mass index in hypothyroid patient undergoing L-thyroxine based on THRA rs939348 T>C genotypes.

Genotypes	BMI=R/S ² , mean±SD			
	Responders	Healthy Individuals	Non-responders	Healthy Individuals
Wild	31.569 ± 2.402	28.317 ± 3.651	31.047 ± 6.664	28.317 ± 3.651
Mutant	33.088 ± 5.895	28.649 ± 3.566	34.452 ± 6.141	28.649 ± 3.566
*Data was presented as mean±SD, $P < 0.05$ was considered significant.				

3.35 Investigate BMI variations among hypothyroid patient by THRA1 rs12393700 C>A polymorphism and treatment response:

Table 3.45 present the data of 100 Hypothyroid patient and 50 healthy individuals. Patients were categorized based on THRA rs12393700 C>A genotypes into wild-type genotype and mutant genotypes and further segmented into responders and non-responders according to TSH levels normalized to those of healthy individuals. For patients with the wild genotype, responders had a mean of BMI 33.035 ± 5.779 Kg/m², while non-responders had a mean of BMI 33.728 ± 6.430 Kg/m²; both groups showed a notable differences $P < 0.01$ compared to the healthy controls with a mean of BMI 28.383 ± 3.600 Kg/m². Among patients with mutant genotypes, for responder patients reported no significant differences in BMI levels (P above the threshold) compared to non-responder patients. however, there was no data available for mutant healthy individuals to compare. These data suggest that patients with the wild-type THRA rs12393700 C>A genotype had BMI levels markedly higher than healthy controls, indicating less responsiveness to the therapy in this genotype group.

Table 3.45: Body mass index in Hypothyroid patient undergoing L-thyroxine based on THRA rs12393700 C>A genotypes.

Genotypes	BMI=R/S ² , mean±SD		
	Responders	Healthy Individuals	Non-responders
Wild	33.035 ± 5.779	28.383 ± 3.600	33.728 ± 6.430
Mutant	32.812 ± 5.700	-	32.815 ± 5.624
*Data was presented as mean±SD, P<0.05 was considered significant.			

Chapter Four

Discussion

4. Discussion

Hypothyroidism is a condition affects a significant percentage of the global population, with L-thyroxine being the dominant prescribed treatment (Staff, 2021). Despite its widespread use, the treatment response to L-thyroxine varies among people due to several unknown factors (Efsthadiadou et al., 2001; Kaur et al., 2021). Recently, scientists suggest that genetic variations in particular gene related to thyroid stimulating hormone may play a crucial role in impacting individual response to hypothyroidism therapy. THRA (thyroid hormone receptor alpha) gene encodes a key protein plays important role in mediating thyroid hormone biological functions (Bochukova et al., 2012; Korkmaz et al., 2019; Paisdzior, Schuelke and Krude, 2022). Therefore, it is worth to examine whether gene variations within THRA gene, in particular rs939348 T>C and rs12393700 C>A genotypes alter receptor function, and consequently, impair thyroid hormone physiological activity. Unmasking the correlation of these specific genetic variants on the therapeutic response of L-thyroxine could improve personalized medicine strategies in hypothyroid patients.

4.1 Demographic analyses of the study participants:

The study compared demographic data collected from female participants grouped to 100 Hypothyroid patient undergoing L-thyroxine treatment and 50 healthy individuals. Interestingly, the patients showed significantly higher BMI, body weight, hypertension prevalence, and blood pressure values compared to the healthy controls. These findings align with previous research indicating that hypothyroidism often leads to overweight, obesity, alter metabolic index and increased cardiovascular disease risk (Jonklaas et al., 2014; Fadeev, 2015). For instance, a study by Smith and his team found similar trends in hypothyroid patients, indicating the importance of monitoring and care improving care during L-thyroxine therapy (Mooijaart et al., 2019). Additionally, scientists observed that L-thyroxine did not significantly improve hypothyroidism symptoms or fatigue in patients with hypothyroidism condition compared to a placebo group. Therefore, it is necessary to understand that individual responses to treatment can vary, necessitating further research to elucidate the underlying mechanisms (Meier, 2001; Meier et al., 2001; Akirov et al., 2019; Mooijaart et al., 2019).

4.2 Analyses of plasma thyroid hormones and metabolic markers in female patients and healthy control group:

TSH and thyroid hormone levels are the primary determinants of clinical hypothyroidism (Leng and Razvi, 2019b). In this study, we found a significant elevation in TSH levels in patients with hypothyroidism ($P<0.001$) compared to healthy people. TSH is secreted by the anterior pituitary gland and activate receptor on the follicular cells in thyroid gland to release intracellular delivery of iodine for synthesis of T3 and T4 (Jansen et al., 2023). Therefore, the most important parameters for diagnoses of thyroid abnormalities with regard to the other diagnosis of thyroid disorders techniques is the change in serum TSH and the consequent magnitude of changes in circulating thyroid hormones. TSH is used by many medical professionals as initial screen of hypothyroidism condition (Sharma and Kakadiya, 2023). The data of this study revealed that serum TSH levels in hypothyroid patients significantly higher than healthy individuals. However, the mean levels of TSH in hypothyroid patients remained within normal established reference range. This may be because hypothyroid patients undergoing L-thyroxine treatment. These data align with many previous studies suggesting that TSH serum levels are the most sensitive and specific marker of systemic thyroid status (Portman, 2008; Mullur, Liu and Brent, 2014; Sinha, Singh and Yen, 2014; Ross et al., 2022; Sharma and Kakadiya, 2023). Additionally, the data showed that FT3 plasma levels were significantly elevated in hypothyroid patients compared to healthy ($P<0.04$). In contrast, FT4 was statistically lower in patients with hypothyroidism (13.38 ± 2.283) compared to healthy individuals (14.60 ± 1.804 , $P<0.001$). Thyroid disorder can be confusing by virtue of being discordant with the clinical symptoms like inability of L-thyroxine to suppress (TSH; thyrotropin) in hypothyroidism, or because using many different assays that contradict each other such as raised thyroid hormones levels without suppress TSH or low thyroid hormones levels with inappropriately normal or low TSH (Vaidya and Pearce, 2008; 2014; Dave et al., 2016; Sharma and Stan, 2019). There are many possible causes of anomalous thyroid function in hypothyroid patients undergoing L-thyroxine including: genetic variation in TSH receptor, inappropriate administration of L-thyroxine, malabsorption of L-thyroxine, change the treatment dosages, resistance to L-thyroxine and poor compliance (Vaidya and Pearce, 2008; 2014; Levy, Koulouri and Gurnell, 2013; Sharma and Stan, 2019). On the other hand, scientists suggested that to avoid thyrotoxicosis or resistance to L-thyroxine treatment, L-thyroxine should be substitute with T3 supplement, which

can be done without reducing or interfering with FT3 levels, there by restoring FT4/FT3 ratio in most patients (Santini et al., 2019). Not surprisingly, in some patients with elevated FT3 and normal FT4 levels, and excessive L-thyroxine intake typically results in a higher FT4/FT3 ratio compared to spontaneously occurring hyperthyroidism (Parvazinia, 2022).

Moving on to the metabolic biomarkers, the data analyses revealed that hypothyroid patients had insulin plasma levels that were twice as high $P<0.0001$ compared to healthy controls. In addition, patients with hypothyroidism showed significantly elevated FPG $P<0.004$ compared to the healthy individuals. Similarly, the hemostasis model assessment of insulin levels was significantly elevated ($P<0.0001$) in hypothyroid patients compared to the healthy control group. These data align with previous findings, by there scientists indicated that thyroid hormones play crucial roles in both glucose metabolism and consumption. Thyroid disorder is closely corelated with diabetes mellitus development or vice versa (Eom, Wilson and Bernet, 2022). Thyroid hormone affects glucose hemostasis by impacting pancreatic β cells, and regulate glucose metabolism through several organs including skeletal muscle, liver and central nervous system. Hypothyroidism may be the result of insulin resistance of peripheral tissues (Ha et al., 2021; Mun et al., 2024). Maratou et al. suggested that patients with hypothyroidism present with decreased expression of insulin-stimulated glucose transport in monocytes, resulting from impaired translocation of glucose transporter, which is essential for glucose utilization (Maratou et al., 2010). Furth more, reduced rate of blood flow in peripheral tissues is associate with the development of insulin resistance, as previously described by Dimitriadis and his research team (Dimitriadis et al., 2006).

4.3 Analyses of blood lipid profile in hypothyroid patients and healthy control group:

In this study patients with hypothyroidism compared to control healthy individuals exhibited significant higher cholesterol levels 188.8 ± 39.51 mg/dl, $P<0.0001$ and significantly elevated TG ($P=0.002$). Unlikely, there were no significant differences were observed in HDL-CHO or LDL-CHO. Surprisingly, healthy individuals had significant higher levels of vLDL-CHO $P=0.011$ compared to hypothyroid patients. Previous researchers have found similar trends. For example, elevation of cholesterol and TG have been well demonstrated in hypothyroidism and agree with our findings (Mavromati and

Jornayvaz, 2021). Hypothyroidism leads to decreased metabolism including breakdown of cholesterol and triglycerides, resulting in elevated TG, Cholesterol and fatty acids in the blood stream (Liu and Peng, 2022). Furthermore, studies have demonstrated that thyroid hormones regulate lipid metabolism by activating specific metabolic pathways in the liver and adipose tissue (Khawwaja et al., 2015). Consequently, hypothyroidism often leads to dyslipidemia, intrahepatic fat accumulation, nonalcoholic fatty liver disease (NAFLD), and the development insulin resistance (Mavromati and Jornayvaz, 2021). Interestingly, recent studies have shown that THRA agonists are effective in treating dyslipidemia (Jakobsson, Vedin and Parini, 2017). While other studies have indicated that THRA variation can lead to dyslipidemia and treatment resistance (Palacio Rojas et al., 2017; Sinha and Kar, 2018).

4.4Duration, distribution and impact of L-thyroxine treatment on thyroid hormones in hypothyroidism:

In this experiment we calculated the proportion of the patients with hypothyroidism (n=100) based on L-thyroxine treatment duration. The majority of the patients (44%) received therapy for 6-10years, followed by 42% received treatment for 1-5years. Smaller percentages were treated for longer periods: 8% for 11-15years, and 3% each for 16-20years and 16-25years. Next, plasma thyroxine hormones were assessed in hypothyroid patients based on the treatment duration, categorized into two segments: 5years or less, and for more than 5years. Notably, TSH levels remained stable over time, and no significant differences were observed in TT3, FT3, TT4, and FT4 levels.

Interestingly, mean TSH levels significantly decreased in hypothyroid patients treated for more than 5years compared to those treated for shorter period, indicating that extended treatment duration may improve the treatment response. This was approved specially when TT3, FT3, TT4 and FT4 levels remained stable regarding of L-thyroxine intake. Previously, scientists shown that TSH levels can be normalized in hypothyroid patients after long term treatment with L-T4. Research indicated that the stability of L-T4 plays an important role in adjusting TSH to reach euthyroidism. For example, patients who used liquid L-T4 formulations showed better improvement in TSH levels compared to those who took L-T4 tablets. However, we did not count the medication formulations in our study, but the findings of these studies are mostly aligned with our findings (Antonelli et al., 2021b). Additionally, in patients with subclinical hypothyroidism, TSH levels often

adjusted to normal range within a few years of treatment. Scientists reported that around 60% of hypothyroid patients over 30years old with elevated TSH but normal FT4 levels reached euthyroidism within five years of L-T4 treatment. However, studies that specifically focus on conducting annual TSH levels testing in treated patients are limited. Despite this, existing reports supported the effectiveness of long-term L-T4 treatment in stabilizing TSH over extended time (Setian, 2007; Wassner, 2017; Antonelli et al., 2021b; Gietka-Czernel and Glinicki, 2021; Wilson, Stem and Bruehlman, 2021).

4.5. Impact the duration of L-thyroxine treatment on the clinical biomarkers in hypothyroid patients:

Next, we examined the impact of L-thyroxine treatment duration on various clinical biomarkers in hypothyroidism. Interestingly, patients treated for more than 5years had higher insulin levels although the difference was not statically significant $P=0.5451$ compared to those with a shorter treatment period. Parallely, FPG was significantly lower in the long-term treatment group $P=0.01$. Regarding, blood lipid profile, total cholesterol showed a slight decrease in the patients treated for more than 5years $P=0.3171$. TG were significantly lower after more than 5years of treatment $P=0.0441$. HDL-Cholesterol and vLDL-cholesterol were stable and similar between the two groups, LDL-Cholesterol was clearly reduced with longer treatment $P=0.0499$. Truly, these data suggests that long-term thyroid hormone replacement enhances metabolic markers and improve glucose hemostasis.

Thyroid hormones play a significant role on hepatic lipid metabolism by regulating transcription factors target specific genes that are involved in lipid hemostasis. Many enzymes, cell-signaling enzymes involved in hepatic lipid hemostasis, cellular energy status and post translational modifications are indeed regulated by thyroid hormones (Singh et al., 2013; 2016). Although, much is known about lipid synthesis and metabolism at the biochemical and physiological levels, more research is needed to unmask the correlation between hypothyroidism, dyslipidemia and glucose hemostasis impairment (Danese et al., 2000a; 2000b).

In this study we observed decrease in FPG levels with longer L-thyroxine therapy indicating potential benefits in glucose hemostasis. Secondly, the significant reduction in LDL-cholesterol levels in the long-term treatment group indicates improved blood lipid profile. It is worth to notice that the lack of significantly changes in HOMA-IR and HDL-cholesterol warrants further investigation. Clinically, studies have shown that hypothyroidism leads to specifically and reversibly increased plasma LDL, TG and

Cholesterol, which are potentially elevating cardiovascular risk (Meier, 2001; Meier et al., 2001). Additionally, scientist found that thyroid replacement therapy for more than six months can maintain euthyroidism, and consequently lower LDL-cholesterol, TG and cholesterol 10-13%. Helsinki Heart Study found that a 7% reduction in LDL-cholesterol in men correlate with L-thyroxine treatment which reduced coronary heart disease incidence by a 15% (Monzani et al., 2004; Mir et al., 2017; Mutlu et al., 2021).

Interestingly, we did not notice changes in the levels of HOMA-IR between the two groups. Similarly, Brenta et al. reported that L-thyroxine therapy had no effect on HOMA-IR (Clyde et al., 2003; Brenta et al., 2007; Teixeira et al., 2009; Lee, Braverman and Pearce, 2014). Additionally, scientists did not report improvement in insulin resistance, HOMA-IR nor in BMI following long term L-thyroxine treatment (Stanická et al., 2005). In Contrast Stanicka et al and Handisurya et al observed a noteworthy reduction in HOMA-IR after L-T₄ treatment. Generally, no significant change in HOMA-IR have been reported in hypothyroid patients before and after L-thyroxine replacement therapy (Nada, 2013; Ostadrahimi et al., 2023).

4.6Study age related variations in thyroid hormones, metabolic biomarkers and blood lipid profiles in hypothyroidism and healthy individuals:

In this experiment we assessed the intricate relationship between thyroid hormones and metabolic biomarkers in particular insulin resistance and glucose hemostasis across different age groups compared to healthy individuals. TSH levels were consistently higher in hypothyroid patients across all ages, with the most significant differences were noticed in the 50-59 years old group $P < 0.01$ compared to healthy individuals. Unlikely, no significant age-related differences were reported in the levels of TT₃ or TT₄. Clearly, FT₄ levels decreased with aged groups ($P < 0.01$). Additionally, FT₃ levels were elevated in hypothyroid patients, specifically in the 60-69 age group with a $P < 0.01$. insulin levels, FPG and HOMA-IR were dramatically elevated in the patients with hypothyroidism compared to healthy controls across all age groups ($P < 0.01$) except we did not have healthy groups with age 70-79 and 80-89 age groups where healthy controls were not available for comparison. These results clearly suggest that hypothyroidism is correlated to significant disruption in thyroid physiological functions and glucose metabolism regulation, with these disruptions becoming more pronounced with age.

Currently, scientists indicate that hypothyroidism condition, marked by increase in the levels of TSH with normal thyroid hormones, are prevalent among adults. Actually,

incidence of hypothyroidism and reference range of TSH increase with age (Leng and Razvi, 2019b). Therefore, there is a recommendation for higher threshold when treating hypothyroidism in older patients, considering the higher TSH targets due to their potentially lower thyroid hormone requirements. Indeed, our data indicates that TSH levels significantly impacted by ageing (Taylor et al., 2023b; 2023a). However, it is unclear what the precise mechanisms are behind the changes in thyroid function that are noticed in older patients and whether these variations are part of ageing or biomarkers of underlying disease (Bremner et al., 2012). Several mechanisms are suggested such as diminished the thyrotrope receptor which impair negative feedback mechanism, or it could be diminished of TSH receptor on the follicular cells in thyroid gland, or genetic variations in the TSH receptor that could lead to limit of TSH responsiveness, which lead to higher TSH levels to normalize T4 concentration (Bremner et al., 2012; Nada, 2013; Ostadrahimi et al., 2023; Taylor et al., 2023b; 2023a). Hence, more research is required to unmask the mechanisms of thyroid changes in older individuals.

Next, we examined the age-related changes in blood lipid profiles in hypothyroid patients and compared the data to healthy controls with the same age, except for the 70-79 and 80-89 age groups, where healthy controls were not available for comparison. Clearly, the data revealed that total cholesterol levels were consistently elevated in hypothyroid patients across different age groups compared to healthy controls. Specifically, in the age group 40-49, patients had significantly higher cholesterol levels $P<0.05$ compared to healthy groups with the same age. Similarly, hypothyroid patients in the 50-59 age showed significantly higher cholesterol levels compared to healthy individuals with the same age $P<0.05$ and in the 60-69 age group the cholesterol levels in hypothyroid patients were dramatically increased $P<0.01$ compared to control groups with the same age. More likely, TG levels were clinically elevated in hypothyroid patients across 40-49 and 50-59 age groups compared to healthy controls $P<0.01$, but not in the 60-69 age group. HDL, LDL and vLDL levels showed variability between the two groups, but remained consistently not significant across all age groups. Interestingly, we did not find conflict in our study findings due to adjusting the data to healthy controls with the same age. The results of this study were similar to the studies conducted in similar context except for HDL which showed both similarity and differences with previous works (Cabral et al., 2011; Bremner et al., 2012; Ding and Li, 2017; Khatri et al., 2021a; Jawzal et al., 2022). Scientists suggested that the most common symptom in hypothyroidism was weight gain and dyslipidemia followed by fatigue, joint pain, dryness of skin, menstrual

abnormalities, constipation, depression and change in voice(Ding and Li, 2017; Khatri et al., 2021b; Terlizese et al., 2021) . Total cholesterol and TG were significantly elevated in hypothyroid patients compared to healthy controls across all ages, in particular in older patients compared to healthy controls. Katri et al, found around 69% of hypothyroid patients had abnormal lipid profiles and among them 10.2% were subclinical hypothyroidism at older age (Khan et al., 2013; Khatri et al., 2021a). Furthermore, significant changes in total cholesterol, TG, HDL, LDL were found among hypothyroidism compared to healthy controls (Khan et al., 2013). Similar changes in lipid profile were reported in our study too (Malik et al., 2018; Yuan et al., 2022). We found an increased in total cholesterol, TG whereas HDL remained variable and consistent. LDL was elevated in hypothyroid patients but was not significant to pronounced. A study was done by Dhaka and his team showed a decrease in HDL levels and significant increase in LDL and vLDL levels. In contrast, Olukoga et al indicated that HDL levels increased significantly in hypothyroid patients compared to healthy individuals (Olukoga et al., 1994; Lin et al., 2019). Hence our findings relatable to some studies and counteract to some studies. A larger sample size and multicenter studies are needed to address these issues. There are several possible explanations behind disturbance in blood lipid profiles; decreased LDL receptor activity may lead to reduced catabolism of LDL. Increased activity of CETP (cholesteryl ester transport protein) and hepatic lipase may lead to elevate total cholesterol levels and decrease HDL levels (Rizos, 2011; Kumar, Mathur and Tuteja, 2019).

4.7Examine the association of THRA rs939348 T>C genotypes with hypothyroid risk: a comparative study:

Molecular biology analysis of THRA rs939348 T>C gene polymorphism in hypothyroidism showed significant differences in genotype distribution between healthy individuals and hypothyroid patients. For instance, the TT genotype (wild allele) was the most common in healthy controls 80% compared to 33% in patients, indicating a crucial effect. Conversely, the CC genotype (mutant homozygote alleles) was more prevalent in patients 48% compared to healthy groups 10%, which may indicate a strong association with increased risk of hypothyroidism $P<0.001$. Additionally, the CT genotype (mutant heterozygous alleles) showed higher proportion in patients 19% than healthy controls 10%, but the differences were not statistically significant $P=0.199$. We used Chi-square calculation for pairwise comparisons revealed substantial differences between wild TT and mutant genotypes CT and CC $P<0.001$, with no significant differences between the

mutant genotypes CT and CC themselves $P=1$. Interestingly, the findings indicated that the mutation alleles (CT and CC) were significantly shifted in hypothyroid patients compared to the wild-type genotype, while in healthy controls, the wild-type TT genotype was the most common frequency than both the mutant alleles and the corresponding frequencies in patients. These results aligned with most of the studies, which also observed significant differences in genotype distribution, even the exact proportions varied (Onda et al., 2002; Pastor et al., 2012; Wibowo, Hastuti and Susanti, 2021b). Furthermore, most of the studies compared the genotypes proportion within the patient groups, whereas our study included a comparison with healthy individuals to understand the baseline of alleles frequencies in the general population. Hence, these findings suggest that mutation strongly associate with the risk of hypothyroidism.

According to Hardy-Weinberg equation, the data exhibited significant deviations. In patients with hypothyroidism, the observed genotype frequencies were as the follow 42.5% for T allele, 57.5% for C allele, while the Hardy-Weinberg expected the genotype frequencies were 18.1% for T allele, 33.1% for C allele and 48.9% for TC/CT. Using Fischer exact test to examine the significantly, we found a significant difference between TT/observed vs TT/expected ($P<0.0002$), CT/observed vs CT/expected ($P<0.0001$) and CC/observed vs CC/expected ($P<0.001$). Similarly, in a healthy control group of 50 individuals, observed frequencies were 85% for T and 15% for C allele. Hardy-Weinberg expected T allele frequency was 72.25% which is significantly lower than the observed frequency $P<0.0002$. Additionally, the expected frequencies of C allele was 2.25% which significant lower than the observed percentage $P<0.0001$, and the expected percentage of CT/TC genotypes were 25.5% which was significantly difference than the observed frequency with a $P<0.001$. These findings indicate that both patients and healthy people exhibit genotypes distribution significantly deviated from Hardy-Weinberg equilibrium, which possibly due to several factors including genetic and epigenetic modifications, environmental factors, sample size, risk factors and geographical distributions.

4.8Examine the association of THRA rs12393700 C>A genotypes with hypothyroid risk: a comparative study:

THRA rs12393700 C>A polymorphism's frequency distribution in healthy individuals and hypothyroid patients revealed 90% of the patients were wild-type genotype (CC), and 10% were mutant-type segmented into 6% mutant heterozygous CA and 4% mutant homozygous AA. While all healthy participants showed wild genotype (CC). Chi-square calculations revealed significant differences in genotype percentages

between the groups $P < 0.001$. Additionally, pairwise comparisons among hypothyroid patients showed a substantial disparity between wild-type genotype and mutant genotypes, but not between the two mutant genotypes. Observed genotype frequencies in hypothyroid patients significantly deviated from expected percentages based on Hardy-Weinberg equilibrium; expectation for CA $P = 0.04$ and AA $P = 0.0012$, indicating hypothyroidism. While healthy individuals genotype completely align with Hardy-Weinberg expectation which further reinforcing a stark contrast between healthy and hypothyroid patients. These findings indicate that THRA rs12393700 C>A polymorphism may play a role in predisposing to hypothyroidism.

The THRA rs12393700 C>A polymorphism was first identified as a novel SNP by Helena et al in 2008. Eventually, Fernández-Rea and his colleague provided further insight into the correlation of this SNP with dyslipidemia and obesity in Spaniards' people. The authors examined blood sample collected from a of large cohort of Spaniards at high risk of heart disease compared to healthy Spaniards. Clearly, the authors discovered that the distribution of rs12393700 C>A was 93% CC, 6.8% CA and 0.2% AA, and those with mutant genotype appeared to be heavier and at higher risk of cardiovascular diseases (Sørensen et al., 2008; Sinha and Kar, 2018). Intriguingly, these findings align with our results, whereas we found that 90% of hypothyroid patients were wild genotype compared to 6% mutant heterozygous CA and 4% mutant homozygous AA. While all healthy participants showed wild genotype (CC).

4.9Demographic and clinical characteristics of participants with THRA rs939348 T>C genotype: a comparative study between Hypothyroid patient and healthy individuals.

The data of this study reported several notable demographic and clinical differences between healthy group and hypothyroid patients based on THRA rs939348 T>C gene variations. The mean age was slightly higher in the patients across all genotypes compared to healthy individuals, with higher percentage of older individuals among patients with wild-type genotype. Previously scientists investigated ageing as risk factor for hypothyroidism. Most of these studies found that prevalence of hypothyroidism increases with age, whereas the incidence of hypothyroidism in younger people remains low and stable. These findings indicated that ageing counts as a potential risk factor for

hypothyroidism (Yadav et al., 2013; Calissendorff and Falhammar, 2020; Wyne et al., 2023).

BMI and mean body weight were significantly higher in hypothyroid patients compared to healthy individuals regardless the genotype groups, although no differences were found within each group based on genotype. Studies have adjusted for BMI and body weight to isolate the effect of gene polymorphisms on hypothyroidism, counting that overweight and obesity are a potential risk factors for this condition (Qiu et al., 2023). Clearly, our results showed the differences of body weight and BMI were more pronounced between healthy and patients independent THRA rs939348 T>C gene variations. Our data align with Al-Azaam et al, who explored the impact of THRA gene polymorphism, specially rs939348, rs2268458 and rs2239610 on L-thyroxine treatment response in hypothyroid patients and they found no differences in BMI and body weight among genotype groups (Al-Azzam et al., 2014; S.I. et al., 2014). In contrast, several studies on animal models have shown that gene variations such as FTO gene polymorphism influence metabolic rate and energy expenditure (Wang et al., 2021c).

SBP was notably elevated in hypothyroid patents with wild genotype, while DBP remain stable and constant across both groups. Prevalence of DM was significantly high among patients $P<0.0396$. Furthermore, hypothyroid patients required longer and higher doses of L-thyroxine treatment.

Goumidi et al for the first time reported that a significant and potential associations between the TT allele of the THRA rs939348 SNP and elevated SBP in two population-based studies, and these findings align with our results (Goumidi et al., 2011c). It is worth to mention that we are the first to report association between THRA rs939348 T>C gene polymorphism and BP in Iraqi population selected from Kerbala city. These results are agreeing with the known links between thyroid dysfunction and BP although the underlying mechanisms are unclear (Saito and Saruta, 1994; Berta et al., 2019b; Wibowo, Hastuti and Susanti, 2021b). Additionally, the same effect was also observed for DBP, and consequently this allele was significantly associated with higher risk of hypertension (Danzi and Klein, 2003). BP regulated by many physiological factors. Atrial and brain natriuretic peptides are directly controlled by thyroid hormones and may play a role in the process of blood pressure stasis. Autonomic nervous system providing an alternate possible mechanism depends on the action of TT3 and FT3 (Mancia and Grassi, 2014; Li et al., 2020; Zhao et al., 2022). Catecholamine secretion are highly elevated in hypothyroidism in particular plasma noradrenaline, which may play a crucial role in

elevated blood pressure (Levey and Klein, 1990; Moghetti et al., 1994a; 1994b). Thyroid gland dysfunction is associated with an increased sympathetic influence on the autonomic cardiovascular system (Cacciatori et al., 2000; Chechekhin et al., 2023). Finally, studies using animal models suggested that thyroid hormone receptor alpha functions to activate parasympathetic nervous system signaling pathway (Goumidi et al., 2011c).

4.10 Demographic and clinical characteristics of participants with THRA rs12393700 C>A genotype: a comparative study between Hypothyroid patient and healthy individuals.

In this experiment we investigated the association between THRA rs12393700 C>A gene polymorphism and demographic and clinical parameters of the patients and healthy individuals. Notably, healthy group only exhibited wild-type CC genotype, necessitating comparison between hypothyroid patients with wild and mutant genotypes and the wild genotype in healthy people. Although there was no significant change in the mean of age between healthy and hypothyroid patients, the mean age was slightly higher in the hypothyroid patients compared to the healthy control group, with a marginally higher proportion of patients in the 50-59 age range. These results do not align with the previous studies that found a positive correlation between hypothyroidism and age. The possible reasons for this discrepancy include the fact that healthy individuals only showed wild genotype, while patients exhibit wild and mutant genotypes. The limited the comparison to only wild-type CC genotype between healthy participants and patients, which may have reduced the statistical power of the analysis. Despite this, the data indicated a higher proportion of hypothyroidism in patients with more than 50 years old, and this was agreed with most of the researches data.

BMI and body weight were significantly higher in patients with wild-type CC genotype compared to healthy individuals with the same genotype. BMI and body weight remained constant and stable across all genotypes in the patients. However, higher prevalence of obesity was observed in the patients with hypothyroidism compared to healthy individuals (33.3 ± 6.015 vs. 28.38 ± 3.6 , $P < 0.0001$). Our data agree with previous publications indicating a significant association between obesity and hypothyroidism. A meta-analysis reported that obesity is correlated with an increased risk of hypothyroidism, with a relative risk of 1.861. Furthermore, obesity and overweight were strongly related to thyroid gland dysfunction such as Hashemites thyroiditis (HT) and positive thyroid peroxidase antibody (TPOAb) (Biondi, 2010; Song et al., 2019; Yan et al., 2022).

Hypothyroid patients with the CC genotype also exhibited a significant prevalence of HT disease ($\chi^2=48.72$, $P=0.001$), while no significant difference was observed for DM between groups ($\chi^2=7.9$, $P=0.162$), despite a higher percentage in patients (32% vs. 16%). Both systolic and diastolic blood pressure were elevated in patients, particularly those with the CC genotype (SBP: 134.1 ± 16.3 mmHg, DBP: 84.58 ± 7.333 mmHg) compared to healthy individuals (SBP: 124.6 ± 7.549 mmHg, DBP: 81 ± 5.425 mmHg). Our results indicate that the disease prevalence increased significantly in hypothyroidism in particular in those with CC genotype. Significant hypertension prevalence in patients with certain genotype such as the CC genotype, may be linked to a higher risk of thyroid autoimmunity and dysfunction. Patients with hypothyroidism particularly those with wild genotype (CC) exhibited elevated in both SBP and DBP compared to healthy control individuals with the same genotype. This is line with research showing that hypothyroidism can lead to increased blood pressure due to its effects on cardiovascular physiological functions (Stabouli, Papakatsika and Kotsis, 2010; Maleki et al., 2016; Giontella et al., 2021; Wang et al., 2021a).

Our data indicated that the majority of patients had been on L-thyroxin for more than 5 years, with the highest percentages among wild genotype patients, and most of them required a 100-mcg dose. This is paralleled with the clinical guidelines that recommend L-thyroxin as a standard therapy for thyroid gland dysfunction with dose normalized according to patient needs. To sum up, our findings supported by existing literature, shedding the complex interplay between obesity, overweight, THRA gene variations, and hypothyroidism. It is worth to mention that this is the first study in Iraq employed to genotype THRA rs12393700 C>A in patients with hypothyroidism compared to healthy controls (Biondi, 2010; Ben-Skowronek and Wisniowiecka, 2012; Budnevsky et al., 2015; Calsolaro et al., 2019a; Song et al., 2019; Yan et al., 2022).

4.11Analyses of plasma thyroid hormones in patients and healthy individuals with THRA rs939348 T>C genotypes.

Next, we explored the influence of THRA rs939348 T>C locus on plasma thyroid hormones levels and metabolic biomarkers in hypothyroid patients compared to healthy individuals. Clearly, patients with mutant-homozygous CC genotype exhibited plasma TSH levels two folds higher than of healthy individuals with the same genotype $P<0.01$. In contrast, TSH levels remained stable and constant across other genotypes between healthy and hypothyroid groups. Although TT3 levels were consistent across genotypes, significant differences in FT3 plasma levels were observed between patients and healthy

controls, but not within each group indicating THRA rs939348 T>C polymorphism is more pronounced when comparing patients to healthy people. Interestingly, there were no clear differences were observed in TT4 levels between the two groups across all the genotypes. FT4 on the other hand was significantly higher in healthy group compared to the patients across all genotypes (TT, TC and CC), supporting the hypothesis that THRA rs939348 T>C gene variations impact thyroid hormone levels in the context of hypothyroidism. These findings line with previous studies examined the association between THRA rs939348 T>C gene polymorphism and thyroid hormones and metabolic biomarkers. For instance, Goumidi et al found that hypothyroidism patients with rs939348 T allele had higher systolic blood pressure, suggesting a potential impact of this locus (Goumidi et al., 2011c). Clinical studies suggested that mutation in either of thyroid hormone receptor (THRA or THR β) can lead to resistance to thyroid hormone, and these mutations can result in variable phenotypes both same receptor isoform and between the two isoforms. Therefore, patients with thyroid hormone resistance can present with unexplained elevated of plasma FT4, high levels of TSH and decreased the ratio of FT4/FT3 (Goumidi et al., 2011c; Singh and Yen, 2017). Hence these explanations were similar to our observations.

4.12Analyses of glucose hemostasis and blood lipid profiles in patients and healthy individuals with THRA rs939348 T>C genotypes:

Advance in areas of cell imaging, cell biology such as autophagy, gene expression and metabolomics have generated solid evidences that thyroid hormones play important role in hepatic lipid regulation. Indeed, hypothyroidism strongly associated with dyslipidemia, increased blood lipid cholesterol and triglycerides as well as NAFLD. Thyroid hormone regulates lipid metabolism through different pathways such as autophagy, β oxidation, cholesterol synthesis and activate cholesterol transporter system (Sinha, Singh and Yen, 2018). Not surprisingly, scientists suggested using thyroid analogues for treatment of metabolic diseases involving the liver, such as hypercholesterolemia and NAFLD (Cordeiro et al., 2013; Sinha, Singh and Yen, 2018; Wang et al., 2021b). This why in this study we explored the effects of THRA rs939348 T>C gene variations on lipid profiles, as blood lipid levels may serve as important indicators for evaluating the response to treatment in hypothyroidism and should be taken into account.

Interestingly, cholesterol levels were significantly elevated in patients with wild-type TT genotype compared to healthy group with the same genotype, while TG levels were

elevated in hypothyroidism patients with TT and CC genotypes compared to healthy controls with the same genotypes. HDL and LDL remained constant and stable between the two groups across all genotypes. We noticed an increase in vLDL levels in healthy participants, with significant increase observed in healthy individuals with TT genotype compared to the patients with the same genotype. It is noteworthy to mention that this is the first time to study the effect of THRA rs939348 SNP on blood lipid profiles.

Previously, studies explored the role of both THR (THRA and THRB) genes encode two isoforms, α THR and β THR respectively on lipid metabolism (Baumann et al., 2001). Mostly, scientists focused on THRB gene variations because it is the major form express in the liver, while THRA is commonly expresses in the other tissues like heart and bone (Chamba et al., 1996a; 1996b; Moran et al., 2014). Generally, THRs are nuclear receptor owning nucleo-cytoplasmic shuttling, in spite of some of the residual available in the cytoplasm. This feature enables THRs to bind directly to their target gene on the promotor area of DNA, forming co-repressor complex with histone deacetylase activity to repress positively regulated transcription genes in response to thyroid hormone elements. Upon ligand binding, co-repressors are released lead to conformation changes in the THR, and activate histone acetyltransferase expression which activate other target gene promotor to trigger transcription (Kawata, 2001; Baumann et al., 2001; Luo et al., 2019). Furthermore, thyroid hormone can regulate several transcription factors play a role in lipid metabolism such as (such as activation of forkhead box protein O1 (FOXO1)), modulating cell-signaling cascades through protein–protein interactions (such as the regulation of phosphoinositide 3-kinase (PI3K) by THR β) or binding to proteins other than THRs (such as binding to α v β 3 integrin) (Singh et al., 2016). Thyroid hormone also can activate other transcription factors that play indirect role in lipogenesis such as SREBP1c and ChREBP. For instance, elevated thyroid hormone leads to activation ChREBP expression by recruiting ChREBP promoters. Studies in animal models found that TH regulates SREBP1c transcription negatively through a putative negative thyroid hormone response element (nTRE) (Furuya, Hanover and Cheng, 2006; Hones et al., 2022). Additionally, TH regulates lipid metabolism by increasing FoxO1 nuclear localization, DNA binding and trigger gene transcription in a THR β dependent manner (Furuya, Hanover and Cheng, 2006). Finally, studies suggested that thyroid hormone activate genes involved in TG synthesis and metabolism mainly due to metabolism of free fatty acids FFAs (Yao et al., 2014). Hence these evidences and facts confirm that THR variations are critical for lipid metabolism and may interfere with one or more of these pathways.

Previously, scientists demonstrated the correlation between THRA rs939348 gene polymorphism and SBP and increased risk of cardiovascular disease to 25% (Goumidi et al., 2011c). Other study reported that THRA variations increased waist circumference in hypothyroid patients specially in those who had T allele. These studies agree with our results, as patients with TT genotype had higher levels of blood cholesterol and TG (Al-Azzam et al., 2014; S.I. et al., 2014). The strength of our study lies in comparing these results to healthy individuals rather than just to other genotypes within the patient group, which solidify the evidence and concludes that people with T alleles are more vulnerable to developing resistance to hypothyroidism treatment.

The analyses of glucose hemostasis revealed significant differences between patients and healthy individuals. Insulin levels were significantly higher in hypothyroid patients with TT and CT genotypes compared to healthy controls indicating a potential impact of THRA rs939348 T>C gene polymorphism on insulin stasis. Similarly, FPG levels were elevated in hypothyroid patients with both TT and CC genotypes compared to healthy participants with the same genotypes. HOMA-IR was dramatically elevated in the patients compared to the healthy individuals across all genotypes. Impairment of glucose metabolism in hypothyroidism patients is supported by many studies, but the uniquely in this study the patients undergoing L-thyroxine which should improve thyroxine hormones and improve glucose hemostasis. Therefore, these results may indicate resistance to the therapy due to gene variations in THRA in particular rs939348 T>C locus. This is consistent with previous studies suggesting THRA polymorphisms influence insulin sensitivity and resistance (Goumidi et al., 2011c; Singh and Yen, 2017).

4.13 Investigate of duration L-Thyroxine treatment on plasma thyroid hormones and clinical parameters in hypothyroid patient with THRA rs939348 T>C genotypes:

Studying the impact of treatment duration and gene variations is essential to understand the interacting between gene variations and treatment response, as can optimize therapeutic outcomes, improve management of thyroid hormones, enhance long-term care by modifying treatment durations to specific genotypes (Al-Azzam et al., 2014; S.I. et al., 2014).

In exploring the influence of THRA rs939348 T>C gene variations on the response to L-thyroxine treatment in patients with hypothyroidism, the samples were divided into two groups: those had treatment for more than 5 years and five years or less. Out of 100 patients, 30% treated for five years or less had mutant genotypes and 12% had wild-type

TT genotype. For those treated for more than five years, 37% had mutant genotypes (CT or CC) and 21% had the TT genotype. No significant differences were found in the treatment durations across all the genotypes ($P=0.1496$). Clearly, TSH levels were higher in patients treated for five years or less compared to those with longer treatment term, with significant increase in patients with CT genotype $P<0.01$. No significant differences were observed in the levels of TT3, FT3, TT4 and FT4 across all the genotypes and treatment durations, indicating that prolonged L-thyroxine treatment did not potentially improved thyroid hormone levels, which may suggest that THRA rs939348 T>C gene polymorphism impact treatment efficiency.

Additionally, no notable differences were reported in glucose hemostasis parameters and lipid profiles markers across different genotypes and treatment durations, suggesting a lack response to treatment or an influence of THRA gene variations on treatment responses that are not covered in clinical studies.

Previously studies shown that thyroid hormone receptor gene variations can impact the effectiveness of L-thyroxine treatment. For instance, mutations and gene polymorphism in genes involved in thyroid hormone signaling such as Thr92Ala-DIO2 gene impair T3/T4 ratio and limit L-thyroxine effectiveness to restore euthyroidism. Consistently, with our observation whereas we did not notice significant improvement in thyroid hormones level in patients with hypothyroidism undergoing L-thyroxine treatment in patients with THRA rs939348 mutation. Furth more, we noticed TSH plasma levels were higher in patients treated for less than five years in particular in those with CT genotype, indicating a potential impact of THRA rs939348 polymorphism on treatment response. The lack noticeable differences in glucose and lipid metabolism profiles across all the genotypes and duration of treatment indicating that THRA rs939348 gene variations may not necessarily impact these metabolic biomarkers. This is aligning with previous some studies that found THRA polymorphisms primely effect thyroid hormone levels and necessary other metabolic parameters (Peeters et al., 2003; Santoro et al., 2014; Penna et al., 2024).

4.14 Measuring the impact of THRA rs939348 mutation on the TSH levels and treatment responsiveness in hypothyroid patient and healthy people:

Several observational studies recommended that TSH is an important indicator of the biochemical response to L-thyroxine, while FT3, FT4, TT3 and TT4 are an additional hypothyroidism treatment target (Midgley et al., 2015). Since we did not find a noticeable

change in the levels of thyroid hormones, we focused on exploring the impact of THRA rs939348 gene polymorphism on hypothyroidism treatment response based on the measurement plasma TSH levels. Indeed, THRA gene plays a crucial role in regulating thyroid hormone physiological and biological functions. Variations in this gene such rs939348 SNP may affect the gens functions and consequently influence the severity of the disease and the patient's response to treatment (Bochukova et al., 2012; Korkmaz et al., 2019; Paisdzior, Schuelke and Krude, 2022).

Interestingly, no notable differences in the TSH levels were observed in hypothyroid patients with wild-type TT genotype compared to healthy individuals. While patients with mutant alleles wither heterozygous or homozygous showed significantly higher TSH levels compared to healthy individuals with the same genotypes, indicating that THRA rs939348 mutation potentially impact hypothyroidism severity and L-thyroxine responsiveness. Additionally, TSH levels in patients with wild allele were stable and constant regardless treatment duration or L-thyroxine dosage, with no significant differences compared to healthy individuals. In contrast, patients with mutant genotypes (CT and/or CC) exhibited elevated TSH levels regardless of treatment term or dosage, suggesting a less effective response to L-thyroxine therapy.

4.15Analyses of plasma thyroid hormones and metabolic biomarkers in patients and healthy individuals with THRA rs12393700 C>A genotypes.

The THRA rs12393700 C>A polymorphism represents a novel area of research and it is the first time this specific locus has been genotyped since the initial study by Helena et al. In this experiment we investigated the impact of THRA rs12393700 C>A polymorphism on plasma thyroid hormone levels and clinical biomarkers including glucose hemostasis and lipid profile. All healthy individuals showed wild-type CC genotype while the patients exhibited mutation and wild genotypes. Therefore, our comparison focused solely on the wild-type CC genotype. Interestingly, hypothyroid patients with wild genotype exhibited significantly higher TSH levels compared to healthy individuals with the same genotype, while no significant differences were observed in TT3, FT3 and TT4 levels. Healthy individuals showed significantly higher levels of FT4 levels than hypothyroid patients.

Hypothyroid patients with wild genotype had markedly elevated plasma insulin levels compared to healthy individuals, alongside increased FPG and HOMA-IR. Blood lipid profile analysis indicated higher levels of cholesterol and TG levels in hypothyroid

patients with wild genotype compared to healthy individuals, with significant differences in HDL, LDL and vLDL levels, the latter being higher in healthy controls compared to the patients with hypothyroidism.

Previously, studies explored the role of THRA gene polymorphisms and a variety of parameters in hypothyroid patients by focusing on different loci (Goumidi et al., 2011c; 2011b; Bufalo et al., 2015). In this study, we assessed the effect of THRA rs12393700 C>A polymorphism. Since this gene locus is novel and explored for the first time, we will compare the data with those from studies on different loci and variations. We found a strong correlation between THRA rs939348 mutation, specifically CC genotype and increased risk of hypothyroidism. In contrast, rs12393700 SNP focuses on the wild-type CC genotype that impacted clinical parameters, THRA rs12393700 gene variations appeared to correlate with disease development. However, previously scientists showed that the THRA gene mutations can lower FT4 and elevated TSH levels in children. These findings line with our suggestions, as hypothyroidism patients with wild-type genotype showed higher TSH levels compared healthy individuals (Le Maire et al., 2020; Zhang et al., 2022).

Interestingly, THRA rs939348 highlighted differences in TT, CT and CC genotypes in the context of glucose hemostasis and lipid metabolism. the disease. More likely, rs12393700 gene variations was used to assess glucose stasis and lipid metabolism by measuring insulin resistance, FPG and HOMA-IR, and assessing blood lipid profiles. THRA rs12393700 showed significant differences in glucose hemostasis (plasma insulin, FPG, HOMA-IR) and lipid profiles (cholesterol, TG, HDL, LDL, vLDL) between hypothyroid patients and healthy individuals. These results align with previous studies suggesting that THRA mutations altered glucose hemostasis and increase risk of insulin resistance and type2 diabetes mellitus (Goumidi et al., 2011c). Indeed, thyroid hormone plays a critical role in lipogenesis on both genomic and non-genomic pathways. Studies have shown that THRA mutation linked to dyslipidemia, characterized by elevation of cholesterol and triglycerides levels. These findings supported our results whereas we found patients with THRA rs12393700 C>A wild genotype had higher levels of cholesterol and TG, along with significant differences in HDL, LDL, vLDL levels were observed (Bianco and Kim, 2018; Guilherme et al., 2020; Mendoza et al., 2021; Penna et al., 2024).

4.16 Investigate of duration L-Thyroxine treatment on plasma thyroid hormones and clinical parameters in hypothyroid patient and healthy controls with THRA rs12393700 C>A polymorphism:

The correlation between THRA rs12393700 C>A polymorphism and L-thyroxine treatment duration, and the effect on thyroid hormones and clinical parameters have been observed. Percentage of patients treated for five years or less were 38% with CC genotype compared to 2% had the CA and 2% had the AA genotype. For those grouped under long term treatment were around 52% with CC genotype, 4% had the CA genotype, and 2% had the AA genotype. Genotype distribution showed significant differences across the patients' groups although the treatment duration did not show significant differences across all the genotypes. Indeed, TSH plasma levels were significantly lower in patients with wild genotype (CC) in particular for those treated for longer time, while patients with THRA rs12393700 did not exhibit improvement in the TSH plasma levels based on the treatment duration. TT3, FT3 levels remained constant and stable across the genotypes based on the treatment duration. TT4 and FT4 showed minimal variations. Additionally, glucose hemostasis and lipid metabolism profiles showed minimal changes across the genotypes and treatment terms, indicating that treatment duration of L-thyroxine does not correlate with these clinical biomarkers, and gene variations may play a role in treatment response in patients with hypothyroidism. These findings agree with previous studies that showed prolonged L-thyroxine treatment may improve TSH plasma levels (Duntas and Yen, 2019; Jiajun, 2023; Zamwar and Muneshwar, 2023). Contrary to other studies that suggesting long term treatment of L-thyroxine does not potentially achieved euthyroidism due to un known mechanism (Gullo et al., 2011; T., Y. and I., 2014). We found L-thyroxine treatment improve TSH plasma levels in patients with THRA rs12393700 wild allele but not in those with mutated alleles. Similarly, earlier studies have reported that TT3, FT3, TT4 and FT4 remained constant and stable regardless of treatment durations. It is well established that TSH plasma levels are the dominant indicator of hypothyroidism progression, while the other thyroid hormones level remains challenging to normalize or detect change in (Gullo et al., 2011; T., Y. and I., 2014; Duntas and Yen, 2019; Jiajun, 2023; Zamwar and Muneshwar, 2023).

4.17 Impact of THRA rs12393700 C>A gene polymorphism on TSH levels and L-thyroxine response in patients with hypothyroidism:

In this experiment we aimed to examine the impact THRA rs12393700 C>A gene polymorphism on TSH levels in hypothyroid patients by comparing the wild genotypes to mutant genotypes (CA and AA). This aimed to group broader categories of genotypes, which help to increase sample size and statistical power. The results showed patients with wild-type CC genotype had significantly higher TSH levels $P < 0.005$ compared to healthy individuals with the same genotypes. However, patients with mutant genotypes exhibited TSH levels similar to healthy controls suggesting that the mutant allele might influence the severity of hypothyroidism disease and effectiveness of L-thyroxine treatment. Indeed, the TSH levels in the patients with wild genotypes remained stable and constantly higher than normal values regardless the treatment durations and dosages. While patients with mutant genotypes exhibited a significant reduction in TSH levels after more than five years of treatment, normalizing levels comparable to healthy people. Worth notes, the study reported that TSH plasma levels increased notably with higher doses L-thyroxine drug in patients with wild genotypes, supporting potential overcompensation or resistance. Contrary, patients with mutant genotypes, showed more responsive to the treatment based on TSH levels.

These findings are unique regarding the rs12393700 SNP, which disagree with prevailing evidences that THRA mutations typically reduce treatment response (A.A. et al., 2013; Van Mullem et al., 2013; van Mullem, Visser and Peeters, 2014). In this study, we discover that rs12393700 novel SNP, explored for the first time (Sørensen et al., 2008), actually improves responsiveness to thyroid hormone treatment. Patients with mutant genotypes showed significantly reduced TSH levels over extended treatment periods normalized to levels comparable to euthyroidism. However, these findings cannot be exclusive because the data does not have healthy individuals with the same genotypes. So, the statistical power of the sample size is weak. While patients with wild genotype exhibited higher TSH levels regardless to the treatment dosages and treatment durations. Hence, the importance of examining rs12393700 specific locus within THRA gene, as may have distinct influences on disease development and management, paving the road for more personalized approaches to hypothyroidism treatment focused on individual genetic profiles. However, more studies are necessary to support these findings and unmask the mechanisms, ultimately improving therapeutic outcomes for patients with hypothyroidism.

4.18 Predicting L-thyroxine responsiveness in hypothyroid patients based on THRA rs939348 T>C and rs12393700 C>A gene polymorphisms:

The impact of THRA rs939348 T>C and rs12393700 C>A gene mutations on treatment responsiveness in hypothyroid patients was examined based on their genotypes and treatment response, measured by normalizing plasma TSH levels. THRA rs939348 T>C polymorphism showed 33% of responders and 11% of non-responders had the wild genotype, while 20% of responders and 36% of non-responders had the mutant genotypes. TSH levels were close to normal in wild-type responders ($2.019 \pm 1.09 \mu\text{U/ml}$) but significantly elevated in wild-type non-responders ($P < 0.0001$). Among mutant genotypes, responders had higher TSH levels compared to healthy individuals ($P < 0.02$), with non-responders showing a six-fold increase. This suggests that the wild-type genotype is associated with better treatment response, while the mutant genotype correlates with poor response. Previously scientists found that THRA rs939348 T>C gene polymorphism is potentially correlated with significant changes in TSH levels and treatment response efficiently. They found patients with mutant genotypes CC alleles had a higher risk of poor response to treatment along with our findings that wild-type TT genotype is associated with better treatment response and lower TSH levels (Kim et al., 2013). In contrast, Zhang and his colleague found no significant differences in treatment outcomes based on the THRA rs939348 T>C gene variations. However, they suggested that genotype variations may impact TSH levels, and treatment response variability may affect by different factors (Pappa and Refetoff, 2021; Wibowo, Hastuti and Susanti, 2021b).

THRA rs12393700 C>A polymorphism exhibited 55% of wild genotype patients and 6% of mutant genotype patients were responders, compared to 35% and 6% of non-responders, respectively, with no significant difference in treatment response between genotypes ($P = 0.9455$). Although mutant non-responders had higher TSH levels, the differences were not statistically significant compared to normal values or mutant-responders. These variabilities may be due to significant variances and small sample size. Therefore, it is challenging to assume that patients with the mutation had a better response since, as the observed differences were not statistically significant.

Studies that explored THRA rs12393700 C>A gene polymorphism are rare. In this study, we discover that rs12393700 novel SNP, explored for the first time (Sørensen et al., 2008), actually improves responsiveness to thyroid hormone treatment. Patients with wild

genotypes showed significantly improvement in therapy responsiveness and reduced TSH levels as comparable to euthyroidism. Hence, the importance of examining rs12393700 specific locus within THRA gene, as may have distinct influences on disease development and management, paving the road for more personalized approaches to hypothyroidism treatment focused on individual genetic profiles. However, more studies are necessary to support these findings and unmask the mechanisms, ultimately improving therapeutic outcomes for patients with hypothyroidism.

4.19 Investigate BMI variations among hypothyroid patient by THRA rs939348 T>C and rs12393700 C>A polymorphisms and treatment response:

According to endocrinologists, obesity, dyslipidemia is a common clinical symptom of hypothyroidism. Thyroid gland dysfunction is responsible for obesity, increased BMI parameter, increased waist circumference. However, linking hypothyroidism and obesity are controversial, and there is a lack of clarity regarding subclinical and clinical hypothyroidism. Pioneer studies suggested that TSH changes could well be secondary to obesity. More studies need to establish normal TSH range in population studies due to increasing prevalence of obesity. Scientists suggested that the most a modest effect of L-thyroxine treatment responses including weight loss or normalize BMI measurement to healthy non-obese people. Therefore, we explored the impact of THRA gene polymorphisms in particular rs939348 T>C and rs12393700 C>A SNPs on hypothyroidism treatment responsiveness based on BMI parameters normalized to the TSH plasma levels.

Our study examined the impact of THRA rs939348 T>C and THRA rs12393700 C>A genotypes on BMI and treatment responsiveness in 100 hypothyroid patients and 50 healthy individuals. For the THRA rs939348 T>C polymorphism, patients with the wild-type genotype had BMI levels similar to healthy controls, indicating a better treatment response, while mutant genotype patients exhibited significantly higher BMI levels regardless of treatment status. Conversely, for the THRA rs12393700 C>A polymorphism, wild-type patients had significantly higher BMI levels compared to healthy controls, indicating less responsiveness to therapy, with no significant BMI differences between responder and non-responder groups among mutant genotype patients. These findings highlight the influence of genetic variations on BMI and treatment outcomes, emphasizing the potential for personalized treatment strategies in hypothyroid patients. These results align with Ghafoor et al, which examined the impact

of genetic polymorphisms on BMI and body weight and metabolic outcomes in thyroid dysfunctions. They highlighted how gene variations can significantly affect BMI and treatment outcomes in thyroid disease, agreeing with our findings that THRA rs939348 T>C variations influences BMI and treatment efficiency(Moran et al., 2022; Qiu et al., 2023; Penna et al., 2024) . Hence, the clinical implications of these findings suggesting that hypothyroidism patients should be identified for THRA genotypes prior to treatment, as they may require higher doses of L-thyroxine. More studies in diverse populations are needed to generalize these findings.

In this study we found no statistical differences in age between patients with hypothyroidism and healthy control population (1.360 ± 1.588 years, p -value = 0.3932). not surprisingly, we found significant differences in weight (13.22 ± 2.470 kg, $p < 0.0001$), Body Mass Index (BMI) (4.872 ± 0.9133 kg/m², $p < 0.0001$), systolic blood pressure (9.210 ± 2.361 , $p < 0.0001$), and diastolic blood pressure (3.610 ± 1.152 , $p < 0.0021$) between the hypothyroidism patients and control populations. These results suggest that despite no significant difference in age, there are significant differences in weight, BMI, and blood pressure between the two groups, which could be influenced by various factors such as lifestyle, diet, and genetics. In general, there are a limited study that explored the effects of hypothyroidism on specific demographic groups (Natarajan and Prakash, 2015; Kumar and Gupta, 2021). Thus, the results of this study regarding the significant variations in blood pressure, BMI, and weight among patients with hypothyroidism may offer insightful new information to the field.

4.20 Analyzing thyroid hormones and metabolic markers in patients and healthy control population:

Hyperthyroidism patients showed a significant difference in the biochemical parameters comparing to the control group. A significant increase in the plasma patients TSH comparing to the healthy control people, indicating potential thyroid dysfunction. Additionally, elevated levels of hyperthyroidism patient's plasma TT3 and FT3 levels indicated hyperactivity of thyroid biological activity. Unlikely, FT4 was dramatically lower in the patients, signaling potential disruptions in free thyroxine regulation. The observed higher insulin levels and fasting plasma glucose in patients imply impaired glucose metabolism. Remarkably, patients have a lower Homeostatic Model Assessment for Insulin Resistance (HOMA-IR) index, suggesting compensatory pathways or complex metabolic interactions. These results highlight the intricate relationship between thyroid

function and metabolic parameters and hyperthyroidism. Contextual factors, such as genetic and lifestyle factors, are important, though, and drawing conclusions about causality based only on correlations found in this study should be done with caution. According to the American Thyroid Association, patients who are hyperthyroid will have an elevated T3 level. In some individuals with a low TSH, only the T3 is elevated and the FT4 or FT3 is normal (Forero-Saldarriaga, Puerta-Rojas and Correa-Parra, 2020; Paczkowska et al., 2020). This aligns with the findings of this study elevated TT3 and FT3 levels in hyperthyroidism patients (Selmer et al., 2012; Forero-Saldarriaga, Puerta-Rojas and Correa-Parra, 2020; Paczkowska et al., 2020; Huang et al., 2022). Another study that found serum TG was positively correlated with TSH and FT3/FT4, and negatively associated with FT4. This is agreed with our results of increased TSH and FT3 levels and decreased FT4 levels in patients (Huang et al., 2022; Carreras-Badosa et al., 2023). Recently, scientists have found that children in the upper baseline TSH tertile showed higher concentrations of FT3, FT3/FT4 ratio, and TSH, which agree with our data for increased TSH and FT3 levels in patients with hyperthyroidism (Carreras-Badosa et al., 2023). However, the findings of this study suggest a link between higher insulin levels, elevated fasting plasma glucose, and impaired glucose metabolism in patients, and also indicates signs of potential compensatory mechanisms through a lower HOMA-IR index. These findings are novel and provide significant value in increasing our knowledge of this area.

4.21 Comparing blood lipid profile in patients and healthy control participants:

Patients with hyperthyroidism showed dramatic elevated of total cholesterol (184.93 ± 42.299 mg/dl) and triglyceride (141.91 ± 63.952 mg/dl) levels compared to the control group (160.28 ± 29.449 mg/dl and 111.22 ± 34.797 mg/dl, respectively), indicating dyslipidemia and altered lipid metabolism. Interestingly, VLDL levels are significantly lower in patients (28.15 ± 13.136 mg/dl) than in controls (33.81 ± 11.799 mg/dl), suggesting potential differences in lipid composition or metabolism. LDL levels are modestly elevated in patients (110.08 ± 35.414 mg/dl) compared to controls (101.91 ± 24.445 mg/dl), while HDL levels show no significant difference. These findings align with previous studies in which scientists reported that hyperthyroidism significantly associated with blood lipid alterations mainly concerning total cholesterol and LDL (Ramulu, Rao and Shaik, 2016; Paczkowska et al., 2020). Other studies suggested that perturbations in the

actions of T3 and T4 influence the normal metabolic pathways (Tan, Shiu and Kung., 1998; Duntas and Brenta, 2018). Recently, scientists have found that TSH was high in 5.2% of the 49.5% of patients who were diagnosed with hyperlipidemia (Iqbal et al., 2021; 2022a; 2022b). Hence, this study represents valuable insight in to the relationship between hyperthyroidism and lipid profiles.

4.22 Exploring the analysis of thyroid hormones in long term L-thyroxine treatment: studying the variations in TSH, TT3, FT3, and TT4 plasma levels across different treatment durations.

A trend toward reduced plasma TSH levels with L-thyroxine use. Those who received treatment for 6-10 years and those who received treatment for 21-25 years showed statistically significant differences, with the latter having slightly lower TSH levels. However, TSH levels in other treatment periods were similar to those who received treatment for 1-5 years, indicating that the observed decrease over time is not consistent across treatment periods. Similarly, scientists suggested that Scientists recognized the crucial role of TSH in FT3 conversion, and more studies have shown that temporarily stopping oral levothyroxine increases plasma TSH levels and alters FT3/FT4 ratios significantly within three days. This suggests a direct correlation between TSH levels and the conversion of FT3 and FT4. The lower TSH levels observed in hyperthyroidism patients undergoing therapy across treatment duration may be attributed to the TSH-lowering effect of the treatment (Carlwe, Schaffer and Sjöberg, 2013; Duntas and Jonklaas, 2019b).

Plasma TT3 levels are examined across L-thyroxine treatment durations using Tukey's multiple comparisons test. The results show statistically significant differences in some comparisons, such as 16–20 years vs. 1–5 years, indicating significant variations in TT3 levels for these particular treatment durations as described previously. The FT3 levels for various L-thyroxine treatment durations (1–25 years) and does not reveal any significant variation between groups. This suggests that FT3 may not be significantly affected by treatment duration within the investigated timeframe. Multiple comparison test indicates that plasma TT4 levels did not change statistically among the treatment duration groups that were being compared.

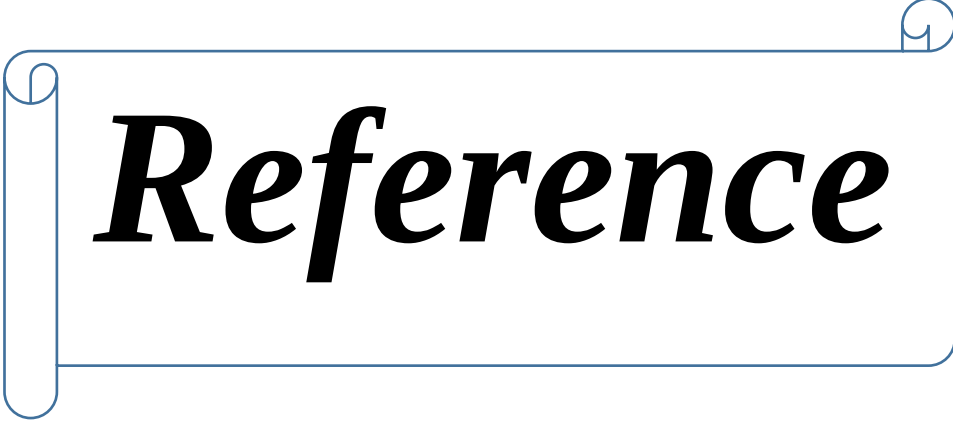
Conclusions

Based on the results that obtained from this study, can be concluded;

1. THRA1 gene was identify with different variable and genotypes in Iraqi femeles with hypothyroidism
2. The wild type TT of rs939348 snp was associated with effective treatment while mutant CC and CT associated with resistance to levothyroxin.
3. The wild type CC of rs12393700 SNP was associated with resistance to the treatment in opposite to mutant type AA and AC.
4. The THRA1 gene SNPs that was detected in Iraqi hypothyroid women was noted to be significantly associated with TSH, fT 4.
5. Both rs939348 and rs12393700 SNPs were associated with the blood pressure parameters or the glycemic parameters in Iraqi hypothyroid female patients.

Recommendations

1. Further Investigations of other SNPs in THRA1 gene along with a large number of hypothyroidism patients and assess their impact on the response to levothyroxine.
2. Investigations other SNPs in THRA1 gene and THRB gene and transporters and enzymes that may affact individual response to the treatment.
3. A large control group needed from populations to determine and compare the results of genetic polymorphysims.



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Appendices

Appendices

Questionnaire for Hypothyroidism Patients Demographic characterization

Name: phone N :

Wight : Hight:

parameters	Vairables	Notes
age		
gender	female	
Occupation		
smoking	Yes no	
History of Family ofhypothyroidism	Yes no	الاب الام
Hypothyodisim complication		
Duration of treatment of levothyroxine		
Drug side effect		
Other disease		
Other medication		
Systolic blood pressure		
Diastolic blood pressure		
Mean BP		
BMI		

Biochemical parameters

parameters	results
TSH	
Total T4	
FreeT4	
TotalT3	
Free T3	
Fasting blood glucose	
Fasting insulin level	
HOMA IR	
HDL	
LDL	
VLDL	
Cholestrol	
Triglysrde	
Results of genotyping	

University of Kerbala

Consent to be in Research

Study Title: The Impact of Genetic Polymorphism of *THRA1* on Therapeutic Response of Levothyroxine in Female Patients with hypothyroidism in Kerbala province

The Researcher Name: Nabaa Zuhair Miran

This is a medical research study, and you do not have to take part. The researcher and doctor, Qusai Baqer Jawad C.D. will explain this study to you. If you have any questions, you may ask me and/or the doctor.

You are being asked to participate in this study because you have primary hypothyroidism and treated with Levothyroxine.

In this study, the researcher are collecting blood samples from you to learn more about the association of genetic polymorphism of *THRA1* with therapeutic response of levothyroxine,

If you agree to be in this study, you will go to the laboratory and give a blood sample for one time only. The blood will be drawn by putting a needle into a vein in your arm. One small tube of blood will be taken. This will take about five minutes.

The risks?

The needle stick may hurt. There is a small risk of bruising and fainting, and a rare risk of infection.

Will my medical information be kept confidential?

We will do our best to protect the information we collect from you and your medical record. Information that identifies you will be kept secure and restricted. If information from this research is published or presented at scientific meetings, your name and other identifiers will not be used. Information that identifies you will be destroyed when this research is complete. You have been given copies of this consent form to keep.

Appendices

The Consent:

If you wish to be in this study, please sign below.

Name of participant:

Date	Participant's Signature for Consent
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Date	Person Obtaining Consent (Researcher)
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الملخص

الخلفية: تعد اضطرابات الغدة الدرقية من بين الاضطرابات الغدد الصماء الأكثر انتشارًا في جميع أنحاء العالم، حيث يعد قصور الغدة الدرقية أحد مظاهرها الشائعة. يتميز قصور الغدة الدرقية السريري بارتفاع مستويات هرمون تحفيز الغدة الدرقية (TSH) بينما يظل هرمون الثيروكسين الحر (T4) أقل من المعدل الطبيعي. يلعب مستقبل هرمون الغدة الدرقية ألفا ١ ($TR\alpha 1$) دورًا حاسمًا في وظيفة الغدة الدرقية، وقد تؤثر الاختلافات الجينية على استجابات العلاج.

الهدف: تبحث هذه الدراسة في تأثير rs939348 و SNPS rs12393700 في جين مستقبل الغدة الدرقية ألفا ١ على الاستجابة العلاجية لعلاج الليفوثايروكسين L-T4 في عينة من مريضات قصور الغدة الدرقية العراقية المقيمت في محافظة كربلاء.

المنهجية: أجريت هذه الدراسة على ١٠٠ مريضة عراقية مصابة بقصور الغدة الدرقية الأولي في سن الأربعين وما فوق، تم علاجهن بالليفوثايروكسين مقارنة بـ ٥٠ مريضة سليمة كمجموعة سيطرة. تم تقييم هرمونات الغدة الدرقية (T4 الحر والكلي، T3 الحر والكلي، TSH). تم تحليل SNP rs939348 باستخدام تفاعل البوليميراز المتسلسل متعدد الأشكال لطول القطعة المقيدة (RFLP-PCR)، في حين تم إجراء الكشف عن SNP rs12393700 باستخدام تفاعل البوليميراز للاليل المتخصص (AS-PCR).

النتائج: بالنسبة لـ SNP rs939348 T>C، كان توزيع النمط الجيني لدى المرضى TT: 33%، CC: 48%، وCT: 19%، مقارنة بـ TT: 80%، 10% لكل من CC، CT في الضوابط. تم العثور على ارتباط مهم بين rs939348 ومستويات TSH ($P < 0.01$) وكذلك إجمالي T3 و T4 الحر ($P < 0.001$) مقارنة بالضوابط.

بالنسبة لـ SNP rs12393700 C>A، كان توزيع النمط الجيني لدى المرضى CC: 90%، AA: 4%، وCA: 6%، في حين أظهرت جميع الضوابط النمط الجيني (CC 100%). تم ملاحظة ارتباط شديد الأهمية ($P < 0.001$) بين rs12393700 ومستويات TSH و T4 الحرة.

الاستنتاج: ارتبط rs939348 T>C وخاصة النمطين الجينيين CT و CC، بمستويات هرمون الغدة الدرقية المتغيرة وقد يساهم في مقاومة علاج L-T4. وعلى نحو مماثل، ارتبط rs12393700 C>A باختلافات هرمون الغدة الدرقية، مما يشير إلى دور محتمل في الاستجابة للعلاج. قد تؤثر الاختلافات الجينية في $TR\alpha 1$ على فعالية الليفوثايروكسين، مما يسلط الضوء على أهمية استراتيجيات العلاج الشخصية في إدارة قصور الغدة الدرقية.



جامعة كربلاء
كلية الصيدلة
فرع الادوية و السموم



تأثير الاشكال الجينية لجين مستقبلات الغدة الدرقية الفا ١ على الاستجابة
العلاجية لعقار الليفوفايرونوكسين عند مريضات قصور الغدة الدرقية في
محافظة كربلاء

رسالة مقدمة الى كلية الصيدلة في جامعة كربلاء كجزء من متطلبات درجة
الماجستير في الادوية والسموم

من قبل

نبأ زهير ميران

(بكالوريوس صيدلة/جامعة كربلاء ٢٠١٤)

بإشراف

د. قصي باقر جواد

١٤٤٦ هجري

أ.م. زهراء عبد الكريم محمد

٢٠٢٤ ميلادي