University of Kerbala

College of Pharmacy

Department of Pharmacology and Toxicology

The Impact of Deiodinase-1 Polymorphisms on the Therapeutic Response of Levothyroxine in Hypothyroidism Patients of 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 of Science in Pharmacology and Toxicology

Written By

Entsar Hadi Jawad

B.Sc. in Pharmacy, University of Baghdad /2005

Supervised by

Prof. Dr. Ban Hoshi Khalaf

Ph.D. Pharmacology and Toxicology

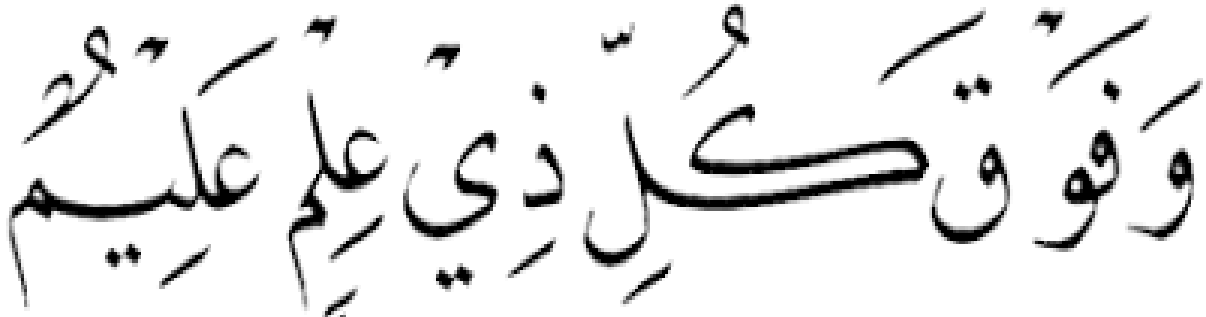
Assist. Prof. Dr. Suzanne Jubair Abbas

Ph.D. Biochemistry

2022 A.D

1444 A.H

بسم الله الرحمن الرحيم



صدق الله العلي العظيم

سورة يوسف: جزء من الآية 76

Supervisor certification

We certify that this thesis was prepared by **(Entsar Hadi Jawad)** under our supervision at the college of Pharmacy / University of Kerbala, as a partial requirement for the degree of Master of Science in Pharmacology and Toxicology

Supervisor
Prof. Dr. Ban Hoshi Khalaf
Ph.D. Pharmacology and Toxicology
University of Kerbala

Supervisor
Assist. Prof. Dr. Suzanne Jubaire Abbas
Ph.D. Biochemistry
University of Kerbala

In the view of the available recommendation, I forward the present thesis for debate by the examining committee

Assist. Prof. Amal Umran Mousa
Chairman of Pharmacology and Toxicology Department
University of Kerbala / College of Pharmacy

Committee Certification

We, the examining committee, certify that we have read the thesis; and have examined the student **(Entsar Hadi Jawad)** in its contents, and find it adequate with standing as a thesis for the degree of Master in Pharmacology and Toxicology.

Thesis Committee Names:

Chairman

Assist. Prof. Sarmad H. Kathem

Ph.D. Pharmacology and Toxicology

Member

Atheer Majid Rashid

Ph.D. pharmacology

Member

Ass. Prof. Mazin Hamid Ouda

M.Sc. Pharmacology and Toxocology

Supervisor

Prof. Dr. Ban Hoshi Khalaf

Ph.D. Pharmacology and Toxicology

Supervisor

Assist. Prof. Dr. Suzanne Jubaire Abbas

Ph.D. Biochemistry

Approved by

College of Pharmacy / Kerbala University

As a thesis for the degree of

Master of Science in Pharmacology and Toxicology

Professor

Dr. Ahmed Salih Sahib

Dean

College of Pharmacy / Kerbala university

Seal

Higher Studies Registration

College of Pharmacy / Kerbala University

Dedication

To

The one who I carry his name proudly, the greatest father in the world

To

The meaning of love and compassion who her prayer is the secret of my success, my mother the angle of my life

To

My best support in all my life, My husband (Dr. Osama alkafagy) for his constant encouragement and support during my study and research

To

My beautiful family, and sisters who have been my source of inspiration and gave me unconditional love, strength, and support every time.

To

Everyone who believes in me and stood by my side with their support, and prayer to complete this thesis.

Entsar

Acknowledgments

*First of all, I render my thankfulness to **ALLAH** who gave me a health, strength and ability to finish this study.*

I owe a deep gratefulness to College of Pharmacy at Kerbala University, Department of Pharmacology and Toxicology for giving me a chance to complete this study.

*I am deeply grateful to my first supervisor **Prof. Dr. Ban Hoshi Khalaf** for her endless support, and for sharing her profound knowledge.*

*My appreciation is conducted to my second supervisor, **Assist. Prof.Dr. Suzanne Jubair Abbas** for her nice support, understanding, and encouragement.*

*I would like to express my deep sense of gratitude to **Prof. Dr. Ahmed Salih Sahib**, the Dean of the College of Pharmacy-University of Kerbala for his continuous support, vast knowledge, and help.*

*My deep appreciation to the chairman of the Pharmacology and Toxicology Department in College of Pharmacy, **Assist. Prof. Amal Umran Mousa** for her help, support, and inducement.*

*I am deeply grateful to **Ass. Prof. Mazin Hamid Ouda AL-Rubaiee**, for his support and help to complete this thesis. And I would like to express my special thanks to **Asst. Prof. Dr. Hassan Mahmoud Mousa,** and **Dr.Jabbar Fadeel Mahdi,** for their helps.*

I wish to express my thanks to all patients participating in this study and to all others who helped me in any way to complete this thesis.

List of Contents

Chapter One: Introduction

List of Tables

List of Figures

List of Abbreviations	
ADME	absorption, distribution, metabolization, and excretion
ANOVA	Analysis of variances
ARMS-PCR	Amplification Refractory Mutation System-Polymerase Chain Reaction
ATP	Adenosine triphosphate
BMI	Body mass index
BMR	Basal metabolic rate
cAMP	Cyclic adenosine monophosphate
CNS	Central nervous system
DBP	Diastolic blood pressure
DIO1	Deiodinase type 1
DIO2	Deiodinase type 2
DIO3	Deiodinase type 3
EDTA	Ethylene diamine tetra acetic acid
ELISA	Enzyme-linked immunosorbent assay
ESS	Euthyroid sick syndrome
FBS	Fasting blood sugar
FSI	Fasting serum insulin
fT3	Free triiodothyronine
fT4	Free thyroxine
HOMA-IR	Homeostasis model assessment of insulin resistance
HPT axis	hypothalamus-pituitary -thyroid axis
IR	Insulin resistance
IRD	Inner ring deiodination
MCT	Monocarboxylate transporter
NTI	Non-thyroidal illness
OATP	Organic anion transporting polypeptide
ORD	Outer ring deiodination
PCR	Polymerase Chain Reaction
PTU	Propylthiouracil
rT3	Reverse triiodothyronine
RXR	Retinoid X receptor
SBP	Systolic blood pressure
SD	Standard deviation
SNPs	Single nucleotide polymorphisms

SPSS	Statistical package for social sciences
T2	Diiodothyronine
T3	Triiodothyronine
T4	Thyroxine
TR	Thyroid hormone receptor
TREs	Thyroid hormone response elements
TRH	Thyrotropin-releasing hormone
TSH	Thyroid stimulating hormone

Abstract

Background: Hypothyroidism is a prevalent condition affecting people worldwide. Levothyroxine is the typical replacement therapy for primary hypothyroidism. Many patients continue to have symptoms and the patient complaints even after a long time of maintaining treatment. Deiodinase type 1 enzyme (DIO1) regulates the metabolism of thyroid hormones. Genetic polymorphisms in the DIO1 gene affect the metabolism of thyroid hormones and could be pathological candidates for hypothyroidism.

This study aims to investigate the impact of two single nucleotide polymorphisms (SNPs) of the DIO1 gene which are rs12095080 (A1814G) SNP and rs11206244 (C785T) SNP on the therapeutic response to levothyroxine replacement therapy in Iraqi hypothyroid female patients.

Methodology: A total of 220 unrelated primary hypothyroidism female patients who were aged 40 years old or older were included in this cross-sectional study**.** All the patients were receiving levothyroxine treatment for at least four months. The rs12095080 (A1814G) SNP and rs11206244 (C785T) SNP in the DIO1 gene were detected using allele-specific polymerase chain reaction method and tetra primers amplification refractory mutation system polymerase chain reaction technique, respectively. The thyroid stimulating hormone (TSH) and thyroid hormones (thyroxin (T4), triiodothyronine (T3), diiodothyronine (T2), and reverse T3 (rT3)) in addition to some glycemic indices and blood pressure parameters were determined.

Result: The genotype frequency of the rs12095080 (A1814G) SNP showed a greater distribution of A allele at the expense of G allele with genotype frequencies of 156 (70.9%) for AA, 61(27.7%) for AG, and 3(1.4%) for GG. At the same time, the

distribution of the rs11206244 (C785T) SNP indicated the prevalence of the C allele with genotypes frequencies 106 (48.2%) for CC, 75 (34.1%) for CT, and 39 (17.7%) for TT.

The patients were divided into three groups depending on the genotype of each SNP. Regarding to the rs12095080 (A1814G) SNP, there were no noticeable variations in the thyroid hormone levels among the three groups of patients.

According to the rs11206244 (C785T) SNP, TSH and rT3 were elevated among the carriers of the T allele (p= 0.018 and p=0.028, respectively), while there were no significant differences on other thyroid hormones (T3, T4, and T2). Besides both SNPs have no association with the glycemic, and blood pressure parameters.

Conclusion: Since the thyroid hormone levels are not affected by the DIO1 gene's rs12095080 (A1814G) SNP, this SNP has no impact on how our sample of Iraqi hypothyroid female patients respond to levothyroxine replacement therapy. Furthermore, since the rs11206244 (C785T) SNP has no impact on the T3 and T4 hormone levels, this SNP dose not contribution with the therapeutic response to levothyroxine. However, this SNP could be associated with the decreased clearance of rT3 from the body.

CHAPTER ONE

Introduction

1. Introduction

1.1Thyroid Gland

The thyroid gland is a part of the endocrine system of the body. It is the largest organ in the human body that conducts endocrine functions. It is a large veined organ that's attached to the front of the throat, near the voice box (1).

The thyroid gland produces two types of hormones, thyroid hormones, and calcitonin. Thyroid hormones including thyroxine (T4), and triiodothyronine (T3) in a significantly lower amount, both are secreted from follicular cells (the functional units of the thyroid). Triiodothyronine has considerably higher biological activity than T4 and is specifically generated at its site of action in peripheral tissues by deiodination of T4. The other hormone, calcitonin, is responsible for regulating calcium levels in the blood which is secreted by the thyroid gland's parafollicular cells (2,3).

The classic understanding of thyroid hormone hemostasis is based on the hypothalamus- pituitary- thyroid axis' (HPT axis) negative feedback mechanism (4,5) as shown in Figures 1-1.

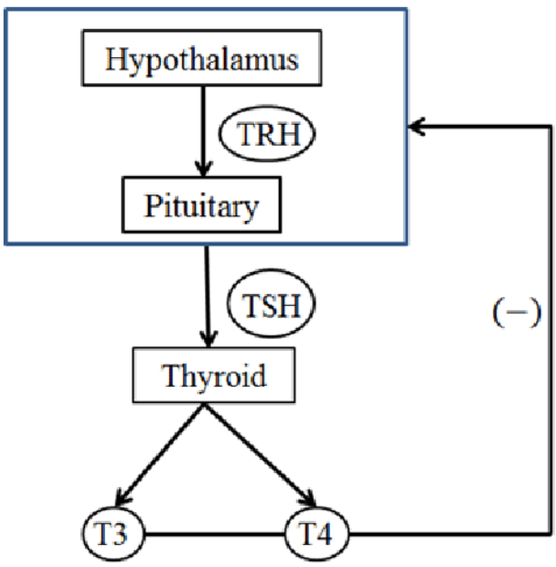


Figure1-1: A negative Feedback Loop of the Hypothalamus-Pituitary-Thyroid (HPT) axis.(6)

This loop begins with the release of thyrotropin-releasing hormone (TRH) from the hypothalamus, which promotes the secretion of thyroid stimulating hormone (TSH) from the anterior pituitary that can stimulate the production of T3 and T4 from the thyroid follicles. The release of TRH and TSH from the

hypothalamus and pituitary is inhibited when the serum levels of thyroid hormones are sufficient (7). Therefore, even a little drop in circulating T4 results in a substantial rise in circulating TSH; a 50% drop in T4 can increase TSH levels by up to 100 times; similarly, increases in T4 in the condition of hyperthyroidism can be easily seen as considerable decreases in circulating TSH levels (8,9).

The thyroid hormones are required for a variety of functions in vertebrate tissues (10), they are responsible for the regulation of a vast range of mental and physiologic processes development that influence nearly most human body tissue at whole stages of life (11).

The thyroid gland plays a crucial role in the management of energy metabolism as well as the regulation of the heart and peripheral vascular function. Thyroid hormones are essential for maintaining healthy levels of glucose and lipids (12). Therefore, T3 is crucial for glucose, lipid, and energy metabolism as well as for thermogenesis (13). When thyroid hormones enter cells, they attach to specific receptors in the nuclei. This cause an increase in the production of different types of mRNA, which leads to the release of large number of enzymes and hormones (14).

Thyroid disorder is the most prevalent metabolic disturbance in the world, after diabetes mellitus (15). Underactive and hyperactive thyroid gland disorders, known as hypothyroidism and hyperthyroidism, respectively, are the most common thyroid dysfunctions. Furthermore, each disease has its own set of etiologies. Certainly, human cases of thyroid hormone excess “hyperthyroidism” are characterized by an acceleration of basal metabolic rate (BMR) and weight loss, whereas thyroid hormone deficiency “hypothyroidism” is presented with decreased BMR and is usually accompanied by weight gain (16).

1.2 Hypothyroidism

Hypothyroidism is a chronic pathological disorder result from insufficient thyroid hormones T3 and T4 levels (17). It can cause serious healthy complications and even death if left untreated. Hypothyroidism is primarily defined biochemically because of the wide diversity of clinical manifestations and the absence of symptoms specificity (18).

It is important to determine the severity of the hypothyroidism as people with severe symptoms may end up in a coma, while moderate cases can go undetected

(19), so many patients with hypothyroidism are completely unaware of their condition (20).

Cardiovascular disease, infertility, musculoskeletal and neurological diseases are all complications of inadequately managed or untreated hypothyroidism (21,22). Additionally, hypothyroidism is strongly linked to a lower quality of life, that is mostly due to weight gain, fatigue, weakness, and depression (17,23).

1.2.1 Diagnosis

Hypothyroidism has a wide range of clinical manifestations and symptoms (24), with no single symptom accurately indicating the occurrence of hypothyroidism, so laboratory testing and clinical examination are required for diagnosis (25).

The measurement of serum TSH level is the best way for determining thyroid function and detection of hypothyroidism (26), since more than 99 % of hypothyroidism is primary hypothyroidism, so the rise in serum TSH level is the first biochemical abnormalities developed in this condition (27). Serum free thyroxine (fT4) should be examined if TSH levels are elevated or decreased (28).

When serum TSH levels are increased above the reference range (most frequently used 0.4–4.0 mIU/L) and T4 levels (free and total T4) are below the overall reference range, primary hypothyroidism is described as overt or clinical (18), while subclinical hypothyroidism which is a type of thyroid dysfunction marked by slightly to moderately high serum TSH levels but T4 level which is still within the normal range (27).

The diagnosis of central hypothyroidism (secondary or tertiary) is more difficult. The existence of free T4 levels lower than normal value in conjunction with decreased or normal TSH levels in individuals have hypothyroidism symptoms suggests central TSH inadequacy, and additional symptoms examination of hypothalamic-pituitary insufficiency is required (29).

Congenital hypothyroidism screening in newborns is critical for diagnosis; but, the diagnosis is difficult because several variables can influence TSH and T4 levels, such as neonate birth weight, age at sample taken, and preterm. Follow-up diagnostic test is recommended for infants with low T4 and increased TSH (30).

It is important to remember that reference ranges are a contentious topic that varies depending on the test utilized, the patient's age, gender, and race. In adults, the upper limit of the TSH normal ranges increases significantly with age (18,31).

Thyroid peroxidase antibody testing isn't technically required to detect hypothyroidism, but it can identify the cause of a thyroid disorder and support the diagnosis of autoimmune hypothyroidism (32).

Other abnormal laboratory values that may be observed in hypothyroid patients include: elevated levels of triglycerides and low-density lipoprotein, decreased high density lipoprotein levels, and normocytic anemia (33). However, the biochemical findings could also reveal increased C-reactive protein, proteinuria, hyponatremia, hyperprolactinemia, and elevated creatine kinase (34).

The clinical diagnoses include signs and symptoms, checking the size of the gland, and physical examination characterized by a long ankle-jerk reflex time which is the best indicator of hypothyroidism severity (32). Two-dimensional ultrasound imaging can also be used to diagnose hypothyroidism (35).

1.2.2 Pathophysiology

Hypothyroidism has complicated pathophysiology that incorporates multiple causative variables. The precise location of malfunction can divide the disease into primary, secondary, and tertiary hypothyroidism (7,36).

The inability of the thyroid gland to synthesize and secrete a sufficient amount of thyroid hormone is known as primary hypothyroidism, this was responsible for about 95% of all cases of hypothyroid patients, the congenital abnormality, autoimmune disease, and iatrogenic effect are the most common underlying mechanism of primary hypothyroidism(33).

Around the world, endemic iodine deficiency in the diet is the major cause of congenital hypothyroidism (uncommon in developed countries). Congenital hypothyroidism may be occurring due to germline abnormalities in genes crucial for thyroid development, such as thyroid agenesis where there is an absence of thyroid parenchyma. Other causes of congenital hypothyroidism are thyroid hypoplasia in which the thyroid gland size could be smaller than usual or the gland is not found in right place (ectopic thyroid) (33,37).

On the other hand, inborn thyroid metabolism defects (dyshormonogenetic goiter) are another rare cause of congenital hypothyroidism which is characterized by an abnormality in multiple stages of thyroid hormone production, like iodine trapping, organification, coupling, transport, thyroglobulin production, and deiodination (38,39).

In iodine-sufficient regions of the world, autoimmune hypothyroidism is still the most common cause of hypothyroidism, Hashimoto thyroiditis accounts for the great majority of autoimmune hypothyroidism patients (36,40). Hashimoto's disease is characterized by the presence of circulating autoantibodies, such as anti-thyroid peroxidase (TPOAb), anti-thyroglobulin (TgAb) antibodies, and an enlarged thyroid (goitrous). It is caused by the destruction of thyroid autoantigen self-tolerance. The existence of circulating autoantibodies against thyroglobulin and thyroid peroxidase is the great majority of Hashimoto patients exemplifies this point. Hashimoto thyroiditis, like other autoimmune disorders, has a substantial hereditary component. Apoptosis-induced loss of thyroid epithelial cells, mononuclear cell infiltration, and fibrosis of the thyroid parenchyma are the hallmarks of thyroid autoimmunity (36).

Thyroid autoantibodies are seen in 5–15 % of women (41). Therefore, women often have a higher rate of thyroid autoantibodies, which may explain why they have a higher rate of thyroid dysfunction (42).

Surgical or radiation-induced ablation can both cause iatrogenic hypothyroidism. Thyroid ablation is the third most common cause, which develops when the thyroid gland is surgically removed in case of thyroid cancer, nodules, or Graves' disease. Developing hypothyroidism involves the destruction of at least 90% of the thyroid gland (5).

Drugs like methimazole and propylthiouracil (PTU), which are used to reduce thyroid secretion, can potentially cause primary hypothyroidism (43). In some situations, agents used to treat non-thyroid disorders can cause hypothyroidism, mainly by interfering with normal thyroid hormone syntheses such as amiodarone, lithium, interferon-alpha, or interleukin-2. These medications are most likely to cause hypothyroidism in people who have a hereditary predisposition to autoimmune hypothyroidism (44,45).

Iodine is abundant in amiodarone, and the Wolff–Chaikoff effect (temporary hypothyroidism) is caused by large levels of iodide generated during amiodarone

metabolism, which inhibits thyroid hormone manufacturing and release. The initial Wolff–Chaikoff will last just a few days until the organification of intrathyroidal iodide returns and normal thyroxine (T4) and triiodothyronine (T3) biosynthesis resumes, according to the so-called "escape" from the Wolff–Chaikoff effect. A minor hormone deficiency that results in increased susceptibility to the inhibitory action of iodine on hormone biosynthesis, a failure to escape the Wolff–Chaikoff effect or both is thought to be the cause of chronic amiodarone-induced hypothyroidism (46).

Iodine deficiency is another factor that leads to primary hypothyroidism, as it is thought to make the thyroid gland more antigenic in addition to being it is an essential component of T3 and T4 (18,47).

The disorder in the hypothalamus-pituitary axis causes secondary or tertiary hypothyroidism which is characterized by lowering TSH or TRH levels, respectively (36,47).Secondary hypothyroidism can develop if a tumor destroys the pituitary, postpartum pituitary necrosis, trauma, or surgery, while tertiary hypothyroidism can develop if the hypothalamus is injured by tumors, trauma, or radiation therapy (36). Those account for less than 5% of hypothyroid patients (33).

1.2.3 Epidemiology

Thyroid dysfunction prevalence rates vary widely across the globe because of various causes, including biological and geographical factors (48). Furthermore, the wide disparity between the studies is expected due to the different features of the people being researched in relation to gender, age groups, iodine intake, racial arrangements, and the prevalence of treated thyroid disease (49).

The most prevalent thyroid disorder associated with age and gender is hypothyroidism, which is 10 times more prevalent in females than in males and more common in older women. According to community research, the frequency of hypothyroidism increases with age by 2% to more than 20% as people get older (50).

Hypothyroidism is a widespread disorder that affects 3–5% of population (23). Subclinical hypothyroidism is common in the general population, with a prevalence of 3–12% (51).

In Iraq the prevalence of primary hypothyroidism, according to a study done in 2005, was found to be 88.9 % as congenital hypothyroidism and 11.1% as Hashimoto thyroiditis. More than half of those patients had parental consanguinity

and a family history of hypothyroidism (52). According to Faisal *et al* study (2010), 14.45% of Iraqi women with thyroid disorders have hypothyroidism (53), while a research done in Kirkuk city in 2021 conducted that 22.7% of the population have hypothyroidism (37).

In the Arab world, the frequency of subclinical hypothyroidism was 2.3 %in Libya (54) while in Makkah region of Saudi Arabia was 47.34% (55).

Hypothyroidism affects multiple organ systems with a prevalence rate of almost 4.6 % of the overall people in the America (56), 3 % of the European population (57,58), and 10.5 % in India (47). According to research done on a large population in Denmark, the most prevalent subtype was autoimmune hypothyroidism (84.4 %) that developed spontaneously, followed by post-partum hypothyroidism and hypothyroidism induced by amiodarone (4.7 % and 4.0 %) respectively (20). Other report was found 2.0% and 5.5% of Iranians have overt and subclinical hypothyroidism, respectively (59). Depending on the population being assessed, the incidence of subclinical hypothyroidism in the Korean population varied from 0.16% to 17.63% (60).

1.2.4 Clinical Features

Many important physiologic processes are regulated by thyroid hormone function, as a result, there are many different clinical signs and symptoms that can be caused by hypothyroidism. The extent of thyroid dysfunction and the development of hypothyroidism typically determine the intensity of these symptoms (24).

Hypothyroidism is often accompanied by a varied range of non-specific symptoms including; weight gain, lethargy, inability to concentrate, depression, widespread muscle soreness, menstruation abnormalities, constipation, proximal muscle weakness, cold sensitivity, vocal changes, goiter, dryness, and loss or brittle hair are all sign that are highly specific for hypothyroidism (20,61)

Hypothyroidism symptoms differ depending on age and gender. Lethargy and growth retardation are more common in infants and children while in elderly patients, cognitive impairment is the main manifestation. In women, hypothyroidism can cause menstrual abnormalities and infertility (24), while hypothyroid men may have lower levels of sex hormone-binding globulin and free testosterone (62).

Hypothyroidism is linked to many other disorders that affect approximately every organ in the body. Hypothyroidism is accompanied by decreased cardiovascular contractility, and its relationship with coronary artery disorder has been established (18), as well as bradycardia, flattened T waves, and low voltage is all common electrocardiography results(24).

Gastrointestinal signs and symptoms may occur due to decreased motility of the viscera, leading to dyspepsia, gastroesophageal reflux, and constipation (63). Dermatologically, glycosaminoglycan will accumulate in the interstitial space of various parts of the body during severe, untreated hypothyroidism resulting in Myxedema which is the swelling of the face, eye, and other parts of the body caused by this accumulation (64). The degree of hypothyroidism influences the skin's changes, such as vitiligo, livedo reticularis, xerosis, reduced sweating, thickening of the skin, loss of the lateral eyebrows (Queen Anne sign), and brittle hair or loss (65).

The neurologic signs of hypothyroidism include sensory polyneuropathy, myopathy, and carpal tunnel syndrome (66).

1.2.5 Complications

Many signs, symptoms, and complications were associated with hypothyroidism, such as: obesity, cardiovascular disease, skin manifestations, various glucose metabolic abnormalities, reproductive system disorders, hyperlipidemia, and depression (67). In general, thyroid hormone functions have distinct effects on endpoints such as body adiposity, glucose levels, cholesterol levels, and blood pressure (68–70).

The regulation of metabolic rate, appetite management, and even sympathetic activity by the thyroid hormone may cause obesity (68,71–73). The thyroid hormone accelerates catabolic and anabolic mechanisms in macronutrient metabolisms, which including lipolysis/fatty-acid oxidation and enhanced protein turnover, via increasing adenosine triphosphate (ATP) use and enhancing the activity of the membrane-bound Na, K-ATPase in many tissues (74)

Subclinical hypothyroidism increases the risk of developing heart failure when TSH levels are more than 10 mIU/L (75). The most obvious symptoms of hypothyroidism include increased systemic vascular resistance, decreases in cardiac output, and contractility, as well as irregular rhythms. A thyroid hormone deficiency increases resistance in peripheral arterioles and reduces tissue thermogenesis

(76,77). Persistent and chronic interstitial edema, which is related to lowered cardiac contractility, could result in cardiac tamponade in its most sever manifestations (12).

Various proteins, polysaccharides, hyaluronic acid, and chondroitin sulfuric acid are typically found in the skin. These complexes accumulate in hypothyroidism, enhancing water retention and the characteristic swelling of the skin (myxedema). When thyroid hormone replacement therapy is administered, the proteins are degraded and the diuresis keeps on until the myxedema is gone (3).

Myxedema coma is a rare but potentially fatal clinical phenomenon that affects patients with untreated, long-term hypothyroidism. Myxedema coma, which involves abnormal mental status, bradycardia, developing lethargies, and bradycardia, can lead to many organ dysfunctions and deaths (78).

Mental processing is delayed and the CSF protein content is increased in hypothyroidism. Since, these changes were reversed by administration of thyroid hormone replacement therapy and high doses cause agitation, irritability and rapid mentation. Thyroid hormones also significantly affect the development of the brain. Consequently, mental retardation, motor stiffness, and deaf-mutism are caused by thyroid hormone deficiency during development (3).

In Hypothyroidism, the T3 deficiency results in decreased insulin binding to receptors and impaired the glucose transporter 4 to the plasma membrane translocation, leading to peripheral insulin resistance (IR), reduce glucose uptake in muscles and adipose tissue which are closely linked to obesity and can contribute to high blood sugar levels (79,80). Triiodothyronine regulates genes involved in insulin signaling, glycogen metabolism and hepatic gluconeogenesis, by acting directly on the liver via thyroid receptor (TR) β (80,81).

Total cholesterol and low-density lipoprotein cholesterol (LDL-C) levels may rise in hypothyroidism due to alterations in lipid production, metabolism, and mobilization (82).

Hypothyroidism has been linked to a variety of reproductive problems, including abnormal sexual development, menstruation abnormalities, and infertility (83,84). Hypothyroidism can disturb fertility in numerous ways, by causing sex hormone imbalances, hyperprolactinemia, luteal phase irregularities, and an ovulatory cycle, but infertility may be easily controlled by taking levothyroxine. According to a study done in India, 76.6% of infertile hypothyroid women were

conceived after administering levothyroxine(85). Additionally, hypothyroidism causes polycystic ovarian morphology, hence hypothyroidism should be ruled out before diagnosing polycystic ovarian syndrome (PCOS) in any woman(86).

Bone growth is reduced and epiphyseal closure is delayed in congenital hypothyroidism because thyroid hormones are required for normal growth and skeletal maturation. The growth hormone release and effect on the tissues are significantly suppressed in the absence of thyroid hormones (3)

1.3 Levothyroxine

1.3.1 General View

The active ingredient, thyroxine, was extracted in 1914 and its structure was ultimately recognized a decade later. Since the 1950s, synthetic thyroxine formulations have been available for usage. Thyroxine is a racemic mixture of Levo and Dextro forms that occur naturally, but the discovery that the Levo form has better absorption and greater physiological activity than the Dextro form led to the introduction of levothyroxine in 1962 (87). For more than 60 years, thyroid hormone replacement therapy with levothyroxine, an exogenous form of T4 that is biochemically and physiologically identical to the natural one, has been the "gold standard" for the management of primary, secondary (pituitary) or tertiary (hypothalamic) as well as congenital or acquired hypothyroidism (32,88). It's also used to treat euthyroid goiters, such as thyroid nodules, subacute or chronic lymphocytic thyroiditis, multinodular goiter, and thyroid cancer patients who had their thyroids removed, as well as a supplement to surgery and radioiodine therapy (89).

Levothyroxine is regarded as the standard of care for hypothyroidism by professional organizations in the United States (90). Levothyroxine sodium was traditionally marketed as a tablet form, however, two novel formulations (a soft gel, and a liquid formulation) have been introduced recently in some countries (88,91).

Treatment with levothyroxine aims to alleviate symptoms and reduce long-term complications (92).

1.3.2 Pharmacokinetics of Levothyroxine

The therapeutic effectiveness of levothyroxine depends on the absorption, distribution, metabolism, and excretion (ADME) of levothyroxine, Figure 1-2 shows

the overall ADME for levothyroxine. At a low PH, oral levothyroxine is dissolved in the stomach, yet after three hours of ingestion, it is mostly absorbed in the small intestine (93)

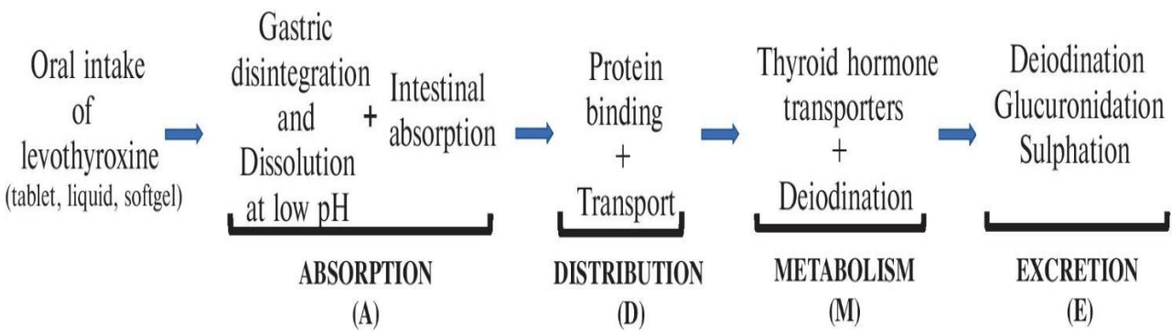


Figure 1-2: An Overview of Absorption, Distribution, Metabolism, and Excretion Processes for Levothyroxine (93)

In hypothyroid patients and fasting euthyroid individuals, levothyroxine bioavailability is 65–80 %, it is about (99.9%) attached to plasma proteins (mostly albumin, high density lipoprotein, transthyretin, and thyroxine-binding globulin) once it was passed the epithelium of intestinal and entered the blood stream (93)

Levothyroxine is metabolized in a variety of glands, organs, and regions of the brain (most importantly the liver, in addition to the anterior pituitary, thyroid, and kidney), as well as muscles (peripheral tissues) (94).

Levothyroxine is a prodrug that is activated by the deiodinase enzyme via outer ring deiodination (ORD) to form active form (T3) as shown in Figure 1-3, which illustrated the chemical structure of T4 and T3 as well as, their main site of deiodination (95).

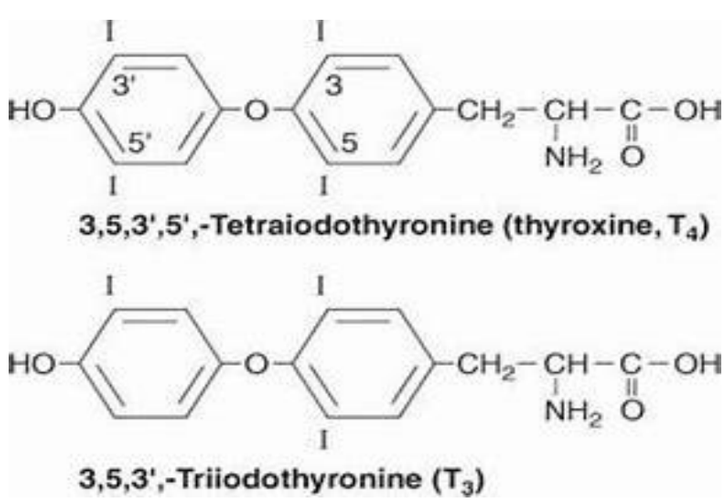


Figure 1-3: Structure of Levothyroxine (T4), Triiodothyronine (T3) and their main site of deiodination (3).

Deiodinases enzymes are selenocysteine-containing enzymes that can eliminate iodine from iodothyronines. Outer ring Deiodination (ORD) of T4 was done by deiodinase type 1 and 2 (DIO1, DIO2) to produce triiodothyronine (T3), the major physiologically active product, while inner ring deiodination (IRD) of T3 and T4 by deiodinase type 3 (DIO3) results diiodothyronine (T2) and reverse T3 (rT3), respectively. Since T2 and rT3 are biologically inactive metabolite for T4 (96).

Thyroid hormone transporters actively carry thyroxine T4 and T3 across the cell membrane; about sixteen transporters in human have been identified as being implicated in the transport of T4 and T3 through the plasma membrane. These transporters are usually categorized into three groups; monocarboxylate transporter (MCT10, MCT8), amino acid transporters, and organic anion transporting polypeptide (OATP1C1), these transporters have tissue-specific expression and are one of the regulatory mechanisms for thyroid hormone function at the cell level. Previous research has found that genetic variations in the enzyme and proteins affect thyroid hormone metabolism, serum thyroid hormone concentrations and levothyroxine bioavailability (93)

Thyroxine T4 and T3 are excreted in the bile after glucuronidation or sulphation in the enterohepatic cycle which accounts for about 20% of the ingested dosage of levothyroxine, which is eliminated in the stools as shown in Figure 1-4, and the other 80% is excreted through the urine (97).

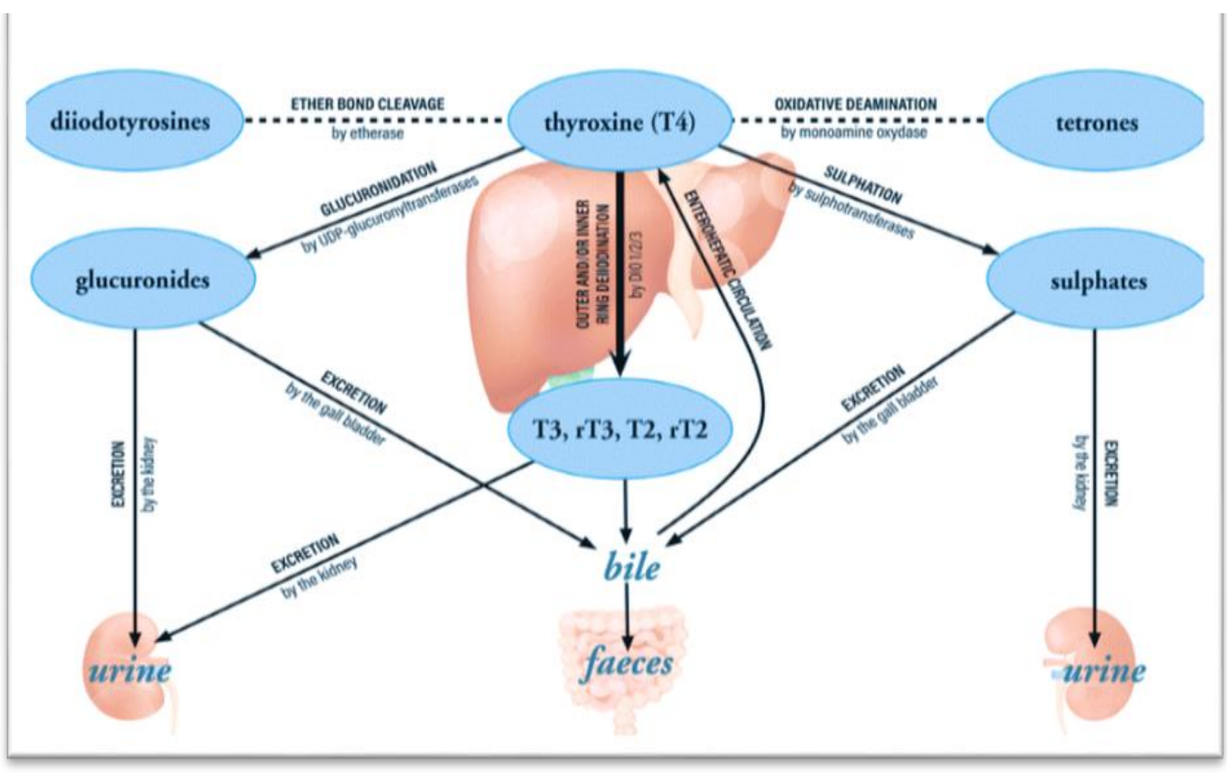


Figure 1-4: Metabolism and Excretion Pathway of Levothyroxine (93).

Levothyroxine is a long-acting medication with a plasma half-life of about 7 days. The drug is usually given as a single oral dose 30 minutes before breakfast in the morning (98).

1.3.3 Dose Adjustment of Levothyroxine

Levothyroxine replacement therapy should be individualized to their specific needs to keep patients euthyroid. In patients with hypothyroidism, the dose to starting treatment with levothyroxine is depending on the patient's body weight (BW). In addition to that many factors can influence the amount of levothyroxine required in each patient including the amount of levothyroxine absorbed in the gastrointestinal tract, which is affected by a variety of disorders, drugs, food, and drink as well as the timing in which levothyroxine is given (99).

Thyroid remaining function, which is dependent on the etiology of hypothyroidism, is another factor that influences levothyroxine dose requirements. Patients with hypothyroidism caused by Hashimoto's thyroiditis or radioiodine ablation for Graves' disease required much lower dosages of levothyroxine than those with a total lack of thyroid tissue (100). The starting dose of levothyroxine is also determined by the presence of co-existing heart illness, and the patient's age, the dose requirements reducing as patients get older (20).

The thyroid-stimulating hormone need to be checked 4 to 6 weeks after starting levothyroxine administration or changing dose, due to the long half-life of levothyroxine. After that, patients' TSH levels must be checked every year (101). Thyroid stimulating hormone level is titrated with levothyroxine until it is normalized between 0.4 and 4.0 mIU/L (102).

Levothyroxine full dose (1.6 μg /kg/day) is often administered orally to healthy adult patients under 50 years of age who have been diagnosed with overt hypothyroidism. However, those patients who are 50–60 years old or those with coronary artery disease are given a lower initial dose of levothyroxine once daily approximately (25-50 μg). Levothyroxine dosage during pregnancy must be adjusted to produce a TSH level in the lower half of the range for each trimester or below 2.5 mlU/L. In patient with subclinical hypothyroidism, 50–75 μg dosages may be enough to return the serum TSH level to normal (20,103).

1.3.4 Mechanism of Action

Levothyroxine exerts its cellular functions through T3 which binds to thyroid hormone nuclear receptors (TRs). Thyroid hormone nuclear receptors are members of the steroid hormone receptor superfamily, however, unlike other steroid hormone receptors, TRs bind to thyroid hormone response elements (TREs) both in the absence and presence of ligand (104).

The expression patterns of the two distinct thyroid hormone receptor TR genes, TRα and TRβ, in developing and adult tissues are different (105,106). TRα is largely found in the brain, heart, and skeletal muscle, while TRβ is abundant in the retina, brain, inner ear, lungs, liver, and kidney (105). Triiodothyronine is further trafficked to the nucleus by these TRs, where it binds to DNA in a complex with thyroid hormone receptors with an affinity 10 times greater than T4 (107,108).

Triiodothyronine binding to thyroid hormone receptors (TRs) in the nucleus of the cell, causing a change in the receptor development and binding, which attaches to DNA and induces thyroid responsive genes transcription. The TR frequently forms a heterodimer with the retinoid X receptor (RXR), and its activation is controlled by co-regulator proteins that bind once T3 binds to the receptor (109). By binding to thyroid receptors (TRs), T3 regulates differentiation and metabolic rate by activating or suppressing particular genes(5).

Thyroid hormone receptors promote transcription when bound to T3, whereas they suppress transcription when not bound to T3. Thyroid hormone, for example, regulates the expression of essential structural and regulatory genes in cardiac myocytes, which is tightly correlated to heart function. Triiodothyronine also regulates β-adrenergic receptors and sodium-potassium ATPase (104). Direct modulation of the transcription rate of certain lipogenic/oxidative genes and changes in metabolite concentrations, cell energy state, and posttranslational changes of proteins involved in hepatic lipid metabolism are all mechanisms of thyroid hormone activity (110).

1.3.5 Drug and Food Interactions

There are a variety of substances that have been shown to affect thyroid hormones levels, and the effect seems to be more distinct in hypothyroid patient receiving exogenous supplementation than in people without thyroid disease, owing

to their intact feedback mechanisms. Additionally, indirect association with levothyroxine could occur through the HPT axis (111).

Various types of food such as soybean, papaya, and grapefruit, can interfere with levothyroxine oral absorption (112). Additionally, coffee can reduce the absorption of some levothyroxine preparations (113). Calcium and iron supplements inhibit absorption, while vitamin C is alone an example of supplementation that may reduce the need for levothyroxine by improving its absorption (114).

Through various mechanisms, medications can change a patient's need for levothyroxine such as: changes in levothyroxine absorption, transport, metabolism, and TSH secretion as well as alteration in thyroid hormone synthesis or release, are examples of these mechanisms' alterations. In most circumstances, estrogen therapy, which is associated with increased levels of thyroxine-binding globulin, is a classic example of a drug that causes an increase in levothyroxine requirement. Tyrosine kinase inhibitors raising the requirement for levothyroxine through increasing levothyroxine metabolism (114).

Thyroid hormone level is reduced by medicines that reduce TSH secretion (dopamine, glucocorticoids, and octreotide), whereas other medications including iodine, lithium, amiodarone, and sulphonamides interfere with the synthesis of thyroid hormone (111).

Because normal stomach acid secretion has important role in levothyroxine absorption, levothyroxine absorption was affected by proton-pump inhibitor drugs (lansoprazole and omeprazole) as evaluated by TSH levels (115). Furthermore, some medications affect DIO1 expression such as amiodarone which inhibits DIO1 activity in peripheral tissues, resulting in a 30% decrease in serum concentrations of T3 and increases of 20-40% in T4 levels and of 20% in reverse T3(116).

1.4 Deiodinases Enzymes

Iodothyronine deiodinases enzymes are a group of selenoproteins that participate in the local and peripheral control of thyroid hormone. There are three deiodinases that facilitate the elimination of iodine from either the inner or outer ring of T4 and its metabolites have been discovered (117).

Type 1 deiodinase and type 2 deiodinase are involved in the conversion of T4 to physiologically active T3 as well as the clearing of rT3 by converting it to T2.

Triiodothyronine inactivation is regulated by the enzyme DIO3, which regulates the conversion of T3 to T2 and T4 to rT3 as shown in Figure 1-5. The expression pattern of deiodinases enzymes differs depending on the tissue; the liver, kidney, thyroid, and pituitary are the main sites of DIO1 protein expression while DIO2 protein is present in the heart, pituitary, thyroid, brown adipose tissue, skeletal muscle, bone and central nervous system (CNS). Although DIO3 is primarily present in fetal tissues throughout life, it is also detected in the adult CNS and placenta. Genetic variations in these enzymes may have an impact on different organ systems because deiodinases regulate thyroid hormones in a variety of tissues (94,118).

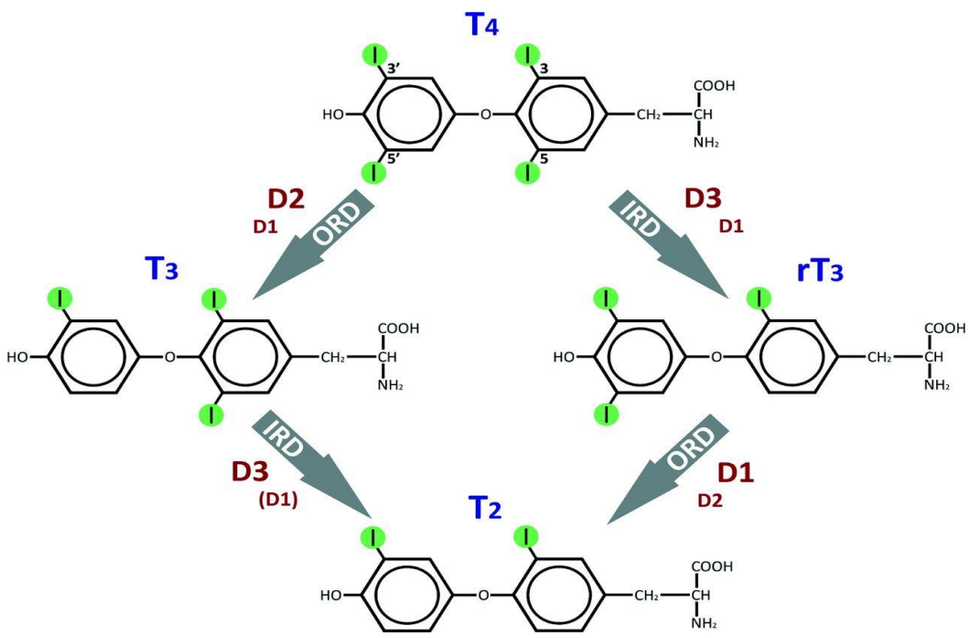


Figure 1-5: Major pathways of thyroid hormone deiodination. D1, D2, D3: deiodinase types (1, 2 and 3), T4: thyroxine, rT3: reverse T3, T2: diiodothyronine, T3: triiodothyronine (119)**.**

1.4.1 Deiodinase type 1 Enzyme

Human deiodinase type 1 is found in many organs, but it is most abundant in the thyroid, liver, and kidneys. Deiodinase type 1 is thought to play a role in the intrathyroidal production of T3 in the thyroid gland (120,121). Deiodinase type 1 is an enzyme that is found in the plasma membrane and is thought to be the main source of the plasma T3 in humans. The amount of T3 released by this pathway is thought to equilibrate with the plasma pool of T3 (94).

Deiodinase type 1 is the only iodothyronine deiodinase that can be active in both the ORD of T4 to produce T3 and reverse T3 (an inactive iodothyronine) to T2, as well as IRD of T4 and T3 to produce rT3 and T2, respectively (122). However, DIO1 is more important in ORD than IRD because it has a limited ability to eliminate

iodine from the inner ring (123). Therefore, DIO1 is essential in both the synthesis of serum T3 and rT3 elimination (124).

Deiodination of the pro-hormone T4 by DIO1 and DIO2 produces nearly 80% of peripheral T3. Since the thyroid hormone regulates the production of DIO1, hypothyroidism is expected to cause a reduction in the DIO1-catalyzed T4 to T3 conversion (121). Deiodinase type 1 enzyme is also found in lymphocytes, affecting its expression by pro-inflammatory cytokines (125).

In normal metabolic conditions, DIO1 creates the majority of circulating T3, and its expression is increased by thyroid hormones, cAMP in thyrocytes and retinoic acid in thyroid and liver cancer cells. Several pathophysiological disorders, including starvation, bacterial sepsis, serious disease, and major surgical interventions, or trauma result in a significant reduction in hepatic T3 production and hepatic DIO1 activity. This condition is called euthyroid sick syndrome (ESS), low-T3 syndrome or non-thyroidal illness (NTI) (126).

The sensitivity of DIO1 to the antithyroid medication propylthiouracil (PTU) is particularly notable. This medication produces an inactive compound and inhibits DIO1 by interacting covalently with the active site of enzyme (124).

Deiodinase type 1 is inhibited and down-regulated in response to physiological and emotional stress, depression, leptin resistance, insulin resistance, obesity, diabetes, inflammation from an autoimmune disorder or systemic illness, and exposure to toxins and plastics (127).

1.4.2 The Effect of Deiodinase Type 1 Gene Polymorphisms on the Therapeutic Response of Levothyroxine.

The human DIO1 gene is present on the short arm of human chromosome 1p32–33 and comprises 4 exons (121,128).

The DIO genetic variants have gotten the major attention to date in patients with thyroid diseases such as hypothyroidism and thyroid cancer. There haven't been any studies looking into the significance of DIO enzyme genetic polymorphisms in patients with non-thyroidal diseases (129). Previous studies have shown that the levels and ratios of thyroid hormones may be impacted by genetic variability in the genes that code for the deiodinase enzyme (130).

Single nucleotide polymorphisms (SNPs) in the deiodinase genes may influence thyroid hormone levels by interfering with the phenotypic expression of these enzymes (131). Many SNPs in the DIO1 gene have been reported to be associated with variations in the amounts of thyroid hormone in the blood. The raised serum T3 concentrations seen in hyperthyroid individuals are due to pathologically enhanced thyroidal DIO1 activity. In the nonthyroidal disease syndrome, however, reduced DIO1 activity is probably related to reduce serum T3 concentration (121).

The rs11206244 (C785T) SNP and rs12095080 (A1814G) SNP are two polymorphisms that have been identified as prospective candidates for physiological and pathological conditions in people. The rs11206244 (C785T) SNP and rs12095080 (A1814G) SNP location is in the 3- untranslated region (UTR) of the mRNA as illustrated in Figure 1-6 (118,132).

The rs11206244 (C785T) SNP polymorphism was linked to elevated fT4 and rT3 serum level, lower fT3 or T3 serum concentrations, and reduced T3 to rT3 ratios or fT3 to fT4 ratios, which indicate that less T4 is being converted to T3. due to decreased DIO1 protein or activity. The synthesis of serum T3 in skeletal muscle by DIO2 may mask a decline in T3 manufacturing in young people but in older people, DIO1 may have a major role in serum T3 production because skeletal muscle size and strength reduce with age in adults, leading to a reduction in DIO2-expressing in skeletal muscle (118).

Figure1-6: Location of the rs11206244 (C785T) SNP and rs12095080 (A1814G) SNP in the 3- untranslated region (3-UTR) of the mRNA (132)**.**

Another DIO1 polymorphism, rs12095080 (A1814G) SNP, has been linked to a markedly greater T3/rT3 ratio, indicating that the G allele may lead to increased DIO1 activity (133,134).

1.5 Aims of the Study

1. To estimate the alleles frequencies of the SNPs (rs11206244 (C785T) and rs12095080 (A1814G)) in the DIO1 gene in the Iraqi hypothyroid female patients.
2. To investigate the possible impact of DIO1 genetic polymorphisms on the therapeutic response to levothyroxine in the Iraqi female patients with hypothyroidism

CHAPTER TWO

Patients, Materials and Methods

2. Patients, Materials and Methods

2.1 Materials

2.1.1 Instruments, Equipment, and their Suppliers

All instruments used in this study are listed in the Table 2-1 accompanied by the manufacturing company.

Table 2-1: Instruments and the manufacturing companies

Types of equipment	Company	Country
Centrifuge	SIGMA	Germany
Cobas e 411	Roche	Germany
Digital camera	Canon	England
Distillator	GFL	Germany
Electrophoresis apparatus	Techinme	England
Hood	LabTech	Korea
Micropipettes	SLAMMED	Japan
PCR machine (Thermocycler)	Verity	USA
Sensitive balance	AND	Taiwan
UV- Trans illuminator	Syngene	England
Vortex mixer	HumanTwist	Germany
Water bath	LabTech	Korea

2.1.2 Chemicals, Kits, and their Suppliers

The chemicals and kits used in this study are mentioned in Table 2-2, along with their manufacturers and countries.

Table 2-2: Chemicals, kits and their Manufacturing Companies

	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	Mindray	China
Hormonal Kits	Free Thyroxine kit	Snibe Diagnostic	China
	Free Triiodothyronine kit	Snibe Diagnostic	China
	Insulin kit	Mindray	China
	TSH kit	Snibe Diagnostic	China
	Total Thyroxine kit	Snibe Diagnostic	China
	Total Triiodothyronine kit	Snibe Diagnostic	China
	Fasting Serum Glucose kit	Mindray	China
Kits for Genetic Study	DNA Extraction Kit from blood Favor Prep	Favogen	Taiwan

	PCR Green Master mix Kit	Promega	USA
	DNA ladder Marker (100 bp)	Bioneer	Korea
	Primer for detection of: 1.rs11206244 (C785T)	Alpha	Canada
	2.rs12095080 (A1814G).	Scientific researcher	Iraq

2.2 Study Patients

Two hundred twenty female patients were enrolled in this study, diagnosed with primary hypothyroidism according to the diagnostic criteria for hypothyroidism by a specialist physician according to the American thyroid association guideline 2022.

All patients have been administered levothyroxine for at least 4 months or more, aged 40 years old or older. All the patients were lived in kerbala province and all of them were unrelated. This study continued from November 2021 to March 2022, when they were visiting a private clinic for medical treatment and advice for their specific cases. All the patients had hypothyroidism symptoms and were non-responders to levothyroxine.

2.2.1 Patients Criteria

2.2.1.A Inclusion Criteria

1. Female patients with primary hypothyroidism.
2. Patients aged 40 years old or older.
3. Patients were taking levothyroxine for at least 4 months

2.2.1.B Exclusion Criteria

1. Patients previously undergoes thyroidectomy.
2. Patients whose age is less than 40 years.

3. Patients received any medications that interact with levothyroxine, such as proton pump inhibitors, cholestyramine, cimetidine, sucralfate, or antacids(111).
4. Patient with hypertension and diabetes mellitus.
5. Male patients.
6. Patients who were taking levothyroxine for less than 4 months.
7. Pregnant patients.

2.2.2 Ethical and Scientific Approval

- The scientific and ethical committee at the college of pharmacy – Kerbala University discussed and approved the proposal of the research.
- All participants were enrolled in the study after signing a written consent form that included a detailed explanation of the study's purpose and a request to complete a specially designed questionnaire.

2.2.3 Study Design

This is a cross-sectional study aims to investigate the impact of DIO1 gene polymorphisms on the therapeutic response to levothyroxine. It was done on two hundred twenty female patients with primary hypothyroidism who were receiving levothyroxine for more than 4 months. Blood samples have been taken from overnight fasting patients for the biochemical, and genetic analysis. Blood pressure were assessed to examine if there is an association between the DIO1 on the personal clinical assessment regarding blood pressure.

Precautions have been taken in clinical settings to prevent infection of COVID-19.

2.3 Methods

2.3.1 Samples Collection

Blood samples were collected from overnight-fasting patients by vein puncture. Blood samples (5 ml) were taken from patients and divided into two parts:

- The first part (2ml) was preserved in an ethylene diamine tetra acetic acid (EDTA) tube for the DNA extraction.
- The other part (3ml) was put in a gel tube for serum isolation that was later used for hormonal and biochemical assays.

2.3.2 Biochemical and Hormonal Assay Methods

2.3.2.1 Estimation of Thyroid Function

2.3.2.1.1 Estimation of Serum Thyroid Stimulating Hormone

In this study, TSH was measured quantitatively in vitro using an immunoassay. The electrochemiluminescence immunoassay "ECLIA" is designed for use with the Cobas e immunoassay analyzer and consists of a sandwich complex formed by two different monoclonal antibodies directed particularly against thyroid-stimulating hormone. The microparticles are magnetically attracted to the electrode's surface. When a voltage is applied to the electrode, chemiluminescent emission occurs, which is detected by a photomultiplier (135).

2.3.2.1.2 Estimation of Serum Total Thyroxine

A competitive chemiluminescence immunoassay is used to determine serum total T4. First, the sample incubated at 37°C, thyroxine in the blood competes with T4 antigen immobilized on magnetic microbeads for a limited number of binding sites, resulting in immunological complex formation.

Finally, a chemiluminescent reaction is started, and the light signal is detected using a photomultiplier that is inversely proportional to the amount of T4 in the sample (136).

2.3.2.1.3 Estimation of Serum Free Thyroxine

As previously stated in total T4 determination, the test for serum-free T4 determination is a competitive chemiluminescence immunoassay (136).

2.3.2.1.4 Estimation of Serum Total Triiodothyronine

As with total T4, the method for serum total T3 measurement is a competitive chemiluminescence immunoassay (136).

2.3.2.1.5 Estimation of Serum Free Triiodothyronine

The assay of serum-free T3 determination is a competitive chemiluminescence immunoassay as explained previously in total T4 (136).

2.3.2.1.6 Estimation of Serum Diiodothyronine

After adding 50 µl of the diluted standard to the standard well and 50 µl of sample to the sample well, 50 µl of biotinylated antigen was added to each well, the plate was sealed, and incubated at 37°C for 60 minutes. After that, the sealer was removed and the plate was washed five times with 300 µl wash buffer, inverting the plate each time.

The avidin-HRP (50 µl) was added to both the standard and sample wells, the plate was sealed and incubated for 60 minutes at 37°C. After removing the sealer and washing again, filled each well with 50 µl of substrate solution A and 50 µl of substrate solution B, and incubated for 10 minutes at 37°C. After that, 50 µl stop solution was added to each well and a microplate reader set to 450 nm and used to determine the optical density (137).

2.3.2.1.7 Estimation of Serum Reverse Triiodothyronine

The assay is the same in diiodothyronine(137)

2.3.2.2 Determination of Glycemic Indices

2.3.2.2.1 Estimation of Fasting Serum Glucose Level

The glucose levels were estimated using the enzymatic reference method with hexokinase. Hexokinase catalyzes the ATP-catalyzed phosphorylation of glucose to

glucose-6-phosphate. Glucose-6-phosphate dehydrogenase converts glucose-6phosphate to gluconate-6-phosphate in the presence of NADP. No other carbohydrate is oxidized.

Photometry determines the rate of NADPH generation during the reaction, which is proportional to the glucose concentration and is measured photometrically by UV spectrophotometer (138).

2.3.2.2.2 Estimation of Fasting Serum Insulin Level

An enzyme-linked immunosorbent assay (ELISA) kit was used to determine the fasting serum insulin level. The ELISA Kit is a solid-phase, sandwich-based enzyme-linked immunosorbent test.

The principle is a two-step incubation method; an aliquot of the patient sample is first incubated in the coat well with enzyme conjugate (which is a monoclonal anti-insulin antibody) that is biotin conjugated. The streptavidin-peroxidase enzyme complex binds to the biotin anti-insulin antibody during the second incubation stage. The amount of the bound peroxidase complex in a sample is related to the amount of insulin present. With suitable insulin standards functioning, the intensity of color created is proportional to the concentration of insulin in the patient sample. At 450 nm, the absorbance was determined spectrophotometrically. The unit IU/ml was used to represent the results (139).

2.3.2.2.3 Estimation of Insulin Resistance

Insulin resistance was measured using the homeostasis model assessment (HOMA IR), which is calculated from fasting serum insulin (FSI) and fasting blood sugar (FBS) using the formula below (140).

HOMA-IR = [FSI (µU/ml) * FBS (mg/dl)]/40

2.3.2.3 Measurement of Body Mass Index

The Body Mass Index (BMI) is a measurement of a person's weight and height. The BMI is determined by divided the body weight on the square of the body height, and it is expressed in kilograms per square meter (kg/m2), with mass in kilograms and height in meters

Weight / (Height) 2 = BMI

The Body Mass Index of 18.5-24.9 is considered normal weight, 25-30 is considered overweight, and greater than 30 is considered obesity(141).

2.3.3 Genetic Analysis

2.3.3.1 Extraction of Genomic DNA from Whole Blood Sample

Favor Prep Genomic DNA Mini kit from Favogen was used to purify total DNA from blood and other biological samples efficiently and simply, yielding pure DNA appropriate for storage and immediate application (142).

Step 1-Sample Preparation

1. A volume 200µl blood transferred to a 1.5ml microcentrifuge tube.
2. Proteinase K enzyme (30µl) was added to the sample and mixed briefly. Then, at 60°C, incubated for 15 minutes.

Step 2 –Cell Lysis

3. FABG buffer (200µl) was added to the sample and mixed by the vortex.
4. To lyse the sample, it was placed in a 70°C water bath for 15 minutes. Then, inverted the sample every 3 minutes during incubation.
5. In a 70°C water bath, the required elution buffer (for Step 5 DNA Elution) was preheated.

Step 3 – Binding

6. The sample was vortexed for 10 seconds after adding 200µl ethanol (96-100%).
7. Into a 2ml collection tube, a FABG column was placed. The sample mixture was carefully transferred to the FABG column (including any precipitate), centrifuged at maximum speed (14,000 rpm or 10,000 x g) for 5 minutes and then the 2ml collecting tube was discarded. The FABG column was placed in a new 2ml collection tube.

Step 4 – Washing

8. The FABG column was washed with 400µl buffer and the flow-through was removed. Then centrifuged for 1 minute at full speed (14,000 rpm or 10,000 x g).

9. The FABG Column was returned to the 2ml collection tube. Wash buffer (600µl) was added to the FABG column and centrifuged at high speed (14,000 rpm or 10,000 x g) for 1 minute. Then the filtrate was discarded
10. The FABG column was placed in the 2ml collection tube once again. To dry the column, it must be centrifuged additional 3 minutes at full speed (14,000 rpm or 10,000 x g).

Step 6 – Elution

11. The dry FABG column was placed in a new 1.5ml microcentrifuge tube.
12. The preheated elution buffer or TE (100µl) was added to the membrane center of the FABG column
13. In an incubator, the FAGB column was incubated for 10 minutes at 37°C.
14. To elute the DNA, it must be centrifuged for 1 minute at full speed (14,000 rpm or 10,000 x g).

The extracted DNA was kept frozen at -20°C (142).

2.3.3.2 Conventional Polymerase Chain Reaction

2.3.3.2.A Primer Preparation

The deiodinase type 1 gene SNPs rs11206244 (C785T) and rs12095080 (A1814G) were amplified using Polymerase Chain Reaction (PCR) with particular primers. These primers were designed by primer–BLAST software, the primers that used to detect rs11206244 (C785T) SNP were obtained as lyophilized products from Alpha DNA, Canada, while the primers that used to detect rs12095080 (A1814G) SNP were obtained from Scientific Researcher Company, Iraq as lyophilized products.

Primers preparation done as following:

1. After dissolving each primer with appropriate amounts of nuclease-free water to generate a stock solution with a concentration of 100 pmol/µL,
2. A diluted working solution (10 pmol/µL) was made by diluting 10 µL of stock solution with 90 µL of nuclease-free water.
3. Until used, this work solution was maintained at -20°C.

Allele-Specific Polymerase Chain reaction is used to detect rs12095080 (A1814G) SNP and its primers is shown in Table 2-3.

Table 2-3: Primers Sequences to Detect the Deiodinase type 1 rs12095080 (A1814G) SNP.

Primer	sequences	Product size (bp)
Allele A	GTTATAAGATGCAGTAAACTAA	169
Allele G	GTTATAAGATGCAGTAAACTAG	169
Reverse primer	TTCTTCCCCCAAAATGAGG	-

Amplification Refractory Mutation System - Polymerase Chain Reaction (ARMS-PCR) was used for rs11206244 (C785T) SNP detection and its primers is shown in Table 2-4.

Table 2-4: Primers Sequences to Detect the Deiodinase Type1 rs11206244 (C785T) SNP.

Primer	sequences	Product size (bp)
Inner Forward	TCTGGACAGATACCTCAATTCTAGGTTAC	191
Inner Reverse	TTGAGAAGCCCTCCCGTGGA	136
Outer Forward	TGATTCGTTTCTCTTGCAGGGTAA	278
Outer Reverse	ACAATTTGTCTTGATTGGGTGCTG	278

2.3.3.2.B Optimization of Polymerase Chain Reaction Conditions

The Polymerase Chain Reaction was optimized after several trials to find the ideal annealing temperature, several amplification cycles for the PCR reaction, and the best concentration of both DNA and primer. Tables 2-5 and 2-6 show the

components of PCR reactions for all amplified fragments and optimized PCR programs, respectively.

2.3.3.2.C Running the Polymerase Chain Reaction

The PCR reaction was performed by combining PCR components with DNA solution and employing the optimized PCR programs as shown in Table 2-7 for the DIO1 gene rs12095080 (A1814G) and Table 2-8 for the DIO1 gene rs11206244 (C785T).

Table 2-5: Polymerase Chain Reaction Mixture for genotyping of Deiodinase Type 1 gene rs12095080 (A1814G) SNP.

Component	Volume (µl)	Total volume (µl)
Allele A or Allele G primer	1	26
Reverse primer	1	
DNA template	4	
Deionized water	7.5	
Master mix	12.5	

Table 2-6: Polymerase Chain Reaction Mixture for genotyping of Deiodinase Type 1 gene rs11206244 (C785T) SNP.

Component	Volume (µl)	Total volume (µl)
Outer Forward	2	35
Outer reverse	2	
Inner Forward	3	
Inner Reverse	3	
DNA template	5	
Deionized water	5	
Master mix	15	

Table 2-7: Polymerase Chain Reaction Program for genotyping of Deiodinase Type 1 gene rs12095080 (A1814G) SNP.

Steps	Temperature/c	Time/sec	Cycle
Initial Denaturation	95	300 (5 min.)	1
Denaturation	95	35	30
Annealing	54.5	30	
Extension	72	40	
Final extension	72	300 (5 min.)	1

Table 2-8: Polymerase chain reaction program for genotyping of deiodinase type 1 gene rs11206244 (C785T) SNP.

Steps	Temperature/c	Time/sec	Cycle
Initial Denaturation	95	300 (5 min.)	1
Denaturation	95	30	40
Annealing	60	35	
Extension	72	55	
Final extension	72	300 (5 min.)	1

2.3.4 Agarose Gel Electrophoresis

- To make an agarose gel, 1.5g of agarose powder was dissolved in 100ml of 1x TBE buffer (PH 8).
- On a hot plate, the solution was heated until it boils.
- After waiting for the solution to cool, 2 µl of ethidium bromide was added to it.
- The comb was fixed to the tray's end to make wells for PCR product loading.
- The agarose was carefully poured into the tray and allowed to solidify for 30 minutes at room temperature.
- The comb was removed out of the tray carefully.
- The tray was fixed in an electrophoresis chamber and filled with a TBE buffer.
- The ladder was put in one well as a marker
- PCR products were directly put into the wells.
- The voltage on the electrophoresis apparatus was set to ensure a 5v/1cm electrical field for the distance between the cathode and anode.
- An ultraviolet trans-illuminator set to 320-336 nm was employed for band detection at the end of the 90-minute run.
- A digital camera was used to photograph the gel (143).

2.4 Statistical Analysis

Participants' data were transferred into a computerized database, checked for mistakes and inconsistencies, and then maintained, processed, and analyzed by using the statistical package for social sciences (SPSS) version 26, IBM, US.

Scale variables are expressed as mean and standard deviation (SD), while nominal (categorical) variables are expressed as frequency (number of participants) and proportion (percentage). Because scale variables like age and BMI have a statistically normal distribution, a parametric test was used.

One-way analysis of variances (one-way ANOVA) was used to compare more than two means. The Chi-square was used to assess the relationship between categorical variables, but when the group size was less than 5, Fisher's exact test was used.

Chi square from goodness of fit test was used to investigate the genotype distributions of the alleles and genotypes of the SNPs according to Hardy-Weinberg equilibrium. The level of significance was established at ($P \leq 0.05$) to be regarded as a significant difference or correlation. Finally, the data and conclusions were presented in tables and/or figures, each with a brief explanation.

CHAPTER THREE

Results

3. Results

3.1 Demographic Characteristics of Hypothyroid patients

The demographic features of the 220 Iraqi females with primary hypothyroidism who participated in the current study are demonstrated in Table 3-1. The age ranged between (40-74) years old for the participants of this study with duration of treatment (0.3-17) years.

Table 3-1: The Demographic Characteristics of the Hypothyroid patients

Demographic characteristics	**Means N= 220**	**Minimum**	**Maximum**
Age (years)	50.7±10.49	40	74
BMI (kg\m²)	31.04±5.69	17.78	48.01
Duration of treatment (years)	4.27±3.71	0.3	17

Data represented by mean ± SD, N: Number of hypothyroid patients, BMI: Body mass index.

3.2 Assessment of Thyroid Laboratory Parameters in the Hypothyroid Patients

The results as shown in Table 3-2 demonstrated the measurement of thyroid laboratory parameters (TSH, total T3 (tT3), free T3 (fT3), total T4 (tT4), free T4 (fT4), reverse T3 (rT3) and T2) in hypothyroid patients.

Table 3-2: Thyroid Laboratory Parameters in Hypothyroid Patients

Parameters	Mean N= 220	Minimum	Maximum
TSH (µIU/mL)	3.05±2.48	0.10	9.80
Total T3 (nmol/L)	1.55±0.41	0.70	3.61
Free T3 (pmol/L)	6.51±1.26	3.20	9.50
Total T4 (nmol/L)	102.88±28.04	54.50	210.50
Free T4 (pmol/L)	15.53±3.44	9.20	30.00
rT3 (pmol/L)	921.31±350.86	261.45	1790.10
T2 (pmol/L)	2039.96±874.06	157.67	4184.86

Data Presented by Mean ± SD, N: Number of hypothyroid patients (220), TSH: Thyroid stimulating hormone, T4: Thyroxine, T3: Triiodothyronine, RT3: Reverse T3, T2: Diiodothyronine.

3.3 Assessment of Blood Pressure in the Hypothyroid Patients

The results in Table 3-3 demonstrated the measurement of blood pressure parameters (systolic pressure, diastolic pressure and mean arterial pressure) in hypothyroid patients.

Table 3-3: Blood Pressure Parameters in Hypothyroid Patients

Parameters	Mean N= 220	Minimum	Maximum
SBP (mmHg)	127.34±14.17	100	180
DBP (mmHg)	82.5±6.89	60	110

Mean arterial pressure(mmHg)	97.45±8.77	73.33	130

Data Presented by Mean ± SD, N: Number of hypothyroid patients (220), SBP: Systolic blood pressure, DBP: Diastolic blood pressure.

3.4 Assessment of Glycemic Parameters in the Hypothyroid patients

The results in Table 3-4 demonstrated the measurement of glycemic parameters (fasting serum glucose, fasting serum insulin, and homeostasis model assessment) in hypothyroid patients.

Table 3-4: Glycemic Parameters in Hypothyroid Patients

Parameters	**Mean N= 220**	**Minimum**	**Maximum**
FSI (mIU/mL)	11.98±5.65	2.83	28.90
FBS (mg/dL)	111.47±38.03	70.00	232.00
HOMA-IR	3.28±1.90	0.61	9.60

Data Presented by Mean ± SD, N: Number of hypothyroid patients (220), FSI: Fasting serum insulin, FBS: Fasting blood sugar, HOMA-IR: Homeostasis model assessment.

3.5 Genetic Analysis

3.5.1 The Frequency and Distribution of the rs12095080 (A1814G) SNP in the Hypothyroidism Patients

For polymorphism detection, allele-specific PCR was employed to assay genotypes of rs12095080 (A1814G) SNP. The amplification product with a size of 169 bp was produced. Three genotypes for rs12095080 (A1814G) SNP, as it is demonstrated in Figure 3-1, were determined after analyzing the amplification results:

- Two 169 bp DNA fragments were appeared in case of the heterozygous mutant type (AG).
- One 169 bp DNA fragment for the wild type (AA) and for the homozygous mutant type (GG).

The distribution of genotype groups of rs12095080 (A1814G) SNP in hypothyroid patients is demonstrated in Table 3-5.

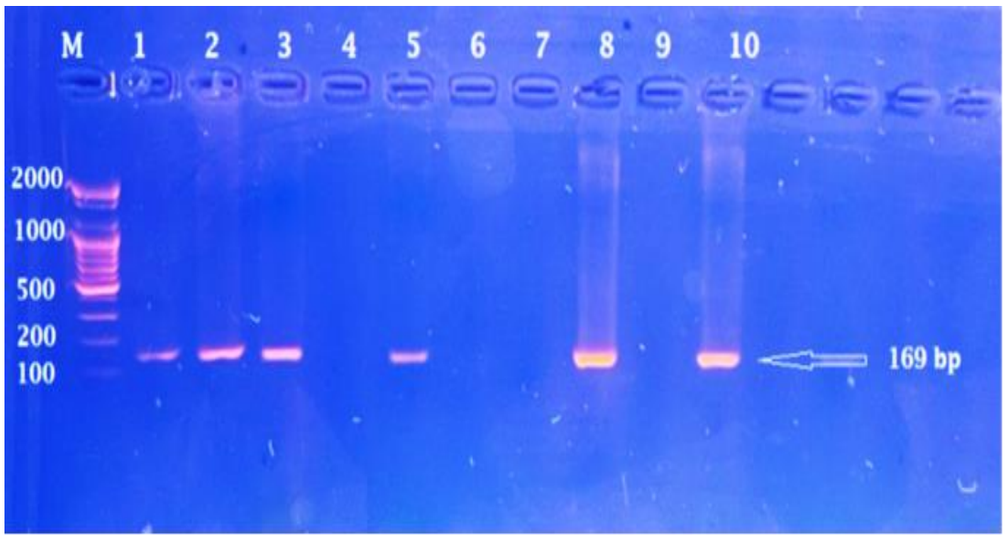


Figure 3-1: The agarose gel electrophoresis of allele-specific polymerase chain reaction to detect rs12095080 (A1814G) SNP. M: 100 bp ladder, 1 and 2 represent the heterozygous mutant type (AG) for one sample, lanes 3, 4, 5, and 6 represent the wild type (AA) for two samples, lanes 7, 8, 9, and 10 represent the homozygous mutant type (GG) for two samples.

The result of the comparison between observed and anticipated values for rs12095080 (A1814G) SNP in the study subjects was shown in Figure 3-2, and Table 3-5. The distribution and percentage of individuals having rs12095080 (A1814G) SNP were statistically not significant (P = 0.547). Since, the number of individuals with the rs12095080 (A1814G) SNP not differs from what would be predicted under Hardy–Weinberg equilibrium (number of observed versus expected)

Our sample of hypothyroid female patients from Iraq showed a high prevalence of the wild type allele (A) for the rs12095080 (A1814G) alleles distribution.

Table 3-5: The Genotypes and Alleles Distribution of the rs12095080 (A1814G) SNP in the Hypothyroidism patients

Genotype (N=220)	Frequency (%)	Allele	Frequency	Chi-square	P-value
AA (Wild type)	156(70.9)	A	0.848	1.208	0.547
AG (Heterozygous mutant type)	61(27.7)	G	0.152		
GG (Homozygous mutant type)	3(1.4)				

Data Presented by numbers and percentage, N: Number of hypothyroid patients (220).

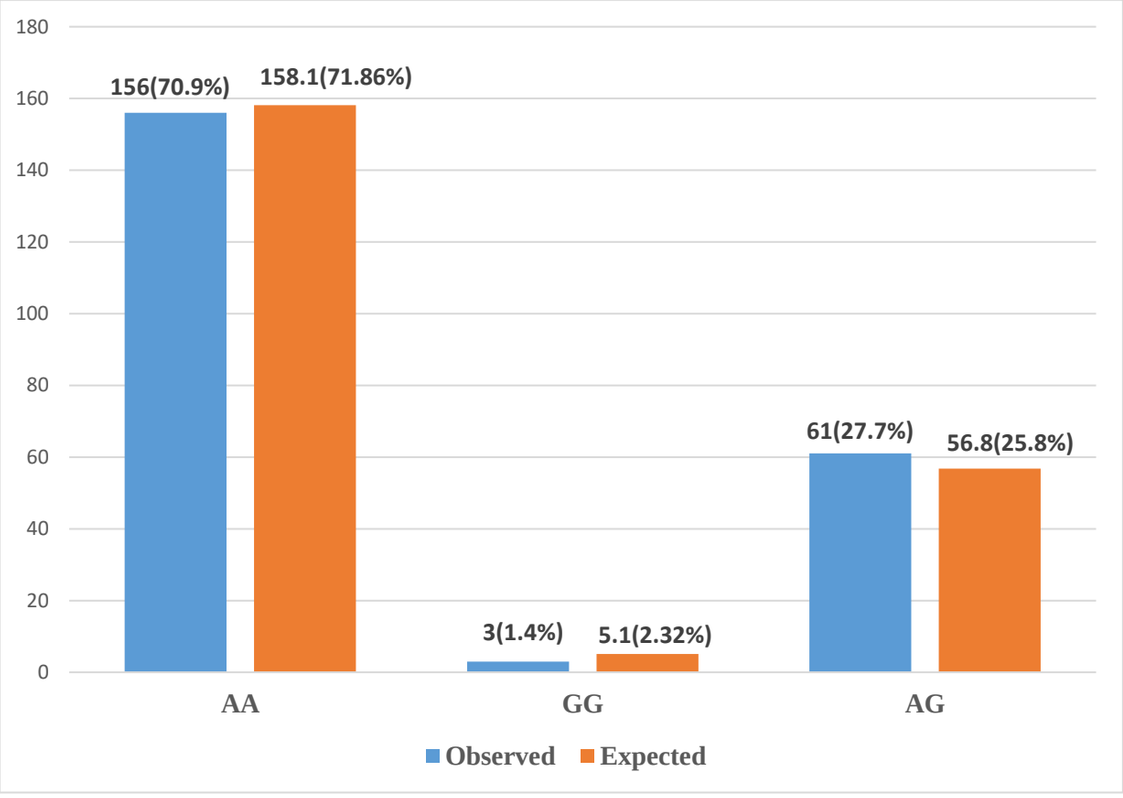


Figure 3-2: Genotype frequencies (the observed and the expected) of rs12095080 (A1814G) SNP among the study sample.

3.5.2 The Frequency and Distribution of the rs11206244 (C785T) SNP in the Hypothyroidism Patients

On the other hand, Tetra ARMS-PCR was used for genotyping of the rs11206244 (C785T) SNP (Figure 3-3). The PCR products were:

- Two DNA fragments for the wild type (CC) each measuring 278 bp and 191 bp.
- Three DNA fragments were obtained for the heterozygous mutant type (CT), each measuring 278 bp, 191 bp, and 136 bp.
- Two DNA fragments for the homozygous mutant type (TT), each measuring 278 bp and 136 bp.

The genotypes and alleles of the rs11206244 (C785T) SNP distribution in hypothyroid patients are listed in Table 3-6.

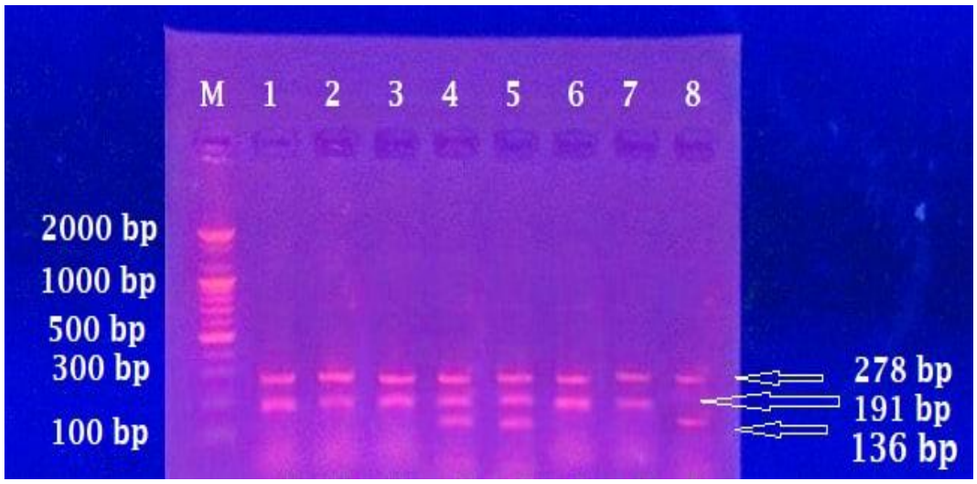


Figure 3-3: The agarose gel electrophoresis of tetra ARMS-PCR to identify rs11206244 (C785T) SNP. M: 100 bp ladder, lanes 1, 2, 3, 6, and 7 represent the wild type (CC), lanes 4 and 5 represent the heterozygous mutant type (CT) and lane 8 represents the homozygous mutant type (TT).

Figure 3-4 and Table 3-6 show the results of the comparison between the observed and the expected values for the genotypes of rs11206244 (C785T) SNP in the tested population. The number of individuals with the rs11206244 (C785T) SNP differs from what would be predicted under Hardy–Weinberg equilibrium (number of observed versus expected, P = 0.001). The distribution of the rs11206244 (C785T) alleles indicated the prevalence of the C allele in our sample of hypothyroid patients from Iraq.

Table 3-6: The Alleles and Genotypes Distribution of rs11206244 (C785T) SNP in the Hypothyroidism Patients

Genotype (N=220)	Frequency (%)	Allele	Frequency	Chi-square	P-value
CC (Wild type)	106 (48.2)	C	0.652	13.58	0.001
CT (heterozygous mutant type)	75 (34.1)	T	0.348		
TT (Homozygous mutant type)	39 (17.7)				

Data Presented by numbers and percentage, N: Number of hypothyroid patients.

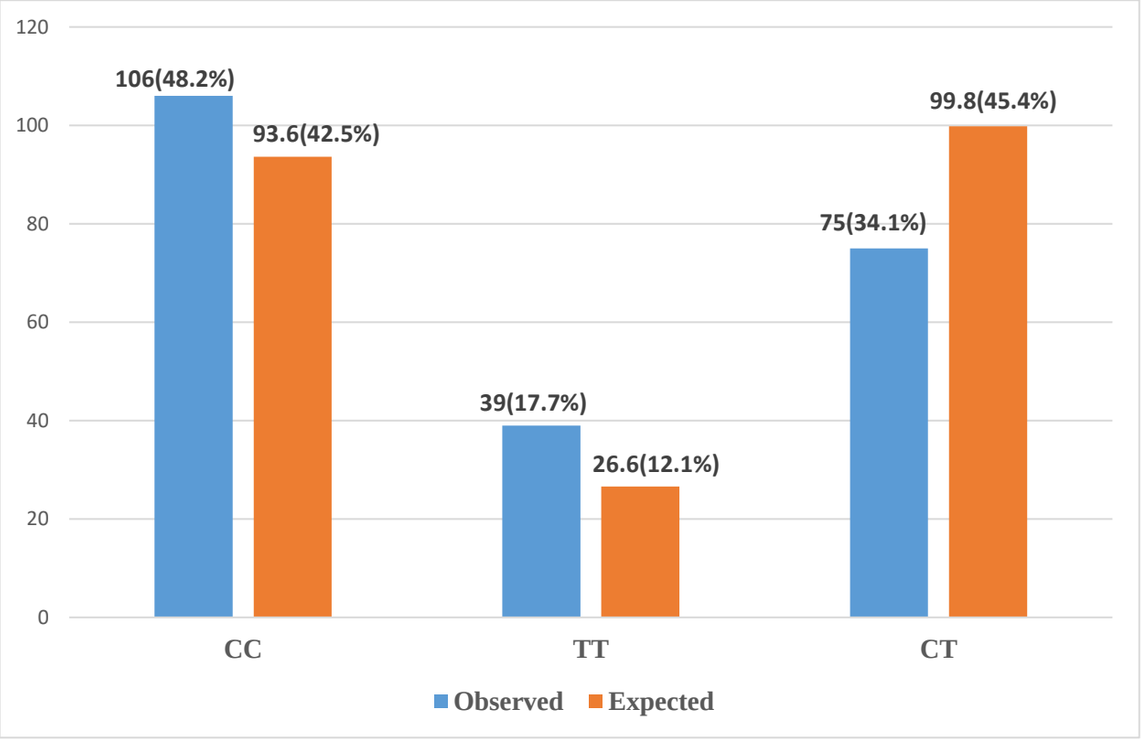


Figure 3-4: Genotype frequencies (the observed and the expected) of rs11206244 (C785T) SNP among the study sample.

3.5.3 The Frequency and Distribution of Both Deiodinase type 1 Enzyme SNPs (rs12095080 (A1814G) SNP and rs11206244 (C785T) SNP) in the Hypothyroidism Patients

The distribution of both DIO1 SNPs (rs12095080 (A1814G) SNP and rs11206244 (C785T) SNP) among the hypothyroidism patients shown no statistically significant difference ($p > 0.05$) as illustrated in Table 3-7.

Table 3-7: The distribution of both rs12095080 (A1814G) SNP and rs11206244 (C785T) SNP among hypothyroidism patients

		rs11206244 (C785T) SNP, (N=220)			Statistical test	P-value
		CC (N=106)	CT (N=75)	TT (N=39)		
(A1814G) SNP (N=220)	AA (N=156)	75 (48.1%)	47 (30.1%)	34 (21.8%)	Fisher's exact= 7.855	0.06
	AG (N=61)	29 (47.5%)	27 (44.3%)	5 (8.2%)		
	GG (N=3)	2 (66.7%)	1 (33.3%)	0 (0%)		

Data is represented by numbers and percentages, N: Number of hypothyroid patients, AA: Wild type, AG: Heterozygous mutant type, GG: Homozygous mutant type, CC: wild type, CT: heterozygous mutant type, TT: homozygous mutant type.

3.6 Association Between Deiodinase Type 1 Polymorphisms and the Demographic Characteristics in Iraqi Female Hypothyroid Patients

3.6.1 Association Between rs12095080 (A1814G) SNP and the Demographic Characteristics in Iraqi Female Hypothyroid Patients

The difference between demographic characteristics (the age, BMI, and duration of treatment) among three groups of patients that were divided according to their genotypes of rs12095080 (A1814G) SNP as shown in Table 3-8 .

The demographic features of the various groups have no statistically significant differences from one another.

Table 3-8: The Association Between rs12095080 (A1814G) SNP and the Demographic Characteristics

Demographic parameters	AA (N=156)	AG (N=61)	GG (N=3)	P-value
Age (years)	50.56±9.35	51.19±13.16	45.33±5.51	0.626
BMI (kg/m^2)	30.49±5.78	32.22±5.32	35.49±3.5	0.051
Duration of Treatment (years)	4.15±3.47	4.71±4.29	1.43±0.98	0.25

The results are represented as mean ± standard deviation, p<0.05 considered significantly different, BMI: Body mass index, N: Number of the hypothyroid patients, AA: Wild type, AG: Heterozygous mutant type, GG: Homozygous mutant type.

3.6.2 Association Between rs11206244 (C785T) SNP and the Demographic Characteristics in Iraqi Female Hypothyroid Patients

Regarding rs11206244 (C785T) SNP, data represented in Table 3-9 demonstrate that the demographic characteristics and the three groups of patients, which divided according to their genotypes, did not produce a significant variation (P > 0.05).

Table 3-9: The Association Between rs11206244 (C785T) SNP and the Demographic Characteristics

Demographic parameters	CC (N=106)	CT (N=75)	TT (N=39)	P-value
Age (years)	51.48±10.28	48.99±9.51	51.64±12.58	0.238
BMI (kg/m^2)	31.92±5.94	30.39±5.35	29.86±5.42	0.075
Duration of Treatment (years)	4.12±3.75	4.69±3.96	3.89±3.09	0.474

The results are represented as mean ±SD, with p<0.05 indicating a significant difference, BMI: Body mass index, N: Number of hypothyroid patients, CC is the wild type, CT is the heterozygous mutant type and TT is the homozygous mutant type.

3.7 Association Between Deiodinase type 1 Polymorphisms and the Thyroid Laboratory Parameters in Iraqi Female Hypothyroidism Patients

There were no statistically substantial correlations ($p > 0.05$) among the genotype of the rs12095080 (A1814G) SNP and the thyroid laboratory parameters as well as the molar ratios of the thyroid hormones (Table 3-10).

Table 3-10: Thyroid Laboratory Parameters According to rs12095080 (A1814G) SNP.

Parameters	AA (N=156)	AG (N=61)	GG (N=3)	P-value
TSH (µIU/mL)	2.99±2.45	3.26±2.59	2.06±1.78	0.6
Total T3 (nmol/L)	1.55±0.46	1.53±0.26	1.67±0.16	0.827
Free T3 (pmol/L)	6.38±1.27	6.83±1.21	7.04±0.74	0.051
Total T4 (nmol/L)	103.57±28.99	101.8±25.76	89.03±25.69	0.635
Free T4 (pmol/L)	15.25±3.63	16.19±2.85	16.34±2.28	0.176
rT3 (pmol/L)	912.27±341.28	955.68±379.84	692.44±62.83	0.376
T2 (pmol/L)	2055.26±866.77	2031.25±886.47	1421.45±1123.26	0.461
T3/ rT3 ratio	1.92±0.86	1.86±0.79	2.41±0.04	0.518
T3 / T4 ratio	1.57±0.51	1.59±0.48	1.95±0.45	0.414
rT3 / T4 ratio	0.94±0.44	1.01±0.49	0.81±0.18	0.567
fT3 / fT4 ratio	0.44±0.12	0.43±0.08	0.44±0.1	0.872
T4 dose (µg)	92.08±38.62	99.59±39.13	91.67±14.43	0.435

The results are presented as mean ± standard deviation, $p<0.05$ considered significantly different, N: Number of the hypothyroid patients, AA: Wild type, AG: Heterozygous mutant type, GG: Homozygous mutant type, TSH: Thyroid stimulating hormone, T4: Thyroxine, T3: Triiodothyronine, rT3: Reverse T3, T2: Diiodothyronine.

Regarding to rs11206244 (C785T) SNP; the results demonstrated that the heterozygous (CT) alleles had considerably higher levels of TSH and rT3 than the wild type (CC) with a $P < 0.05$. However, other thyroid laboratory parameters did not significantly differ between the genotype's groups. In addition, the T3/rT3 molar ratio of the heterozygous (CT) is much lower than that of the wild type (CC) and the homozygous mutant type (TT). As well as, there was a substantial variation in the rT3/T4 ratio among the genotype's groups. The patients with the heterozygous mutant type (CT) have a higher rT3/T4 ratio than wild-type (CC) (Table 3-11)

Table 3-11: Thyroid Laboratory Parameters According to rs11206244 (C785T) SNP.

parameters	CC (N=106)	CT (N=75)	TT (N=39)	P-value
TSH (µIU/mL)	2.67±2.26	3.7±2.8	2.86±2.17	0.018 [a]
Total T3 (nmol/L)	1.62±0.46	1.49±0.34	1.47±0.35	0.055
Free T3 (pmol/L)	6.43±1.3	6.75±1.17	6.29±1.28	0.125
Total T4 (nmol/L)	105.72±30.59	100.31±24.98	100.1±26.19	0.351
Free T4 (pmol/L)	15.72±4.14	15.46±2.66	15.13±2.57	0.649
rT3 (pmol/L)	872.17±336.89	1008.64±340.11	886.91±384.2	0.028 [a]
T2 (pmol/L)	2078.09±791.21	2105.22±1006.98	1810.83±795.09	0.193
T3/rT3 ratio	2.07±0.83	1.65±0.65	2.00±1.05	0.003 [b]
T3 /T4 ratio	1.6±0.49	1.57±0.52	1.55±0.47	0.865
rT3 / T4 ratio	0.88±0.4	1.08±0.49	0.95±0.48	0.012 [a]
FT3 / FT4 ratio	0.43±0.12	0.44±0.09	0.43±0.12	0.622
T4 dose (µg)	96.46±38.95	94.2±37.84	87.82±39.27	0.491

The results are presented as mean ± SD, $p<0.05$ considered significantly different, N: Number of hypothyroid patients, CC: wild type, CT: heterozygous mutant type, TT: homozygous mutant type, TSH: Thyroid stimulating hormone, T3: Triiodothyronine, T4: Thyroxine, rT3: Reverse T3, T2: Diiodothyronine. a: CT>CC, b: CT<CC and TT.

3.8 Association Between Deiodinase type 1 Polymorphisms and the Blood Pressure Parameters in the Iraqi Female Hypothyroid Patients

There is no significant difference between all genotypes of rs12095080 (A1814G) SNP according to blood pressure parameters (P > 0.05) as shown in Table 3-12.

Table 3-12: Blood Pressure Parameters According to rs12095080 (A1814G) SNP.

Blood Pressure Parameters	AA (N=156)	AG (N=61)	GG (N=3)	P-value
SBP (mmHg)	127.95±14.66	125.8±13.02	126.67±11.55	0.604
DBP (mmHg)	82.8±6.98	81.72±6.78	83.33±5.77	0.572
Mean arterial pressure (mmHg)	97.85±9.01	97.78±7.7	96.41±8.2	0.555

The result represented as mean ± SD, p<0.05 considered significantly different. N: Number hypothyroid patients, AA: Wild type, AG: Heterozygous mutant type, GG: Homozygous mutant type, SBP: Systolic blood pressure, DBP: Diastolic blood pressure.

Regarding the rs11206244 (C785T) SNP, there was no difference that had been statistically significant (P >0.05) in the blood pressure parameters among the three genotypes groups of patients as demonstrated in Table 3-13.

Table 3-13: Blood Pressure Parameters According to rs11206244 (C785T) SNP.

Blood pressure parameters	CC (N=106)	CT (N=75)	TT (N=39)	P-value
SBP (mmHg)	128.71±15.18	125.42±11.95	127.29±15.16	0.308
DBP (mmHg)	83.07±7.22	81.63±5.37	82.69±8.48	0.383
Mean arterial pressure (mmHg)	98.28±9.24	97.56±10.14	96.23±7.15	0.301

The results represented as mean ± SD, p<0.05 considered significantly different, N: Number hypothyroid patients, CC: wild type, CT: heterozygous mutant type, TT: homozygous mutant type, SBP: Systolic blood pressure, DBP: Diastolic blood pressure.

3.9 Association Between Deiodinase type 1 Polymorphisms and the Glycemic Parameters in the Iraqi Female Hypothyroid patients

No substantial variations were found (P > 0.05) in the glycemic parameters (FSI, FSG, and HOMA-IR) among the groups of the patients that were obtained based on the genotypes of rs12095080 (A1814G) SNP Table 3-14

Table 3-14: The Glycemic Parameters According to rs12095080 (A1814G) SNP.

Glycemic Parameters	AA (N=156)	AG (N=61)	GG (N=3)	P-value
FSI (mIU/mL)	11.69±5.87	12.58±5.14	14.73±2.13	0.413
FBS (mg/dL)	111.35±38.92	112.02±36.78	106.67±18.58	0.97
HOMA-IR	3.21±2	3.43±1.66	3.85±0.57	0.656

The results represented as mean ± SD, p<0.05 considered significantly different, N: Number of hypothyroid patients, AA: Wild type, AG: Heterozygous mutant type, GG: Homozygous mutant type, FSI: fasting serum insulin, FBS: Fasting blood sugar, HOMA-IR: homeostasis model assessment.

The results indicated insignificant statistically variation (P > 0.05) in the glycemic parameters among the different groups of patients that were divided according to rs11206244 (C785T) SNP as illustrated in Table 3-15.

Table 3-15: The Glycemic Parameters According to rs11206244 (C785T) SNP.

glycemic parameters	CC (N=106)	CT (N=75)	TT (N=39)	P-value
FSI (mIU/mL)	12.63±5.86	11.64±5.46	10.9±5.32	0.215
FBS (mg/dL)	112.58±38.93	108.73±33.68	113.75±43.79	0.736
HOMA-IR	3.45±1.97	3.14±1.8	3.09±1.91	0.429

The results represented as mean ± SD, p<0.05 considered significantly different, N: Number of hypothyroid patients, CC: wild type, CT: heterozygous mutant type, TT: homozygous mutant type, FSI: fasting serum insulin FBS: Fasting blood sugar, HOMA-IR: homeostasis model assessment

3.10 The Impact of the Presence of both rs12095080 SNP and rs11206244 SNP on the Study Parameters

The effect of both the two SNPs on the different estimated parameters and characteristics in the hypothyroid patients demonstrated in Table 3-16. The results showed a significant difference in the duration of treatment ($p < 0.05$) in the patients' group who have both rs12095080 (A1814G) and rs11206244 (C785T) SNPs than those with only one SNP.

A statistically significant higher total T3 level ($p< 0.05$) was found among the patients who have no polymorphisms than patients with only one SNP.

The HOMA-IR was found to significantly higher ($p< 0.05$) among the patients who have both the two SNPs compared to the group of patients who have only one SNP.

No statistically significant difference ($p> 0.05$) was found in the other parameters (demographics characteristics, other thyroid hormones, blood pressure and glycemic parameters).

Table 3-16: The Impact of the Presence of both rs12095080 SNP and rs11206244 SNP on the Study Parameters

Parameters	Patient Genotype (N=220)			P value
	No polymorphism (N=75)	Either rs12095080 SNP or rs11206244 SNP (N=112)	Both rs12095080 SNP & rs11206244 SNP (N=33)	
Age (years)	50.94±8.47	50.91±11.27	49.17±12.03	0.676
BMI (kg/m^2)	31.4±6.06	30.61±5.65	31.63±5.01	0.526
Duration of Treatment (years)	4.52±4.08	3.63±2.74	5.89±5.05	0.006 [c]
TSH (µIU/mL)	2.62±2.25	3.19±2.51	3.57±2.76	0.126
Total T3 (nmol/L)	1.66±0.52	1.47±0.34	1.56±0.26	0.007 [a]
Free T3 (pmol/L)	6.28±1.3	6.56±1.25	6.87±1.14	0.067
Total T4 (nmol/L)	108.12±31.78	99.51±25.94	102.41±24.64	0.119
Free T4 (pmol/L)	15.57±4.5	15.27±2.78	16.32±2.51	0.304
rT3 (pmol/L)	882.84±329.43	913.73±354.49	1034.45±372.52	0.111
T2 (pmol/L)	2123.25±771.51	1985.81±914.89	2034.46±960.24	0.576
SBP (mmHg)	129.75±16.04	126.26±12.97	125.52±13.18	0.186
DBP (mmHg)	83.57±7.7	82.03±6.06	81.74±7.54	0.259
Mean atrial pressure	98.96±9.89	96.77±7.82	96.33±8.93	0.181
FSI (mIU/mL)	12.79±6.2	11.11±5.32	13.11±5.11	0.063
FBS (mg/dL)	114.10±39.67	108.83±37.89	114.48±35.09	0.577
HOMA-IR	3.56±2.12	2.97±1.76	3.69±1.70	0.046 [c]

The results represented as mean ± SD, $p<0.05$ considered significantly different, N: Number of hypothyroid patients, TSH: Thyroid stimulating hormone, T4: Thyroxine, T3: Triiodothyronine, RT3: Reverse T3, T2: Diiodothyronine, SBP: Systolic blood pressure, DBP: Diastolic blood pressure, FSI: fasting serum insulin FBS: Fasting blood sugar, HOMA-IR: homeostasis model assessment. c: Both rs12095080 SNP & rs11206244 SNP group > either rs12095080 SNP or rs11206244 SNP group, a: No polymorphism group > either rs12095080 SNP or rs11206244 SNP group.

CHAPTER FOUR

Discussion

4. Discussion

Hypothyroidism is considering as one of the most prevalent endocrine diseases, caused by inability of thyroid hormones to perform their essential functions. These functions include regulating energy metabolism, promoting development, differentiation, and other physiological processes. The normal circulating levels of fT3 and fT4 are reduced greatly in hypothyroidism (144).

The mainstay of hypothyroidism treatment is levothyroxine, which is one of the World Health Organization's essential medications needed for routine medical care. Levothyroxine is used to treat hypothyroidism in an attempt to decrease symptoms and avoid long-term consequences, however, it is one of the drugs that is used most commonly worldwide (20).

It is expected that the modifications in the DIO1 genes will result in abnormalities in the hormone metabolism since DIO1 functions as a scavenger by eliminating iodo group from both the inner and outer rings (145).

The levels and bioactivity of thyroid hormones may be significantly impacted throughout life by polymorphisms in one or more gene related to thyroid hormone metabolism (134).

Clinical evidence in the community indicates that patients with hypothyroidism who were administrating thyroid hormone replacement therapy continue to experience disease-related symptoms. Not all patients receiving treatment have the same standards for quality of life (146). As a result, and as the personal medicine continue to grow in the medical community, it is important to investigate the impact of the DIO1 polymorphisms on the response to levothyroxine.

This study is the first in Iraq and Middle East that investigate the effect of the DIO1 gene polymorphism rs12095080 (A1814G) SNP and rs11206244 (C785T) SNP on the clinical management of levothyroxine-treated hypothyroid female patients.

The current study chose SNPs that accounted for a significant fraction of the common polymorphism among several DIO1 genes.

4.1 The Frequency of Deiodinase type 1 Polymorphisms in the Hypothyroid Female Patients

Genes involved in thyroid hormone metabolism, such as the DIOs enzyme gene, may have genetic variations that alter the protein levels, expression, or activity, as well as the outcomes of various pathways (147). The current study detects two polymorphisms, rs12095080 (A1814G) SNP and rs11206244 (C785T) SNP, within the DIO1 encoding gene which is localized in the mRNA's 3`-UTR of the DIO1 gene (118)

4.1.1 The Frequency and distribution of rs12095080 (A1814G) SNP in the Hypothyroid Female Patients

As demonstrated in the Table 3-5, the frequency of the wild type (AA) of the rs12095080 (A1814G) SNP was 70.9%, which is the most prevalent genotype corresponding to the other genotypes (AG and GG which were represented 27.7% and 1.4% respectively) among the study participants. In a large Danish Twin population, the AA genotype was also the predominant type (78.0%) (148). In addition, another two studies on the Netherlands population found a higher frequency of the AA genotype (79.5% and 81.0%) than other genotypes of the rs12095080 (A1814G) SNP (134,149). This genotype (AA) was also the most common type (69.7%) in a hypothyroid patient in Poland (147).

The high frequency of (A) allele fits with many previous studies (132,134,147–149), besides it obeys Hardy-Weinberg equilibrium (X^2 = 1.208, P = 0.547) as shown in Table 3-5 and Figure 3-2.

4.1.2 The Frequency and distribution of rs11206244 (C785T) SNP in the Hypothyroid Female Patients

The genotype distribution of the rs11206244 (C785T) SNP revealed that the wild type (CC) was more common and accounted for 48.2% of the study subjects while the heterozygous mutant type (CT) was followed with a frequency of 34.1% and the homozygous mutant type (TT) frequency was 17.7% (Table 3-6). This distribution is compatible with the results of many previous studies, one study was achieved on Danish twins where the distribution of CC genotype was (44.6%) and the CT and TT frequencies were 43.3% and 12.1%, respectively (148).

Another study was performed on hypothyroid Turkish patients where the wild type CC is predominant (52.2 %) followed by the heterozygous mutant type CT (38.9 %) and homozygous mutant type TT (8.9%) (144). Another study done on the Poland population illustrated the same pattern of genotype distribution that the frequency of CC, CT and TT was 50.0%, 43.3% and 6.7%, respectively (147). Similar results were also found in another research that was performed on white European heritage in England (130).

On the other hand, De Jong *et al* and Peeters *et al* found that the distribution of CT (46.2%, 48.7%) was more than other genotypes CC (41.1%, 41.7) and TT (12.6%, 15%) respectively, in the Netherland population (134,149).

The alleles frequencies of rs11206244 (C785T) SNP were out of Hardy-Weinberg equilibrium (X^2 = 13.58, P = 0.001, Table 3-6 and Figure 3-4) and this could be related to the small size of the study participants rather than a population bias.

4.2 The Effect of Deiodinase type 1 Polymorphisms on the Demographic Characteristics in Hypothyroid Female Patients

Despite receiving levothyroxine medication, many hypothyroid patients may still experience a variety of symptoms that have varying effects on their quality of life (150).

Three groups were obtained when the patients were divided according to their genotypes regarding each one of both the two SNPs. There were no appreciable differences in the demographic features among the groups of patients (Table 3-8 and Table 3-9). This result is consistent with Peeters *et al* and Verloop *et al* study's; they demonstrated that BMI in the Dutch population was unaffected by the rs12095080 (A1814G) SNP and rs11206244 (C785T) SNP (118,132). Arice *et al* also reported that there was no statistically significant difference in BMI among the carriers of the rs11206244 (C785T) SNP (144).

According to Gałecka *et al* findings, which were in line with the outcome of the current study, the distribution of demographic trials for the various genotypes of rs12095080 (A1814G) SNP and rs11206244 (C785T) SNP did not show any detectable variations (147).

4.3 The Effect of Deiodinase Type 1 Polymorphisms on the Thyroid Laboratory Parameters in Hypothyroid Female Patients

Since DIO1 is crucial for peripheral T3 generation and exhibits an optimal activity in the ORD of rT3 to produce T2, the enzyme is frequently referred to as an ORDase. As a result, the DIO1 is significantly more efficient in the ORD of the inactive metabolite rT3 even though it is regarded to be very important for peripheral T3 synthesis by ORD of T4 (151).

The ratios of the various iodothyronines were assessed since they reported to be more accurately represent the metabolism of peripheral thyroid hormones (132).

4.3.1 The Effect of rs12095080 (A1814G) SNP on Thyroid Laboratory Parameters in Hypothyroid Female Patients

The GG carriers had higher total T3 (tT3) (1.67±0.16), fT3 (7.04±0.74), and lower tT4 (89.03±25.69) and rT3 (692.44±62.83) than AA carriers (1.55±0.46, 6.38±1.27, 103.57±28.99 and 912.27±341.28) and AG carriers (1.53±0.26, 6.83±1.21, 101.8±25.76 and 955.68±379.84) (Table 3-10). The differences between the three groups of patients did not reach to a significant level, this could be due to the GG genotype's low frequency as shown in Table 3-10.

Although the pattern of the elevation of tT3 and fT3, and the decreasing of rT3 in the three groups was worthy to be noticed. This pattern indicates that rs12095080 (A1814G) SNP could have an impact on the DIO1 enzyme activity and thyroid hormones levels. Increasing the T3 levels and decreasing the T4 and rT3 levels among the GG carriers could indicate the marginally increased DIO1 activity in the presence of the mutant allele.

The same results were found by Van Der Deure *et al* who studied the Danish twins, they illustrated that the rs12095080 (A1814G) SNP was associated with greater tT3 and fT3 and lower rT3 levels, however, the associations failed to reach to a statistically significant level (148). Other genes, such as DIO2, DIO3, TSH receptor gene, or even thyroid transporters gene could be involved in the regulating and controlling the metabolism of these hormones. The lack of a significant association between the rs12095080 (A1814G) SNP and thyroid parameters may be due to the polymorphism in these genes.

Since several loci play roles in regulating serum thyroid parameters (148), it is worthy to mention that rT3 is also generated or eliminated by the activity of DIO3 enzyme (122), this could mask the impact of DIO1 on rT3 levels.

As demonstrated in the Table 3-10, the GG carriers in this study had higher T3/T4 and T3/rT3 ratios (1.95±0.45 and 2.41±0.04, respectively) and a lower rT3/T4 ratio (0.81±0.18). This is attuned with De Jong *et al* and Peeters' *et al* findings who estimated that the GG carriers had greater T3/T4 ratio and T3/rT3 ratio as well as lower rT3/T4 ratio. These two studies reported that the carriers of G allele might have higher enzyme activity (134,149). The same results, higher T3/ rT3 ratio and T3/T4 ratio and lower rT3/T4 ratio, was found in a large cohort study on healthy Danish twins (148). The increased T3/T4 and T3/rT3 ratios suggests that G allele carriers may have marginally increased activity of DIO1. Although further investigation is required with greater sample size to insure a greater number of the GG carriers.

It has to be mentioned that the deiodination of thyroid hormones by DIO1 is not the only mechanism that the body has to metabolize these hormones; there are numerous mechanisms for T3 and T4 metabolism, including glucuronidation, sulfation, and deiodination by DIO2 or DIO3. Furthermore, there are three sources for plasma T3 production, including thyroidal T3 secretion, ORD of T4 by DIO1, and ORD of T4 by DIO2 (152). Therefore, any disruption of these mechanisms is probably going to affect T4 and T3 levels.

The diiodothyronine levels among the carriers of the GG genotype were lower than its levels among the carriers of AA and AG genotypes, but this difference didn’t reach to a significant level as illustrated in Table 3-10. Diiodothyronine is produced from T3 by IRD activity of DIO1 and DIO3, and from rT3 by ORD activity of DIO1 and DIO2 (119).

As a consequence of the small differences in the levels of T4 and T3 among the three groups, the TSH (P= 0.639) has a negligible difference between these groups as well (Table 3-10). This finding comes along with Van Der Deure *et al* who reported the same result in the Dutch population (148).

4.3.2 The Effect of rs11206244 (C785T) SNP on Thyroid Laboratory Parameters in Hypothyroid Female Patients

According to rs11206244 (C785T) SNP, the current study found that the fT3 and fT4 level in CC, CT, and TT groups are not significantly varied, however tT4 was higher among the wild type group, but to a non-significant level (Table 3-11). These results are in line with Arici *et al*, who found that fT3 and fT4 values in patients with wild type and with the mutant type were within close limits (144). Van Der Deure *et al* also demonstrated that no considerable variation in fT3 and fT4 levels were found among the wild and the mutant types groups during his study on the Dutch population (148).

The statistical confidence in some previous studies' apparent connections between rs11206244 (C785T) SNP and the levels of fT4 and fT3 was substantially low (134,149). The most important human plasma T3 source has been the subject of debate for a very long time. Even though it was first believed that DIO1 generated the major amount of plasma T3 in humans, but recent researches indicate that DIO2 had a significant contribution. Accordingly, PTU therapy (which blocks DIO1 activity) only results in a 30% drop in T3 in individuals receiving constant exogenous T4 doses for primary hypothyroidism, indicating a potential significant role for DIO2 in the synthesis of plasma T3 (94). This finding could be a possible explanation for why that the rs11206244 (C785T) SNP does not significantly affect the T3 level.

This study revealed that rs11206244 (C785T) SNP has an impact on plasma rT3 levels (Table 3-11), as the CT carriers have a significantly higher rT3 level ($p=0.028$) than the CC carrier. This result is consistent with the result of De Jong *et al*, Verloop *et al*, and Peeters *et al*, which revealed that the T-allele carrier patients had higher plasma rT3 levels (118,133,149).

Reverse T3 is produced by DIO3-catalyzed IRD of T4 and removed by DIO1-catalyzed ORD. The favored substrate for the DIO1 is rT3 (134). It has been reported that the DIO1 efficiency in the ORD of rT3 was 100 times greater than that of the ORD of T4 (132). This indicates the significance role of DIO1 in the breakdown of thyroid hormone. As a result, polymorphisms in DIO1 could be expected to be associated with rT3 levels. This could explain why the rs11206244 (C785T) SNP

variation had no significant association with plasma T3 and T4 but did have a high association with rT3 levels in our study sample.

As a consequence of the higher rT3 level in the CT carriers, those carriers had a lower T3/rT3 ratio (1.65±0.65) than CC and CT carriers (2.07±0.83, 2.00±1.05, respectively), as well as they, had higher rT3/T4 ratio (1.08±0.49) than CC and TT carrier (0.88±0.4, 0.95±0.48) respectively (Table 3-11). This finding is compatible with the result of De Jong *et al* and Peeters *et al*, which reported a significant correlation between T allele and a decreased in T3/rT3 ratio (134,149).

In addition, the findings of Van Der Deure *et.al*, who investigated this SNP among Danish twin were consistent with this study results (148). Furthermore, according to this result, a lower T3/rT3 ratio and higher rT3/T4 ratio were related to the T allele.

As previously mentioned, because the ratios of the various iodothyronines are more indicative of peripheral thyroid hormone metabolism rather than variations in serum thyroid hormones (132), the differences in serum iodothyronines ratios could be more indicative of the impact of polymorphisms in DIO1 (133).

The thyroid hormone levels in serum are regulated by thyroid function, the capacity of thyroid hormone binding and deiodinase activity. The ratio of iodothyronines in serum describes the various deiodinases activity most accurately due to the confusing influence of different quantities of thyroid hormone-binding proteins (132).

For rs11206244 (C785T) SNP carriers, the ratio of T3/rT3 is lower whereas rT3/T4 ratio is higher among the carriers of CT genotype compared with the CC and TT carriers in the same pattern (Table 3-11). This could indicate that the CT genotype has a greater impact on DIO1 enzyme activity and the levels of thyroid hormones. The heterozygous mutant type of the rs11206244 (C785T) SNP could be associated with the decreased clearance of rT3 in other words this mutant type could decrease the enzyme activity.

The diiodothyronine did not show a significant correlation with different genotype of rs11206244 (C785T) SNP (Table 3-11), but as it is mentioned above, to prove this, further research is required.

However, TSH level was found to be higher in CT carriers than in CC and TT carriers (Table 3-11), it is important to mention that the values in the three groups

were within the normal range value (0.4 – 4 mIU/L). Complex feedback loops are the base of thyroid hormone homeostasis. Thyroid hormone feedback, for instance, inhibits the release of TSH, and evidence suggests that, in addition to controlling thyrotropin-releasing hormone (paracrine action), TSH also controls its secretion (autocrine action) (8). The current study finding about TSH level is compatible with the result of a large cohort study on Danish twin participates which reported that the CT carriers had higher TSH levels than CC and TT carriers (148).

4.4 The Association between Deiodinase Type 1 Polymorphisms and Levothyroxine Dose in Hypothyroid Female Patients

In recent years, it has become clinically essential to use personalized medicine rather than conventional management to improve patient responses (153). Levothyroxine is the best drug used for the treatment of hypothyroidism. Levothyroxine is given orally to adults at an estimated dosage of 1.6 μg/kg/day, which is equivalent to 100–125 μg per day. Concurrent drugs, gender, age, genetic background, patient compliance, etc. may affect the efficacy of levothyroxine treatment (20).

The results in a Table 3-10 demonstrated that no statistical difference in levothyroxine dose was found among the three groups regarding the rs12095080 (A1814G) SNP, there is a lake of studies on this SNP to compare with.

The findings in Table 3-11 show that there is no association between the rs11206244 (C785T) SNP and the dose of levothyroxine among the groups of patients, this is compatible with Santoro *et al* who reported the same result (95). Furthermore, Arici *et al* in their research on the Turkish population, also found that there was no association between the dose of levothyroxine and the three genotypes groups of patients (144).

4.5 The Association between Deiodinase Type 1 Polymorphisms and Blood Pressure in Hypothyroid Female Patients

According to previous studies, thyroid hormones have considerable impacts on the peripheral vascular system, including preventing atherosclerosis and relaxing vascular smooth muscle cells (154).

Deiodinase Type 1 enzyme expression in the kidney, liver, pituitary and thyroid, but not in vascular smooth muscle (94) could explain our result, that there

is no significant effect of rs12095080 (A1814G) SNP and rs11206244 (C785T) SNP on the blood pressure parameters (diastolic, systolic and mean arterial pressure, Table 3-12 and Table 3-13). Furthermore, due to the DIO2 expression in vascular smooth muscle cells, there is a hypothesis that the genetic variations in DIO2 would affect the levels of thyroid hormone parameters and possibly lead to hypertension since T3 has a vasodilation impact (154).

4.6 The Association between Deiodinase Type 1 Polymorphism and Glycemic Parameters in Hypothyroid Female Patients

In a large number of experimental and epidemiological research, insulin resistance and glucose homeostasis are significantly affected by thyroid hormones. As a result, certain deiodinase profiles influencing the local or peripheral level of thyroid hormones could contribute to the development of diabetes mellitus type 2 or increase the risk of insulin resistance. The research emphasized particularly the importance of the DIO2 polymorphism on glucose homeostasis (118).

To the best of our knowledge, this study is the first to investigate the effect of the rs12095080 (A1814G) SNP and rs11206244 (C785T) SNP on the glycemic indices, and it was found that the FSG, FBS, and HOMA-IR did not significantly differ among the three groups of patients regarding both the studied SNPs of the DIO1 gene (Table 3-14 and Table 3-15). Accordingly, the rs12095080 (A1814G) SNP and rs11206244 (C785T) SNP may have no impact on insulin resistance or diabetes mellitus in our sampling of Iraqi women with hypothyroidism.

4.7 The Impact of the Presence of both rs12095080 SNP and rs11206244 SNP on the Study Parameters

To investigate the impact of the presence of both SNPs in the patients on the demographic characteristics, thyroid laboratory parameters, blood pressure and glycemic parameters, therefore the patients were divided into three groups; patients with no polymorphism, patients who have either rs12095080 (A1814G) SNP or rs11206244 (C785T) SNP and patients who have both the two SNPs.

There was a statistically significant difference in the duration of treatment; the patients who had both the two SNPs had the longer duration of treatment than carriers of one SNP, although there was no significant difference in the age of the three groups (Table 3-16). This result suggests that the presence of these two SNPs might be one causes of hypothyroidism incidence.

The significantly higher total T3 levels in the group of patients who not have any SNPs in compare with patients have only one SNP, indicates that the presence of both the two SNPs has no impact on the levels of the total T3 (Table 3-16).

This study found that the HOMA-IR is significantly different among the three groups of patients but it still with in normal range as illustrated in Table 3-16. The patients who had both SNPs had higher HOMA-IR than carriers of only one SNP.

There were no significant variations in the other parameters (demographics characteristics, thyroid laboratory, blood pressure and glycemic Parameters), this indicates that there is no effect for the presence of both the two SNPs on these parameters.

5. Conclusions

According to the current study findings, the following conclusion could be obtained**:**

1. Two DIO1 gene polymorphisms were detected in different genotypes frequencies in Iraqi hypothyroid female patients. The wild type of the both SNPs (rs12095080 (A1814G) and rs11206244 (C785T)) were the most frequent genotype.
2. The rs12095080 (A1814G) SNP and rs11206244 (C785T) SNP have no significant association with T3 and T4 hormone levels. Therefore, no significant impact of these two SNPs was detected on how well the patients responded to levothyroxine therapy.
3. The present result demonstrated that there was no effect of rs12095080 (A1814G) SNP and rs11206244 (C785T) SNP on the blood pressure and hypertension.
4. The rs12095080 (A1814G) SNP and rs11206244 (C785T) SNP have no association with the glycemic parameters of Iraqi hypothyroid female patients.

6. The Recommendations and Future study

1. To elucidate the role of DIO1 gene (rs12095080 (A1814G) SNP and rs11206244 (C785T) SNP) on therapeutic response to levothyroxine in Iraqi hypothyroid female patients, investigations with larger sample size of patients are preferably required.
2. Investigation of the impact of other SNPs of DIO1 gene on the responsiveness to levothyroxine.
3. Investigation of the genetic differences in other genes such as DIO2, DIO3, thyroid transporter and TSH receptor that are responsible for metabolism of thyroid hormone in individuals receiving levothyroxine, and find out how they effect on responsiveness to this drug.
4. To clearly establish if the genetic variation contributes in the development of hypothyroidism, a healthy control group from the general population is added to future investigation.
5. Hypothyroid male patients could be involved in a future study.
6. Additional researches are required to determine T2 association with the DIO1 gene and DIO1 enzyme activity.
7. Measurements of lipid profile could be involved in a future study.

REFERENCES

References

1. Alaa H, Alsayed R, Yousif E. Thyroid Gland and Its Rule in Human Body Research Journal of Pharmaceutical, Biological and Chemical Sciences Thyroid Gland and Its Rule in Human Body. Journal of Pharmaceutical, Biological and Chemical Sciences. 2016;7(11):6.
2. Vitti P, Hegedüs L. Thyroid diseases: pathogenesis, diagnosis, and treatment. Springer; 2018:1-426.
3. Barrett KE, Barman SM, Boitano S, Brooks HL, York N, San C, et al. Ganong' s Review of Medical Physiology. 2012: 339-351.
4. Sellitti DF, Suzuki K. Intrinsic regulation of thyroid function by thyroglobulin. Thyroid. 2014;24(4):625–38.
5. Brent GA. Mechanisms of thyroid hormone action. Vol. 122, Journal of Clinical Investigation. 2012. p. 3035–43.
6. Pandiyan B, Merrill SJ. A model of the cost of delaying treatment of Hashimoto's thyroiditis: Thyroid cancer initiation and growth. Mathematical Biosciences and Engineering. 2019;16(6):8069–91.
7. Halawani MS, Nughays RO, Altemani AF. Causes, Diagnosis and Management of Hypothyroidism. Egypt J Hosp Med. 2018;71(1):2250–2.
8. Gottwald-Hostalek U, Kahaly GJ. Triiodothyronine alongside levothyroxine in the management of hypothyroidism? Curr Med Res Opin. 2021;37(12):2099–106.
9. Razvi S, Bhana S, Mrabeti S. Challenges in interpreting thyroid stimulating hormone results in the diagnosis of thyroid dysfunction. J Thyroid Res. 2019;2019.
10. Mullur R, Liu YYY, Brent GA. Thyroid hormone regulation of metabolism. Physiol Rev. 2014;94(2):355–82.
11. Bianco AC, Kim BS. Pathophysiological relevance of deiodinase polymorphism. Curr Opin Endocrinol Diabetes Obes. 2018;25(5):341–6.
12. Corona G, Croce L, Sparano C, Petrone L, Sforza A, Maggi M, et al. Thyroid and heart, a clinically relevant relationship. J Endocrinol Invest. 2021;44(12):2535–44.
13. Abdi H, Kazemian E, Gharibzadeh S, Amouzegar A, Mehran L, Tohidi M, et al. Association between Thyroid Function and Body Mass Index: A 10-Year Follow-Up. Ann Nutr Metab. 2017;70(4):338–45.
14. Anita P. Sensitivity and Specificity of Diagnostic Biomarkers in Thyroid Diseases: From circulating chemistry to molecular cytogenetics. BLDE (Deemed to be University); 2019.
15. Mahdavi M, Amouzegar A, Mehran L, Madreseh E, Tohidi M, Azizi F. Investigating the prevalence of primary thyroid dysfunction in obese and overweight individuals: Tehran thyroid study. BMC Endocr Disord. 2021;21(1):1–13.

16. Kim MJ, Cho SW, Choi S, Ju DL, Park DJ, Park YJ. Changes in body compositions and basal metabolic rates during treatment of Graves' disease. Int J Endocrinol. 2018;2018.

17. Guglielmi R, Grimaldi F, Negro R, Frasoldati A, Misischi I, Graziano F, et al. Shift from levothyroxine tablets to liquid formulation at breakfast improves quality of life of hypothyroid patients. Endocrine, Metabolic & Immune Disorders-Drug Targets (Formerly Current Drug Targets-Immune, Endocrine & Metabolic Disorders). 2018;18(3):235–40.

18. Chaker L, Bianco AC, Jonklaas J, Peeters RP. Hypothyroidism. The Lancet. 2017;390(10101):1550–62.

19. Kostoglou-Athanassiou I, Ntalles K. Hypothyroidism-new aspects of an old disease. Hippokratia. 2010;14(2):82.

20. Chiovato L, Magri F, Carlé A. Hypothyroidism in context: where we've been and where we're going. Adv Ther. 2019;36(2):47–58.

21. Peeters RP. Subclinical hypothyroidism. New England Journal of Medicine. 2017;376(26):2556–65.

22. Magri F, Buonocore M, Oliviero A, Rotondi M, Gatti A, Accornero S, et al. Intraepidermal nerve fiber density reduction as a marker of preclinical asymptomatic small-fiber sensory neuropathy in hypothyroid patients. Eur J Endocrinol. 2010;163(2):279.

23. Dew R, Okosieme O, Dayan C, Eligar V, Khan I, Razvi S, et al. Clinical, behavioural and pharmacogenomic factors influencing the response to levothyroxine therapy in patients with primary hypothyroidism—protocol for a systematic review. Syst Rev. 2017;6(1):1–7.

24. Gaitonde DY, Rowley KD, Sweeney LB. Hypothyroidism: An update. Am Fam Physician. 2012;86(3):244–51.

25. Canaris GJ, Tape TG, Wigton RS. Thyroid disease awareness is associated with high rates of identifying subjects with previously undiagnosed thyroid dysfunction. BMC Public Health. 2013;13(1):1–7.

26. Ross DS. Laboratory assessment of thyroid function. UpToDate, Cooper, DS (Ed), UpToDate, Waltham, MA. 2013;

27. McDermott MT. Hypothyroidism. Ann Intern Med. 2020;173(1):ITC1–16.

28. Pearce SHS, Brabant G, Duntas LH, Monzani F, Peeters RP, Razvi S, et al. 2013 ETA guideline: management of subclinical hypothyroidism. Eur Thyroid J. 2013;2(4):215–28.

29. Grunenwald S, Caron P. Central hypothyroidism in adults: better understanding for better care. Pituitary. 2015;18(1):169–75.

30. DeMartino L, McMahon R, Caggana M, Tavakoli NP. Gender disparities in screening for congenital hypothyroidism using thyroxine as a primary screen. Eur J Endocrinol. 2018;179(3):161–7.

31. Surks MI, Hollowell JG. Age-specific distribution of serum thyrotropin and antithyroid antibodies in the US population: implications for the prevalence

of subclinical hypothyroidism. J Clin Endocrinol Metab. 2007;92(12):4575–82.

32. Almandoz JP, Gharib H. Hypothyroidism: Etiology, Diagnosis, and Management. Vol. 96, Medical Clinics of North America. 2012. p. 203–21.

33. Agabegi S. Step-Up to Medicine 5th Edition (2019). 2019:419-436.

34. Ladenson PW, Singer PA, Ain KB, Bagchi N, Bigos ST, Levy EG, et al. American Thyroid Association guidelines for detection of thyroid dysfunction. Arch Intern Med. 2000;160(11):1573–5.

35. Loy M, Cianchetti ME, Cardia F, Melis A, Boi F, Mariotti S. Correlation of computerized gray-scale sonographic findings with thyroid function and thyroid autoimmune activity in patients with Hashimoto's thyroiditis. Journal of Clinical Ultrasound. 2004;32(3):136–40.

36. Kumar V, Abbas AK, Fausto N, Aster JC. Robbins and Cotran pathologic basis of disease, professional edition e-book. Elsevier health sciences; 2014:1082-1100.

37. Salih SM, Kamel WA, Abbas MT, Abass KS. Prevalence of Hyperthyroidism and Hypothyroidism and its Correlation with Serum Antithyroglobulin among patients in Kirkuk-Iraq. Journal of Advanced Pharmacy Education and Research. 2021;11(2):57–60.

38. Núñez A, Bedregal P, Becerra C, Grob LF. Neurodevelopmental assessment of patients with congenital hypothyroidism. Rev Med Chil. 2017;145(12):1579–87.

39. Braun D, Schweizer U. Thyroid hormone transport and transporters. Vitam Horm. 2018;106:19–44.

40. Laufgraben MJ, Gopalakrishnan G, others. Tarascon Adult Endocrinology Pocketbook. 2013:63-69.

41. Kroopnick JM, Kim CS. Overview of hypothyroidism in pregnancy. In: Seminars in Reproductive Medicine. Thieme Medical Publishers; 2016. p. 323–30.

42. Strieder TGA, Prummel MF, Tijssen JGP, Endert E, Wiersinga WM. Risk factors for and prevalence of thyroid disorders in a cross-sectional study among healthy female relatives of patients with autoimmune thyroid disease. Clin Endocrinol (Oxf). 2003;59(3):396–401.

43. Mirali S, Seneviratne A. Toronto Notes 2020. Toronto Notes. 2020. 243–246 p.

44. Feldt-Rasmussen U, Klose M, Rasmussen ÅK. Causes of hypothyroidism. Encyclopedia of Endocrine Diseases. 2018;600–3.

45. Sweeney LB, Stewart C, Gaitonde DY. Thyroiditis: an integrated approach. Am Fam Physician. 2014;90(6):389–96.

46. Cohen-Lehman J, Dahl P, Danzi S, Klein I. Effects of amiodarone therapy on thyroid function. Nat Rev Endocrinol. 2010;6(1):34–41.

47. Prasuna SG, Chary BH. HYPOTHYROIDISM–A MANIFESTATION OF AVARANA. International Journal of Ayurveda and Pharma Research. 2020;47–50.

48. Sajjadi-Jazi SM, Sharifi F, Varmaghani M, Meybodi HA, Farzadfar F, Larijani B. Epidemiology of hyperthyroidism in Iran: a systematic review and meta-analysis. J Diabetes Metab Disord. 2018;17(2):345–55.

49. Leng O, Razvi S. Hypothyroidism in the older population. Thyroid Res. 2019;12(1):1–10.

50. Al-Deresawi MS, Al-Rekaabi AR, Ahmed HS. Downregulation of TPO gene and it's related with Iodine deficiency in women with hypothyroidism. Journal of Pharmaceutical Sciences and Research. 2019;11(4):1223–6.

51. Kim WG, Kim WB, Woo G, Kim H, Cho Y, Kim TY, et al. Thyroid stimulating hormone reference range and prevalence of thyroid dysfunction in the Korean population: Korea National Health and Nutrition Examination Survey 2013 to 2015. Endocrinology and Metabolism. 2017;32(1):106–14.

52. Nasheiti NA. Childhood hypothyroidism in Iraq: a retrospective study. 2005; 3:136-139.

53. Faisal AH. Study of thyroid hormones among female Iraqi thyroid disorders. In: The 2nd Annual International Conference of Northeast Pharmacy Research, Thailand. 2010. p. 250–5.

54. Ghawil M, Tonutti E, Abusrewil S, Visentini D, Hadeed I, Miotti V, et al. Autoimmune thyroid disease in Libyan children and young adults with type 1 diabetes mellitus. Eur J Pediatr. 2011;170(8):983–7.

55. Lamfon HA. Thyroid disorders in Makkah, Saudi Arabia. Ozean J Appl Sci. 2008;1(1):52–8.

56. Inoue K, Leung AM. Thyroid disease awareness among U.S. adults with hypothyroidism, 1999-2014. Endocrine Practice. 2018;24(10):932–3.

57. Taylor PN, Albrecht D, Scholz A, Gutierrez-Buey G, Lazarus JH, Dayan CM, et al. Global epidemiology of hyperthyroidism and hypothyroidism. Nat Rev Endocrinol. 2018;14(5):301–16.

58. Garmendia Madariaga A, Santos Palacios S, Guillén-Grima F, Galofré JC. The incidence and prevalence of thyroid dysfunction in Europe: a meta-analysis. J Clin Endocrinol Metab. 2014;99(3):923–31.

59. Amouzegar A, Mehran L, Takyar M, Abdi H, Azizi F. Tehran thyroid study (TTS). Int J Endocrinol Metab. 2018;16(4 Suppl).

60. Ha J, Lee J, Jo K, Lim DJ, Kang M Il, Cha BY, et al. Sex differences in risk factors for subclinical hypothyroidism. Endocr Connect. 2018;7(4):511–22.

61. Hennessey J, Espaillat R. Diagnosis and Management of Hypothyroidism. Journal of the American Geriatrics Society.2015:63(8):1663-1673.

62. Bhasin S, Enzlin P, Coviello A, Basson R. Sexual dysfunction in men and women with endocrine disorders. The Lancet. 2007;369(9561):597–611.

63. Ebert EC. The thyroid and the gut. J Clin Gastroenterol. 2010;44(6):402–6.

64. Widmaier EP, Raff H, Strang KT. Vander's Human Physiology THE MECANISM OF BODY FUNCTION. McGraw-Hill Education. 2016. 351–354 p.

65. Kasumagic-Halilovic E. Thyroid disease and the skin. Annals Thyroid Res. 2014;1(2):27–31.

66. Scott KR, Simmons Z, Boyer PJ. Hypothyroid myopathy with a strikingly elevated serum creatine kinase level. Muscle & Nerve: Official Journal of the American Association of Electrodiagnostic Medicine. 2002;26(1):141–4.

67. Todd CH. Management of thyroid disorders in primary care: challenges and controversies. Postgrad Med J. 2009;85(1010):655–9.

68. Iwen KA, Schröder E, Brabant G. Thyroid hormones and the metabolic syndrome. Eur Thyroid J. 2013;2(2):83–92.

69. Bianco AC, Dumitrescu A, Gereben B, Ribeiro MO, Fonseca TL, Fernandes GW, et al. Paradigms of Dynamic Control of Thyroid Hormone Signaling. Endocr Rev. 2019;40(4):1000–47.

70. Gaur A, Pal GK, Ananthanarayanan PH, Pal P. Role of Ventromedial hypothalamus in high fat diet induced obesity in male rats: association with lipid profile, thyroid profile and insulin resistance. Ann Neurosci. 2014;21(3):104.

71. Fontenelle LC, Feitosa MM, Severo JS, Freitas TEC, Morais JBS, Torres-Leal FL, et al. Thyroid function in human obesity: underlying mechanisms. Hormone and Metabolic Research. 2016;48(12):787–94.

72. Gereben B, McAninch EA, Ribeiro MO, Bianco AC. Scope and limitations of iodothyronine deiodinases in hypothyroidism. Nat Rev Endocrinol. 2015;11(11):642–52.

73. McAninch EA, Bianco AC. Thyroid hormone signaling in energy homeostasis and energy metabolism. Ann N Y Acad Sci. 2014;1311(1):77–87.

74. Sayre NL, Lechleiter JD. Fatty acid metabolism and thyroid hormones. Curr Trends Endocinol. 2012;6:65.

75. Gencer B, Collet TH, Virgini V, Bauer DC, Gussekloo J, Cappola AR, et al. Subclinical thyroid dysfunction and the risk of heart failure events: an individual participant data analysis from 6 prospective cohorts. Circulation. 2012;126(9):1040–9.

76. Rhee CM, Curhan GC, Alexander EK, Bhan I, Brunelli SM. Subclinical hypothyroidism and survival: the effects of heart failure and race. J Clin Endocrinol Metab. 2013;98(6):2326–36.

77. Gerdes AM, Iervasi G. Thyroid replacement therapy and heart failure. Circulation. 2010;122(4):385–93.

78. Wiersinga WM, De Groot LJ, Chrousos G, Dungan K, Feingold KR, Grossman A, et al. Myxedema and coma (severe hypothyroidism). Endotext. MDText. com. Inc, South Dartmouth, MA. 2000;

79. Sengupta S, Jaseem T, Ambalavanan J, Hegde A. Homeostatic Model Assessment-Insulin Resistance (HOMA-IR 2) in Mild Subclinical Hypothyroid Subjects. Indian Journal of Clinical Biochemistry. 2018;33(2):214–7.

80. Teixeira P de F dos S, dos Santos PB, Pazos-Moura CC. The role of thyroid hormone in metabolism and metabolic syndrome. Ther Adv Endocrinol Metab. 2020;11:204201882091786.

81. Hasan Al-bayati Dr. Ali Abdulateef, MJ AL-Khateeb DrS. The Effects of Thyroid Hormones and Their Abnormalities on Intestinal and Hepatic Glucose Metabolism. Scholars International Journal of Biochemistry. 2021;4(3):26–36.

82. Pearce EN. Update in lipid alterations in subclinical hypothyroidism. Journal of Clinical Endocrinology and Metabolism. 2012;97(2):326–33.

83. VAQUERO E, DE CAROLIS C, VALENSISE H, ROMANINI C, LAZZARIN N, MORETTI C. Mild Thyroid Abnormalities and Recurrent Spontaneous Abortion: Diagnostic and Therapeutical Approach 1. American Journal of Reproductive Immunology. 2000;43(4):204–8.

84. Bercovici JP. Menstrual irregularities and thyroid diseases. Feuill Biol. 2000;74:1063–70.

85. Verma I, Sood R, Juneja S, Kaur S. Prevalence of hypothyroidism in infertile women and evaluation of response of treatment for hypothyroidism on infertility. Int J Appl Basic Med Res. 2012;2(1):17.

86. Singla R, Gupta Y, Khemani M, Aggarwal S. Thyroid disorders and polycystic ovary syndrome: An emerging relationship. Vol. 19, Indian Journal of Endocrinology and Metabolism. Medknow Publications; 2015. p. 25–9.

87. Bryan J. Levothyroxine: from sheep thyroid injections to synthetic formulations. Pharm J. 2013;291:90.

88. Elmor R, Sandulli W, Carter CA. The Economic Impact of Changing Levothyroxine Formulations in Difficult-to-Treat Hypothyroid Patients: An Evidence-Based Model. Pharmacoeconomics: Open Access. 2017;2(2).

89. Haynes RC. Thyroid and antithyroid drugs. The pharmacological basis of therapeutics. 1990;1361–83.

90. Madan R, Celi FS. Combination Therapy for Hypothyroidism: Rationale, Therapeutic Goals, and Design. Front Endocrinol (Lausanne). 2020;11(July):1–11.

91. Hennessey J V. The emergence of levothyroxine as a treatment for hypothyroidism. Endocrine. 2017;55(1):6–18.

92. Okosieme O, Gilbert J, Abraham P, Boelaert K, Dayan C, Gurnell M, et al. Management of primary hypothyroidism: statement by the British Thyroid Association Executive Committee. Clin Endocrinol (Oxf). 2016;84(6):799–808.

93. Caron P, Grunenwald S, Persani L, Borson-Chazot F, Leroy R, Duntas L. Factors influencing the levothyroxine dose in the hormone replacement therapy of primary hypothyroidism in adults. Vol. 23, Reviews in Endocrine and Metabolic Disorders. Springer; 2022. p. 463–83.

94. Paragliola RM, Corsello A, Concolino P, Ianni F, Papi G, Pontecorvi A, et al. Iodothyronine deiodinases and reduced sensitivity to thyroid hormones. Vol. 25, Frontiers in Bioscience. 2020 Jan 1;25(2):201-28.

95. Santoro AB, Vargens DD, De Camargo Barros Filho M, Bulzico DA, Kowalski LP, Meirelles RMR, et al. Effect of UGT1A1, UGT1A3, DIO1 and DIO2 polymorphisms on L-thyroxine doses required for TSH suppression in patients with differentiated thyroid cancer. Br J Clin Pharmacol. 2014;78(5):1067–75.

96. Luongo C, Dentice M, Salvatore D. Deiodinases and their intricate role in thyroid hormone homeostasis. Nat Rev Endocrinol. 2019;15(8):479–88.

97. Peeters RP, Visser TJ. Metabolism of Thyroid Hormone - Endotext - NCBI Bookshelf. 2017.

98. Hoermann R, Midgley JEM, Larisch R, Dietrich JW. Individualised requirements for optimum treatment of hypothyroidism: Complex needs, limited options. Drugs Context. 2019;8(August).

99. Devdhar M, Drooger R, Pehlivanova M, Singh G, Jonklaas J. Levothyroxine replacement doses are affected by gender and weight, but not age. Thyroid. 2011;21(8):821–7.

100. Gordon MB, Gordon MS. Variations in adequate levothyroxine replacement therapy in patients with different causes of hypothyroidism. Endocrine Practice. 1999;5(5):233–8.

101. Brenta G, Vaisman M, Sgarbi JA, Bergoglio LM, Andrada NC de, Bravo PP, et al. Clinical practice guidelines for the management of hypothyroidism. Arquivos Brasileiros de Endocrinologia & Metabologia. 2013;57:265–91.

102. Jonklaas J, Bianco AC, Bauer AJ, Burman KD, Cappola AR, Celi FS, et al. Guidelines for the treatment of hypothyroidism: prepared by the american thyroid association task force on thyroid hormone replacement. thyroid. 2014;24(12):1670–751.

103. Alexander EK, Pearce EN, Brent GA, Brown RS, Chen H, Dosiou C, et al. 2017 Guidelines of the American Thyroid Association for the diagnosis and management of thyroid disease during pregnancy and the postpartum. Thyroid. 2017;27(3):315–89.

104. Klein I, Danzi S. Thyroid disease and the heart. Circulation. 2007;116(15):1725–35.

105. Cheng SY, Leonard JL, Davis PJ. Molecular aspects of thyroid hormone actions. Endocr Rev. 2010;31(2):139–70.

106. Oetting A, Yen PM. New insights into thyroid hormone action. Best Pract Res Clin Endocrinol Metab. 2007;21(2):193–208.

107. Takeshige K, Sekido T, Kitahara J ichirou, Ohkubo Y, Hiwatashi D, Ishii H, et al. Cytosolic T3-binding protein modulates dynamic alteration of T3-mediated gene expression in cells. Endocr J. 2014;61(6):561–70.

108. Zevenbergen C, Meima ME, Lima de Souza EC, Peeters RP, Kinne A, Krause G, et al. Transport of iodothyronines by human L-type amino acid transporters. Endocrinology. 2015;156(11):4345–55.

109. panicker V. Genetics of thyroid function disease. Clinical Biochemist Reviews. 2011;32(4):165–75.

110. Sinha RA, Singh BK, Yen PM. Direct effects of thyroid hormones on hepatic lipid metabolism. Nat Rev Endocrinol. 2018;14(5):259–69.

111. Colucci P, Yue CS, Ducharme M, Benvenga S. A review of the pharmacokinetics of levothyroxine for the treatment of hypothyroidism. Eur Endocrinol. 2013;9(1):40–7.

112. Liwanpo L, Hershman JM. Conditions and drugs interfering with thyroxine absorption. Best Pract Res Clin Endocrinol Metab. 2009;23(6):781–92.

113. Vita R, Saraceno G, Trimarchi F, Benvenga S. A novel formulation of L-thyroxine (L-T4) reduces the problem of L-T4 malabsorption by coffee observed with traditional tablet formulations. Endocrine. 2013;43(1):154–60.

114. Duntas Jacqueline Jonklaas LH. Levothyroxine Dose Adjustment to Optimise Therapy Throughout a Patient's Lifetime. Advances in therapy. 2019 Sep;36(2):30-46.

115. Sachmechi I, Reich DM, Aninyei M, Wibowo F, Gupta G, Kim PJ. Effect of proton pump inhibitors on serum thyroid-stimulating hormone level in euthyroid patients treated with levothyroxine for hypothyroidism. Endocrine Practice. 2007;13(4):345–9.

116. Vagenakis AG, Braverman LE. Drug induced hypothyroidism. Pharmacology & Therapeutics Part C: Clinical Pharmacology and Therapeutics. 1976;1(1–2):149–59.

117. Canani LH, Capp C, Dora M, Meyer ELS, Wagner S, Harney JW, et al. The Type 2 Deiodinase A / G (Thr92Ala) Polymorphism Is Associated with Decreased Enzyme Velocity and Increased Insulin Resistance in Patients with Type 2 Diabetes Mellitus. 2005;90(6):3472–8.

118. Verloop H, Dekkers OM, Peeters RP, Schoones JW, Smit JWA. Genetics in endocrinology: Genetic variation in deiodinases: A systematic review of potential clinical effects in humans. Vol. 171, European Journal of Endocrinology. BioScientifica Ltd.; 2014. p. R123–35.

119. Darras VM, Van Herck SLJ. Iodothyronine deiodinase structure and function: From ascidians to humans. Journal of Endocrinology. 2012;215(2):189–206.

120. Germain DLS, Galton VA. The Deiodinase Family of Selenoproteins The availability of cDNAs for these enzymes provides important reagents for pursuing additional studies aimed at defining their biochemical features and roles in thyroid hormone economy. 1997;7(1).

121. Maia AL, Goemann IM, Meyer ELS, Wajner SM, Souza Meyer EL, Wajner SM. Type 1 iodothyronine deiodinase in human physiology and disease. Journal of Endocrinology. 2011;209(3):283–97.

122. Larsen PR, Zavacki AM. Role of the Iodothyronine Deiodinases in the Physiology and Pathophysiology of Thyroid Hormone Action. Eur Thyroid J. 2012;232–42.

123. Bianco AC, Salvatore D, Gereben B, Berry MJ, Larsen PR. Biochemistry, cellular and molecular biology, and physiological roles of the iodothyronine selenodeiodinases. Endocr Rev. 2002;23(1):38–89.

124. Kuiper GGJM, Kester MHA, Peeters RP, Visser TJ. Biochemical mechanisms of thyroid hormone deiodination. Thyroid. 2005;15(8):787–98.

125. Gałecka E, Anna KK, Orzechowska A, Maes M, Górski P, Szemraj J. Assessment of type 1 and type 3 deiodinase expression levels in depressive disorders. Acta Neurobiol Exp (Wars). 2017;77(3):225–35.

126. Köhrle J. Local activation and inactivation of thyroid hormones: The deiodinase family. Mol Cell Endocrinol. 1999;151(1–2):103–19.

127. Holtorf K. Peripheral Thyroid Hormone Conversion and Its Impact on TSH and Metabolic Activity. J Restor Med. 2014 Jun 26;3(1):30–52.

128. Köhrle J. The deiodinase family: Selenoenzymes regulating thyroid hormone availability and action. Cellular and Molecular Life Sciences. 2000;57(13–14):1853–63.

129. Bunevicius A, Laws ER, Saudargiene A, Tamasauskas A, Iervasi G, Deltuva V, et al. Common genetic variations of deiodinase genes and prognosis of brain tumor patients. Endocrine. 2019 Dec 1;66(3):563–72.

130. Panicker V, Cluett C, Shields B, Murray A, Parnell KS, Perry JRB, et al. A common variation in deiodinase 1 gene DIO1 is associated with the relative levels of free thyroxine and triiodothyronine. Journal of Clinical Endocrinology and Metabolism. 2008;93(8):3075–81.

131. Dora M, Machado WE, Rheinheimer J, Crispim D, Maia AL. Association of the type 2 deiodinase Thr92Ala polymorphism with type 2 diabetes : case – control study and meta-analysis. 2010;427–34.

132. Peeters RP, van den Beld AW, van Toor H, Uitterlinden AG, Janssen JAMJL, Lamberts SWJ, et al. A polymorphism in type I deiodinase is associated with circulating free insulin-like growth factor I levels and body composition in humans. Journal of Clinical Endocrinology and Metabolism. 2005 Jan;90(1):256–63.

133. Peeters RP, van der Deure WM, Visser TJ. Genetic variation in thyroid hormone pathway genes; polymorphisms in the TSH receptor and the iodothyronine deiodinases. Eur J Endocrinol. 2006;155(5):655–62.

134. Peeters RP, van Toor H, Klootwijk W, de Rijke YB, Kuiper GGJM, Uitterlinden AG, et al. Polymorphisms in thyroid hormone pathway genes are associated with plasma TSH and iodothyronine levels in healthy subjects.

Journal of Clinical Endocrinology and Metabolism. 2003 Jun 1;88(6):2880–8.

135. Patersone N, Biggart E M, chapman R S et al. Evaluation of a time-resolved immunofluorometric assay for serum thyroid stimulating hormone. Ann. Clin. Biochem. 1985: 22: 606-661.

136. Marsden P, Facer P, Acosta M, Howorth PJN. A comparative study of serum total thyroxine estimation on unextracted serum by radioimmunoassay and by competitive protein binding. J Clin Pathol. 1975;28(8):608–12.

137. Meinhold H, Schürnbrand P. Radioimmunological Measurement of 3,3'-Diiodothyronine in Serum and Amniotic Fluid. Vol. 15, J. Clin. Chem. Clin. Biochem. 1977.

138. Schlenker S. Standard operating procedure. Text Chem Color. 1997;29(7):283–6.

139. Lidofskyt SD, Hinsberg WD, Zare RN. Enzyme-linked sandwich immunoassay for insulin using laser fluorimetric detection (insulin detection in serum samples/enzyme amplification/laser fluorimetry). Vol. 78, Medical Sciences. 1981.

140. Katsuki A, Sumida Y, Gabazza EC, Murashima S, Furuta M, Araki-Sasaki R, et al. Homeostasis model assessment is a reliable indicator of insulin resistance during follow-up of patients with type 2 diabetes. Diabetes Care. 2001;24(2):362–5.

141. Rahman M, Berenson AB. Accuracy of current body mass index obesity classification for white, black and Hispanic reproductive-age women. Obstetrics and gynecology. 2010;115(5):982.

142. Material Safety Data Sheet Plasmid DNA Extraction Mini Kit.

143. Meyers JA, Sanchez D, Elwell LP, Falkow S. Simple Agarose Gel Electrophoretic Method for the Identification and Characterization of Plasmid Deoxyribonucleic Acid [Internet]. Vol. 127, JOURNAL OF BACTERIOLOGY. 1976. Available from: https://journals.asm.org/journal/jb

144. Arici M, Oztas E, Yanar F, Aksakal N, Ozcinar B, Ozhan G. Association between genetic polymorphism and levothyroxine bioavailability in hypothyroid patients. Endocr J. 2018;65(3):317–23.

145. AlRasheed MM, AlAnzi A, AlShalhoub R, Abanmy N, Bakheet D. A study of the role of DIO1 and DIO2 polymorphism in thyroid cancer and drug response to therapy in the Saudi population. Saudi Pharmaceutical Journal. 2019 Sep 1;27(6):841–5.

146. Hegedüs L, Bianco AC, Jonklaas J, Pearce SH, Weetman AP, Perros P. Primary hypothyroidism and quality of life. Nat Rev Endocrinol. 2022;18(4):230–42.

147. Gałecka E, Talarowska M, Maes M, Su KP, Górski P, Szemraj J. Polymorphisms of iodothyronine deiodinases (DIO1, DIO3) genes are not associated with recurrent depressive disorder. Pharmacological Reports. 2016 Oct 1;68(5):913–7.

148. Van Der Deure WM, Hansen PS, Peeters RP, Uitterlinden AG, Fenger M, Kyvik KO, et al. The effect of genetic variation in the type 1 deiodinase gene on the interindividual variation in serum thyroid hormone levels: an investigation in healthy Danish twins. Clin Endocrinol (Oxf). 2009;70(6):954–60.

149. de Jong FJ, Peeters RP, Heijer T den, van der Deure WM, Hofman A, Uitterlinden AG, et al. The association of polymorphisms in the type 1 and 2 deiodinase genes with circulating thyroid hormone parameters and atrophy of the medial temporal lobe. Journal of Clinical Endocrinology and Metabolism. 2007;92(2):636–40.

150. Tan NC, Chew RQ, Subramanian RC, Sankari U, Koh YLE, Cho LW. Patients on levothyroxine replacement in the community: Association between hypothyroidism symptoms, co-morbidities and their quality of life. Fam Pract. 2018;36(3):269–75.

151. Morenoa M, Berryc MJ, Horstd C, Thornad R, Harney JW, Larsen PR, et al. Activation and inactivation of thyroid hormone by type I iodothyronine deiodinase. 1994;344:143–6.

152. Visser TJ. Sulfation and glucuronidation pathways of thyroid hormone metabolism. Thyroid hormone metabolism: molecular biology and alternate pathways. 1994;85–117.

153. Yardley L, Ainsworth B, Arden-Close E, Muller I. The person-based approach to enhancing the acceptability and feasibility of interventions. Pilot Feasibility Stud. 2015;1(1):1–7.

154. Kasahara T, Tsunekawa K, Seki K, Mori M, Murakami M. Regulation of iodothyronine deiodinase and roles of thyroid hormones in human coronary artery smooth muscle cells. Atherosclerosis. 2006;186(1):207–14.

APPENDIX

Questionnaire for Hypothyroidism Patients

Demographic characterization

الاسم: رقم الهاتف:

الوزن: الطول:

Parameters	variable	Notes
Age		
Gender	Female	
Duration of treatment of levothyroxine		
Other diseases		
Other medication		
Systolic blood pressure		
Diastolic blood pressure		
Mean B.P		
BMI		
Levothyroxine dose		

University of Kerbala
Consent to be in Research

Study Title: The Impact of Deiodinase-1 Polymorphisms on the Therapeutic Response of Levothyroxine in Hypothyroidism Patients of Kerbala Province

The Researcher Name: Entsar Hadi Jawad

This is a medical research study, and you do not have to take part. The researcher will explain this study to you. If you have any questions, you may ask me.
You are being asked to participate in this study because you have hypothyroidism and treated with levothyroxine.
In this study, the researcher is collecting blood samples from you to learn more about the association of genetic polymorphism of DIO1 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.

The Consent:

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

Name of participant:

Date Participant's Signature for Consent

Date Person Obtaining Consent (Researcher)

Ministry of Higher Education
and Scientific Research
University of Karbala
College of Pharmacy
Department of Pharmacology & Tox.

جمهورية العراق
وزارة التعليم العالي والبحث العلمي
جامعة كربلاء
كلية الصيدلة
شعبة الدراسات العليا

Issue No.:
Date:

العدد: د.ع/ 6 /2072
التاريخ: 19 / 12 / 2021

امر اداري

استناداً الى الكتاب الصادر من جامعة كربلاء/ امانة مجلس الجامعة ذي العدد ج/1568 بتاريخ 2020/11/21 والمتضمن المصادقة على محضر الجلسة الثالثة لمجلس كلية الصيدلة للعام الدراسي 2021-2022 المنعقدة بتاريخ 2021/11/3 واستنادا الى الصلاحيات المخولة لنا تقرر اقرار بحوث طلبة الدراسات العليا /ماجستير في فرع الادوية والسموم والمدرجة تفاصيلهم في الجدول ادناه :

ت	أسم الطالب	عنوان البحث	المشرف
1	آلاء هاشم محمد	The Impact of Deiodinase-3 gene Polymorphisms on the Therapeutic Response of Levothyroxine in Hypothyroidism patients of Kerbala province	المشرف الاول :- أ.د. بان حوشي خلف المشرف الثاني:- أ.م.د. سوزان جبير عباس
2	انتصار هادي جواد	The Impact of Deiodinase-1 Polymorphisms on the Therapeutic Response of Levothyroxine in Hypothyroidism patients of Kerbala province	المشرف الاول :- أ.د. بان حوشي خلف المشرف الثاني:- أ.م.د. سوزان جبير عباس
3	عبير حسين هليجي	Effects of follicle stimulating hormone receptor gene polymorphism on response to FSH hormone therapy in infertile women	المشرف الاول :- أ.م. مازن حامد عودة المشرف الثاني:- د. حميدة هادي عبد الواحد
4	محمد شهيد ساجت	Role GATM gene polymorphism in the incidence of myopathy in patients treated with statins	المشرف الاول :- أ.م. مازن حامد عودة المشرف الثاني:- أ.م.د. حسن محمود موسى
5	هالة يونس كاظم	Role of COQ2 gene polymorphism on incidence of myopathy in patients treated with statins	المشرف الاول :- أ.م. مازن حامد عودة

Email: Pharmacy@uokerbala.edu.com
العراق- محافظة كربلاء- مكتب بريد كربلاء- ص ب 1125

rs11206244 (C785T) ليس له أي تأثير هو الاخر على مستويات هرمونات T3 و T4 ، فيمكن الاستنتاج بانه لا يساهم في الاستجابة العلاجية لليفوثايروكسين ومع ذلك ، يمكن أن يترافق هذا التغاير الجيني الاحادي مع انخفاض في سرعة التخلص من ال rT3 من الجسم.

الخلاصة

الخلفية: قصور الغدة الدرقية هو حالة منتشرة تؤثر على الناس في جميع أنحاء العالم. يعد الليفوثيروكسين العلاج القياسي لقصور الغدة الدرقية الأولي. ورغم الاستمرار بأخذ هدا العلاج لفترة طويلة، لا يزال العديد من المرضى يعانون من الأعراض ويشتكون من المرض. يقوم إنزيم الديأيودينيز من النوع 1 (DIO1) بتنظيم عملية التمثيل الغذائي لهرمونات الغدة الدرقية. تؤثر الاختلافات الجينية في جين الديأيوديني على استقلاب هرمونات الغدة الدرقية ويمكن أن تكون مرشحة كسبب مرضي لقصور الغدة الدرقية.

تهدف هذه الدراسة إلى التحقق لاثنين من الأشكال الجينية احادية التغاير في جين DIO1 و هما التغاير الجيني الاحادي (A1814G) rs12095080 و التغاير الجيني الاحادي (C785T) rs11206244 على الاستجابة العلاجية للعلاج بالليفوثيروكسين في مريضات قصور الغدة الدرقية العراقيات.

المنهجية: اشتركت 220 مريضة من مريضات قصور الغدة الدرقية الأولي في هذه الدراسة المستعرضة. حيث لايوجد بين المريضات صلة قرابة وأعمارهن كانت 40 عاما أو أكثر. جميع المريضات كانوا يتلقين علاج الليفوثيروكسين لمدة أربعة أشهر على الأقل.

كشف عن التغاير الجيني الاحادي (A1814G) rs12095080 و التغاير الجيني الاحادي rs11206244 (C785T)في جينDIO1 باستخدام طريقة تفاعل البلمرة المتسلسل الخاصة بالأليل وتقنية تفاعل البلمرة المتسلسل لنظام الطفرة الحرارية للتضخيم الرباعي على التوالي. حددت مستويات هرمونات الغدة الدرقية (هرمون الغدة الدرقية (T4) وثلاثي ايودوثيرونين (T3) وثلاثي يودوثيرونين العكسي (rT3) وثنائي ايودوثيرونين) (T2) وهرمون تحفيز الغدة الدرقية (TSH) بالإضافة إلى بعض مؤشرات نسبة السكر في الدم ومعلمات ضغط الدم.

النتائج: أظهر تردد النمط الجيني للتغاير الجيني الاحادي (A1814G) rs12095080 توزيعا أكبر للأليل A على حساب أليل حيث كانت ترددات الانماط الجينية 156 (70.9٪) ل AA و 61 (27.7٪) ل AG و 3 (1.4٪) ل GG. في الوقت نفسه، أشار توزيع التغاير الجيني الاحادي (C785T) rs11206244 إلى انتشار أليل C حيث كانت ترددات الأنماط الجينية 106 (48.2٪) لCC ، و75 (34.1٪) ل TC ، و39 (17.7٪) لTT.

قسمت المرضى إلى ثلاث مجموعات وفقًا للنمط الجيني لكل تغاير جيني وفيما يتعلق بـ التغاير الجيني الاحادي (A1814G) rs12095080، لم تكن هناك اختلافات كبيرة في مستويات هرمونات الغدة الدرقية بين مجموعات المرضى الثلاث.

وفقًا لـ التغاير الجيني الاحادي (C785T) rs11206244 ، وجد ان مستويات ال TSH و rT3 كانت مرتفعة بين حاملي الاليل T ($p = 0.018$ و $p = 0.028$ ، على التوالي) ، بينما لم تكن هناك فروق ذات دلالة إحصائية في هرمونات الغدة الدرقية الأخرى (T3 و T4 و T2) كما لايوجد ارتباط بين هذه التغايرات الجينية الاحادية ومعلمات نسبة السكر في الدم او معلمات ضغط الدم.

الأستنتاج: نظرًا لأن مستويات هرمون الغدة الدرقية لم تتأثر بالتغاير الجيني الاحادي rs12095080 (A1814G) في جين DIO1 ، فإنه لا يمكن القول بأن له تأثير على كيفية استجابة عينة النساء العراقيات المصابات بقصور الغدة الدرقية لعلاج الليفوثايروكسين. علاوة على ذلك ، نظرًا لأن التغاير الجيني الاحادي

جامعة كربلاء

كلية الصيدلة

فرع الادوية والسموم

تأثير تعدد الأشكال الجينيه في الديأيوديناز 1على الاستجابة العلاجية لليفوثيروكسين لدى مرضى قصور الغدة الدرقية في محافظة كربلاء

رسالة مقدمة الى

كلية الصيدلة في جامعة كربلاء كجزء من متطلبات درجة الماجستير في

الادوية والسموم

من قبل الطالبة

انتصار هادي جواد

(بكالوريوس صيدلة \ جامعة بغداد 2005)

باشراف

أ.د. بان حوشي خلف

دكتوراه ادوية وسموم

أ.م.د. سوزان جبير عباس

دكتوراه كيمياء حياتية

1444هجري

2022 ميلادي