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College of Pharmacy

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The Impact of Deiodinase-3 gene Polymorphisms on the Therapeutic Response of Levothyroxine in Hypothyroidism Female Patients in Kerbala Province

A Thesis

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

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سورة المجادلة الاية 11

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Dedication

TO….

My Parents

My Husband

My Brothers, Sisters, Son& daughter.

With deep appreciation

Alaa Hashim Mohammed

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List of Abbreviations	
ANOVA	Analysis of Variance
4-AAP	4-Aminoantipyrine
ADME	Absorption,Distribution,Metabolism,Excretion
ARMS-PCR	amplification refractory mutation system-polymerase chain reaction
BMI	Body Mass Index
CETP	Cholesterol Ester Transfer Protein
DUOX2	Dual Oxidase2
DIO1	Deiodinase type-1
DIO2	Deiodinase type-2
DIO3	Deiodinase type -3
DIOS	Iodothyronine Deiodinase Enzymes
DLK1	Delta-Like homolog 1
DIO3os	Deiodinase type-3 Opposite Strand
DTE	Desiccated Thyroid Extract
ELISA	Enzyme-Linked Immunosorbant Assay
EDTA	Ethylene Diamine Tetraacetate
FSG	Fasting Serum Glucose
FSI	Fasting Serum Insulin
FT3	Free Triiodothyronine
FT4	Free Tetraiodothyronine
GLIS3	GLI similar 3
GWAS	Genom-Wide Association Study
GOD	Glucose Oxidase
HPT	Hypothalamus - Pituitary gland-Thyroid gland
HOMA-IR	Homeostatic Model Assessment for Insulin Resistance
IR	Insulin Resistance
IYD	Iodotyrosine Deiodinase
LDL	Low Density Lipoprotein
L-T4	Levothyroxine

MCT8	Monocarboxylate8
MAP	Mean Arterial Pressure
PCR	Polymerase Chain Reaction
POD	Peroxidase
rT3	Reverse triodothyronine
SPSS	Statistical Package for the Social Sciences
SECIS	Seleno-cysteine Insertion Element
SeCys	Selenocysteine
SNP	Single Nucleotide Polymorphism
T4	Tetra-iodothyronine
T3	Triiodothyronine
THs	Thyroid Hormones
TSH	Thyroid stimulating Hormone
TRH	Thyroid Releasing Hormone
TBG	Thyroid Binding Globulin
TTR	Transthyretin
TSHR	Thyroid Stimulating Hormone Receptor
TR	Thyroid Receptor
TPO	Thyroperoxide
TG	Thyroglobuline
TD	Thyroid Disorder
T2	Diiodothyronine
TBE	Tris-borate-EDTA
3-UTR	3- Untraslated Region

Abstract

Background: Different factors influence obtaining the target of treatment with levothyroxine (L-T4) replacement therapy, therefore, many patients remain have persistent symptoms of hypothyroidism even when they are taking this treatment. Deiodinase type-3 enzyme converts thyroxine (T4) to reverse triiodothyronine (rT3) and triiodothyronine (T3) to diiodothyronine (T2). Consequently it plays important role in regulating thyroid hormones homeostasis.

Aim: This study investigates the effects of rs945006; T>G and rs1190716; C>T single nucleotide polymorphisms (SNPs) in the deiodinase type-3 gene on the therapeutic response to L-T4 in a sample of Iraqi hypothyroidism female patients living in Kerbalaa province.

Methodology: Two hundred twenty hypothyroid female patients aged 40 years or older who were on L-T4 treatment for at least 4 months were enrolled in this cross-sectional study. Thyroid hormones (T4, T3, rT3 and T2) and thyroid stimulating hormone were assessed. The genetic analysis to detect rs945006; T>G SNP and rs1190716; C>T SNP was done using the tetra primers amplification refractory mutation system- polymerase chain reaction technique.

Results: Genotypes distribution of rs945006; T>G SNP was 25 (11.4%) for the wild type (TT), 50 (22.7%) for the heterozygous mutant type (TG), and 145 (65.9%) for the homozygous mutant type (GG). The patients were divided into three groups according to their genotypes. Non significant differences was found in the serum thyroid hormones levels among the groups of the three genotypes carriers. The genotypes distribution of rs1190716; C>T SNP was 10 (4.5%) for the wild type (CC), 50 (22.7%) for the heterozygous mutant type (TC), and 160 (72.7%) for the

homozygous mutant type (TT). Significant differences ($P \leq 0.05$) were found in the levels of T4, T3 and T2 among the groups of the patients ($P=0.019$, $P=0.039$, $P= 0.032$, respectively)

Conclusion: Since the rs945006 SNP is not associated with the serum thyroid hormones levels, it might not affect the response to L-T4 treatment. The rs1190716; C>T SNP could affect the activity of the deiodinase type-3 enzyme and the metabolic homeostasis of the thyroid hormones, therefore rs1190716; C>T SNP could have an impact in the therapeutic response to L-T4 in Iraqi female patients with primary hypothyroidism. This is a novel finding, hence further studies are needed to confirm it.

Chapter one

Introduction

1. Introduction

1.1 Thyroid Gland

An endocrine gland is located at the front of the neck. The thyroid gland secreted hormones that are important in regulating development, growth, and metabolism. These hormones are thyroxin (T4) and triiodothyronine (T3) [1]. T4 is the most common hormone generated by the thyroid gland generating very little T3 [2,3]. Only around 20% of T3 in peripheral tissue comes from the thyroid gland, while the remainder comes through enzymatic T4 to T3 conversion in the target tissues [4].

Tyrosine amino acids from follicular thyroglobulin, iodine molecules from the diet, thyroperoxidase enzyme in the apical surface of follicular cells, and hormonal stimulation represented by thyroid-stimulating hormone (TSH) from the anterior pituitary glands were required for the synthesis of thyroid hormones by follicular cells [5]. Thyroid hormones (THs) are lipophilic and cause effects by both nuclear and membrane receptors [6].

Thyroid hormone production is 85 g/day for T4 and 6.5 g/day for T3. T4 deiodination, primarily via deiodinases, produces the majority of the estimated total amount of 30 g T3/day which is produced outside the thyroid parenchyma [6]. Thyroid hormones are transported in the blood by transport proteins, for T4 about 75% is bound to thyroxin binding globulin (TBG), 20% to transthyretin (TTR, pre albumin), and 5% to serum albumin. Apolipoprotein B and apolipoprotein A1-containing lipoproteins, contribute to TH transport with 3% for T4 and 6% for T3 [7].

The hypothalamic-pituitary-thyroid (HPT) axis controls thyroid hormone production by the thyroid gland [8]. Thyroid hormone is secreted in response to TSH, which is produced and released from the pituitary

gland and mediates its impact via binding to the TSH receptor (TSHR). Hypothalamic thyrotropin-releasing hormone (TRH), in turn, stimulates TSH production. Thyroid hormones can inhibit the production of TRH and TSH, by a mechanism known as negative feedback control [9].

Age and gender have an impact on THS levels; in men, free TH but not TSH concentrations drop with age, but in women, free TH levels remain constant but TSH levels rise with age [10].

1.2 Thyroid Disorders.

Thyroid disorders (TDs) are prevalent illnesses that can have serious health repercussions for people all over the world. Both hypothyroidism and hyperthyroidism have overt, clinically exhibited manifestations that are caused by thyroid gland hormone serum abnormalities for free thyroxine (FT4) and free triiodothyronine (FT3), while the subclinical forms of TDs show normal serum levels of free thyroid hormones with inverse feedback changes in thyroid-stimulating hormone (TSH) levels [11,12].

Hypothyroidism is a condition characterized by a decrease in thyroid hormone production (or activity). Its clinical manifestations range from modestly raised thyrotropin (TSH) levels in asymptomatic persons to severe hypothyroidism, which can lead to myxedema coma on rare occasions [13].

When there is low thyroid gland activity, it is classified as primary hypothyroidism while secondary hypothyroidism is caused by low levels of TSH secretion by the anterior pituitary, and tertiary hypothyroidism is caused by low levels of TRH secretion by the hypothalamus [14].

Peripheral hypothyroidism, which comprises the thyroid hormone resistance syndrome due to thyroid hormone transport and metabolism abnormalities, is a significantly less prevalent cause [15]. Over 99% of hypothyroid cases are primary hypothyroidism due to inadequate function of the thyroid gland [16, 17].

Hypothyroidism is associated with poor quality of life, most likely related to symptoms such as changes in body weight, fatigue, weakness, and depression [18,19]. Hypothyroidism is also associated with coronary artery disease [20], contributes to infertility, and can cause reversible dementia, as well as neurosensory, musculoskeletal, and gastrointestinal symptoms [21].

1.2.1 Epidemiology of Hypothyroidism.

Thyroid defects were reported worldwide in over 110 countries with about 1.6 billion people at risk as they are from iodine-deficient areas. These areas are mostly developing countries like Asia, Africa, and Latin America [22]. Earlier studies reported that the prevalence of thyroid diseases throughout the world is 25% in women and 0.6% in men [23].

In the general population, the prevalence of overt hypothyroidism varies between0.3% and 3.7% in the USA and between 0.2% and 5.3% in Europe [24,25]. The prevalence of hypothyroidism increases with age. It is 10 times more common in women than in men, predominantly after the age of 40 years [26,27]. According to the National Institute of Health, approximately 4.6% of the United States population aged 12 years and older has hypothyroidism, but most of these cases are mild. In the United States and other areas of adequate iodine intake, autoimmune thyroid disease (Hashimoto’s disease) is the most common form of

hypothyroidism [28]. Environmental iodine deficiency remains the primary cause of thyroid disorders [29].

In Iraq, the female to male ratio diagnosed with hypothyroidism was 1.6:1[30], and about 3.2% were overt hypothyroid (22.4% males and 77.6% females), moreover, 14.1% were subclinical hypothyroid cases (19.7% males and 80.3% females) [31]. Other study in Kirkuk province established that 22.7% of population have hypothyroidism [32], while 4% of female had hypothyroidism in Erbil [33].

1.2.2 Pathophysiology of Hypothyroidism

There are many different causes of thyroid hypofunction. Congenital defects can fail the thyroid gland to develop normally (dysgenesis) or failure of a structurally normal thyroid gland to produce normal quantities of thyroid hormone (dyshormonogenesis) [34]. Thyroid dysgenesis—which encompasses the spectrum of thyroid agenesis, hypoplasia, and ectopy—is the most common cause of congenital hypothyroidism. Thyroid dysgenesis usually occurs sporadically, with only 2–5% of cases being attributable to identifiable genetic mutations. The thyroid-stimulating hormone receptor (TSHR) and the transcription factors PAX8, NKX2–1, and FOXE1 are all expressed in the developing thyroid. Disruption of any of these genes can lead to failure ofnormal thyroid gland formation [35]. Mutations in GLIS3 underlie a complex syndrome of congenital hypothyroidism. GLIS3 is highly expressed in the thyroid, and GLIS3 mutations may be associated with either thyroid dysgenesis or an ectopic but histologically abnormal thyroid gland [36]. GLIS3 may act as a transcriptional activator or repressor, but its precise role in thyroid development and function remains to be determined. GLIS3 has an additional effect on the central regulation of the hypothalamic-pituitary-thyroid (HPT) axis [37].

Known genetic causes of dyshormonogenesis include mutations in thyroglobulin (TG), thyroperoxidase (TPO), dual oxidase 2 (DUOX2), the sodium-iodide symporter (SLC5A5), pendrin (SLC26A4), and iodotyrosine deiodinase (IYD) [38].

Any thyroid hormone pathway gene mutation may affect TH production and secretion [39].

The thyroid gland may be destroyed by a disruption in blood flow, infection, inflammation, autoimmune responses [40], or neoplastic growth. Thyroiditis is generally caused by an attack on the thyroid resulting in inflammation and damage to thyroid cells due to the malfunction of the immune system. In chronic autoimmune thyroiditis, around 95% of people had high anti-TPO antibody levels antithyroglobulin antibodies are present in 60% of patients. The viral and bacterial infection works similarly to antibodies to cause inflammation in the gland [41].

Aging may cause a reduction in function, or the gland may atrophy due to medication therapy or other unidentified causes. Some thyroid gland dysfunction conditions are linked to receptor abnormalities, including hormone receptor absence, poor hormone binding to receptors, and decreased cellular reactivity to hormones. It is believed that in some circumstances, a gland may create a physiologically inert hormone or that circulating antibodies may destroy an active hormone before it can function [42].

Inadequate dietary intake of iodine is affected hormone synthesis and secretion also exposure of the gland to excess iodide suppresses hormone formation and secretion and reduces the capacity of the gland to accumulate more iodide. This affects several thyroid follicular cell

metabolism levels, such as iodide transport and organification, adenylyl cyclase activity, proteolysis, and hormone release [43].

1.2.3 Clinical Presentation of Hypothyroidism

Hypothyroidism affects various organ systems in the body, including the central and peripheral nervous systems, the cardiovascular, integumentary, gastrointestinal, respiratory, muscular, hematopoietic systems, and electrolyte metabolism. Its manifestations are mostly independent of the underlying condition but are functionally related to the amount of hormone deficiency [1].

For individuals with longer-term unrecognized and untreated hypothyroidism, signs and symptoms are more pervasive and involve multiple body symptoms, in many cases, however, signs and symptoms of primary hypothyroidism are often insidious and subtle. They may include

- Cold intolerance
- Constipation, [44].
- Forgetfulness, and depressed mood.
- Modest weight gain is usually the result of decreased metabolism and fluid retention [45].
- Paresthesias of the hands and feet are common, often because of carpal-tarsal tunnel syndrome, which is caused by the deposition of proteinaceous ground substance in the ligaments surrounding the wrists and ankles [46].
- Females with hypothyroidism may develop menorrhagia or secondary amenorrhea.

- The patient's facial expression will be dull, the voice will sound hoarse, and the speech will slow.
- Facial puffiness and periorbital swelling occur because of infiltration with mucopolysaccharides known as hyaluronic acid and chondroitin sulfate [47].
- The eyelids droop due to decreased adrenergic drive.
- The hair is coarse, dry, and sparse.
- The skin is coarse, dry, scaly, and thickened.
- The relaxation phase of the deep tendon reflexes becomes slowed, and hypothermia is common [48].
- Dementia or frank psychosis (myxedema madness) may develop [49].

Patients with hypothyroidism may present with myxedema caused by diminished clearance of complex glycosaminoglycans and hyaluronic acids from the reticular layer of the dermis [50].

Symptoms more accurately predict overt hypothyroidism in men than in women [51], and in younger than older people, particularly in younger men compared with older women [52].

1.2.4 Diagnosis of Hypothyroidism

Primary hypothyroidism is characterized by an elevation in TSH concentration over the reference range (0.4-4.0 mlU/L) and free thyroxin (FT4) concentration under the reference range which is different according to the type of used assay as well as patient age, sex, and ethnic origin [53]. The higher limit of TSH reference ranges increases with age in adults and age-dependent reference ranges gave variant results in younger individuals in studies from the UK and Australia [54,55].

TSH measurement is the cornerstone in the diagnosis of thyroid dysfunction [56]. Measuring TSH and T4 concentrations in the blood is the most efficient way to diagnose hypothyroidism. Certain drugs that may interfere with serum T4, birth control pills, clofibrate, and estrogen are known to increase T4; lithium, propranolol, and androgens are known to decrease T4 [57].

Autoimmune primary hypothyroidism in which autoantibodies are produced against TPO and Tg; therefore, it is diagnosed by additional tests that identify the presence of these autoantibodies. The normal range for TPO autoantibodies is between 42 and 100 IU/mL, whereas the normal range for Tg autoantibodies is between 67 and 115 IU/mL. Values greater than these are considered positive [58].

For patients who are hospitalized for a nonthyroidal illness that occurs in a different disease state, serum FT4 is the key test for the diagnosis of suspected hypothyroidism in these patients [59].

The diagnosis of hypothyroidism is based on repeated biochemical findings [60, 61, 62].

Serum total thyroid hormone (TT4, TT3) measurement can be recommended no longer as the initial screening test of thyroid function because many conditions affect serum TT4 andTT3 concentration [63].

1.2.5 Complications of Hypothyroidism

Hypothyroidism causes a systemic drop in metabolism, which can lead to a decrease in cardiac output due to reduced contractility and a lower heart rate [64]. Thyroid hormones have genetic effects on the heart, including regulating the expression of certain enzymes involved in myocardial contractility and relaxation [65]. In hypothyroid patients without any other underlying cardiac illness, however, signs and symptoms of heart

failure are frequently absent but when hypothyroidism develops in patients with cardiac disease, it might aggravate heart failure or angina pectoris. Pericardial effusion, diastolic hypertension associated with increased peripheral vascular resistance, hypercholesterolemia, and hyperhomocysteinemia are further changes that can occur with hypothyroidism and contribute to cardiovascular disease [66, 67, 68].

Thyroid hormones govern the activities of cholesterol ester transfer protein (CETP), liver lipase, and lipoprotein lipase, all of which affect cholesterol metabolism. The flow of bile acids and the quantity and activity of LDL receptors in the liver are all controlled by these hormones [69]. As a result, dyslipidemia is a prevalent metabolic side effect of hypothyroidism. Also with hypothyroidism, reduced lipoprotein lipase activity is likely to be the main cause of hypertriglyceridemia [70]. Using statins for the treatment of hypercholesterolemia may be associated with myopathy, which is exacerbated if coexists with undiagnosed hypothyroidism [71]. As a result, it's best to rule out hypothyroidism in all individuals with dyslipidemia before initiating statin therapy [72].

Hypothyroidism has a variety of consequences on the hematopoiesis, blood cells, and coagulation processes [73]. Because erythropoietin production is reduced in hypothyroidism, normocytic anemia is prevalent, and pernicious anemia affects roughly 10% of autoimmune thyroiditis patients, whereas iron deficiency anemia is caused by the menstrual cycle irregularities (polymenorrhagia and hypermenorrhea) or malabsorption [74].

Myxedema coma is a life-threatening condition caused by severe deficiency of thyroid hormone resulting from chronic undiagnosed or untreated hypothyroidism. It may also be caused by infection, trauma, or surgery. A patient with a coma or altered mental status who is also

hypothermic, hyponatremic, and/or hypercapnia should be suspected to have a myxedema coma [75].

Thyroid gland dysfunction causes numerous neurological problems, including anxiety, depression, and cognitive impairment [76]. In dementia patients, a positive relationship with thyroid gland dysfunction has been observed [77, 78]. Several studies have identified that hypothyroidism can change the neuronal function that is involved in cognition processes [79,80]. The study suggested that the central brain region of cognitive performance is the hippocampus [81, 82], hypothyroidism increased inflammatory response in hippocampal brain regions and encourages spatial memory loss [83, 84].

1.2.6 Treatment of Hypothyroidism (levothyroxine)

Levothyroxine is thyroid hormone replacement therapy, it is the exogenous form of T4, and is being the ‘‘gold standard’’ for the treatment of primary hypothyroidism for more than 60 years [85,86]. Levothyroxine is one of the most commonly given drugs in the world, and it is one of the top two in the United States [87]. The World Health Organization considers it an important drug for basic health care [88]. The synthetic hormone has almost completely replaced earlier preparations derived from desiccated thyroid extracts (DTE) of bovine or porcine origin [89, 90].

Generally, the goal treatment can be defined as the substitution of hormonal deficit, aiming to return to the prior euthyroid state [91].

Levothyroxine is available as tablets and soft-gel caps, intravenously, and, more recently, in liquid formulations [92].

1.2.6.1 Initial Dosing of levothyroxine

Levothyroxine has a narrow therapeutic index, therefore any differences in dose or blood concentration may fail treatment or cause adverse drug reactions [93] .

Levothyroxine's beginning dose is influenced by the following factors:

- Patient age,
- Lean body mass of the patient
- The presence of co-existing cardiac disease
- The etiology and the severity of the patient's biochemical hypothyroidism[94].

To calculate dose requirements different formulae have been proposed. The simplest formulae are based on body weight or body mass index (BMI) while the more complex formulae used other factors such as patient sex [95,96]. Levothyroxine dosage is increased gradually until TSH levels fall within the acceptable range of 0.4 to 4.0 mIU/L. [97].

When a healthy adult patient is diagnosed with overt hypothyroidism at a young age (less than 50 years), they often take the full replacement dose of levothyroxine (1.6µg/kg/day) orally, while those between the ages of 50 and 60 and those with coronary artery disease receive a lower initial dose (25 to 50 µg once daily) [98]. While in a patient with subclinical hypothyroidism, dosages between 50 and 75µg may be sufficient to normalize the serum TSH.

Because the half-life of levothyroxine is long (1 week), it is necessary to measure TSH level after 4–6 weeks from initiation of therapy or dosage change. After that, patients with stable normal serum TSH levels should be monitored every 12 months[99].

1.2.6.2 Dose Adjustment

After ,some time if the disease proceeds or the patient develops other situation that influences the metabolism of the thyroid, the levothyroxine replacement dose may need an adjustment [26].

The most common cause of therapeutic failure is poor patient adherence [100]. Poor adherence to levothyroxine is generally associated with an increased TSH level. However, if the missed doses are not reported by the patient, a high dose of levothyroxine can lead to a decreasein TSH level when the drug has later adhered to. [101, 102].

Factors potentially contributing to the need for levothyroxine dose adjustments throughout a patient's life span, include; pregnancy, hormonal change, and aging [103,104,105]. These adjustments need to be handled with caution and take into account the many contributing factors, as multiple levothyroxine dose adjustments result in a greater burden on healthcare resources [106].

1.2.6.3 Pharmacokinetics of Levothyroxine

In the stomach oral LT4 (typically formulated as sodium salt) dissolves at low pH, but is mainly absorbed in the small intestine (the jejunum and ilium) during three hours of ingestion [107].

Optimal administration of LT4 is required to empty the stomach and abstinence from eating or drinking for thirty minutes after ingestion [108]. The bioavailability of LT4 in fasting euthyroid subjects and hypothyroid patients is 65-80%. [109].

The liquid formulations show the best absorption when ingested with food, and have been developed to get better adherence [110].

When the LT4crosses the intestinal epithelium and reaches the circulation, it is entirely (~99.9%) bound to plasma proteins mainly albumin, thyroxine-binding globulin (TBG), transthyretin, and high-density lipoprotein [111].

The mean distribution volume of LT4 is 11–12 L in euthyroid subjects and up to 15 L in hypothyroid patients due to fluid retention [112] Metabolism of T4 occurs in several organs such as the liver, kidney, glands, areas of the brain, and peripheral tissues (muscles) [113]. LT4 is a prodrug that is activated through metabolization by enzymatic 5'-deiodination into the biologically more active derivative T3, due to the actions of two types of iodothyronine deiodinases, type 1 and type 2, which are differentially expressed by various organs [114]

While the other deiodinase (type 3) catalyzes the inner ring deiodination of T4, inactivating it to reverse triiodothyronine (rT3), and is also responsible for T3 degrading to 3,3'-diiodothyronine (T2) [115]. After glucuronidation or sulphation in the enterohepatic cycle, T4 and T3 are excreted in the bile and this compromises 20% of ingested dose that is mainly eliminated in stools, and the remaining 80% is excreted in the urine [93].

However, the distribution, metabolism, and excretion of exogenous LT4 are different from those of endogenous T4, and the correlations between TSH, free T4 (FT4), and free T3 (FT3) levels in LT4-treated patients are not the same to those in healthy and euthyroid persons [116].

1.2.6.4 Factors Affect the Clinical Effectiveness of Levothyroxine

Many factors may be affected the adequacy of levothyroxine therapy in hypothyroid patients, including the factors that can interfere with absorption, distribution, metabolism, and excretion (ADME) [117], these factors can be grouped into three broad categories;

- Pharmaceutical factors; pharmaceutical formulation, administration route, dosing regimen [118,119], concomitant administration of another thyroid hormone likeLT4pluse LT3 combination therapy.
- Pathophysiological factors; thyroid disorder (type, degree, and progressing) and etiology (auto-immune disease, thyroid surgery, radioiodine treatment), comorbidities [120,121], age, sex, body mass index, pregnancy, genetic variants, malabsorption, and changes in the underlying residual thyroid function.
- Behavioral; concomitant intake of (medication, foodstuffs, and food supplements) [122,123], poor compliance pseudo malabsorption, poor quality of life.

1.3 Iodothyronine Deiodinase Enzymes

Iodothyronine deiodinases (DIOs) are small, highly homologous, integral membrane enzymes that modify thyroid hormone signaling [124]. Three enzymes act as deiodinases (DIO1, DIO2, and DIO3), they are dimeric integral membrane proteins of about 60 kDa, with a transmembrane domain involving the first 30 to 40 amino acids as shown in figure (1-2)

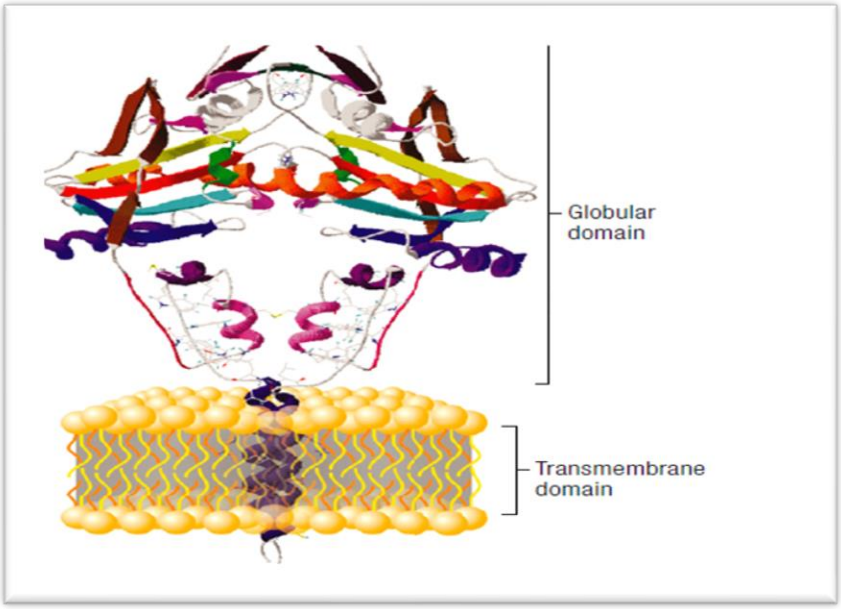

Figure (1-2) Molecular structure of deiodinase enzyme [125].

Iodothyronine deiodinases characterized by the modified amino acid selenocysteine (SeCys) in the active center, which confers catalytic activity, at physiological pH, SeCys is ionized and acts as a very efficient electron donor, making the deiodination reaction possible [126]. The catalytic center contains a highly conservative core sequence that is important for dimerization, which is mediated by disulfide bonds [127]. The three enzymes have different subcellular locations ; DIO2 is located at the endoplasmic reticulum, which is close to the nucleus, while DIO1 and DIO3 are found at the plasma membrane [128]. DIO1 and DIO2 catalytic globular domains face the cytoplasm, while the catalytic domain of DIO3 molecules protrudes primarily into the extracellular space [129].

Given the topology of all three deiodinases, thyroid hormones need to enter the cell to be metabolized by these enzymes, cell entry is mediated by membrane transport proteins, monocarboxylate 8 (MCT8) being the most relevant one [130]. Once inside the cell, the concentration of thyroid

hormone is tightly controlled by iodothyronine deiodinase enzymes (DIO1, DIO2, and DIO3), which catalyze the removal of iodine atoms at the phenolic ring (activation pathway) or the tyrosyl ring (inactivation pathway) of T4 and T3 [131].

1.3.1 Iodothyronine Deiodinase Type -3

Iodothyronine deiodinase type-3 (DIO3), an essential inner-ring deiodinase with a 12-hour half-life, is crucial for the inactivation of the synthesis of circulating TH [132].

Iodothyronine deiodinase type-3 the physiological terminator of thyroid hormone activity, inactivates both T3 and T4 through tyrosyl ring deiodination, DIO3 catalyzes the physiologically inert conversion of T4 to rT3 and T3 to 3,3' diiodothyronine (T2) [133],and is the predominant deiodinase in developing tissues and throughout the embryonic stage which shields those tissues from excessive TH synthesis. The central nervous system [134] and skin [135] are the two adult tissues with the highest levels of DIO3 expression, while the majority of other tissues exhibit very little or no DIO3 expression under physiological conditions. Furthermore, the high levels of DIO3 expression in the placenta and pregnant uterus imply that this enzyme is crucial to the development process [136]. Only instances of TH overloading seen in a few serious disorders linked to hypoxic/ischemic circumstances have been established the protective role of this enzyme in maturity [137].Through endosome vesicles, DIO3 is quickly internalized into cells, and it is then recycled back to the cell surface [138]. The immunohistochemical studies suggest that DIO3-catalyzed process may take place inside the endosome vesicles where thyroid hormones are actively trafficked [139].

Expression and activity of DIO2 and DIO3 offer an inverse reciprocal relationship during hypothyroidism or hyperthyroidism [125]. Whereas DIO2 is negatively regulated by TH, the opposite is observed for DIO3.

1.3.2 Deiodinase Type 3 Gene and the Impact of its Polymorphisms

Although just a few genes have been identified to affect the thyroid function, it is now known that genes involved in thyroid hormone action can have clinically noticeable effects without affecting the levels of circulating thyroid hormones [140]. Since numerous factors can influence the final binding of T3 to the TR in the cell nucleus, circulating (measurable) quantities of thyroid hormones may not be an accurate representation of the levels in specific tissues. This might be especially important for organs like the brain, which have mechanisms in place to shield local tissue from fluctuations in circulating levels [141].

As previously established, the deiodinase type-3 enzyme catalyzes deiodination on the inner rings of T3 and T4 to create T2 and rT3, respectively. This enzyme is the physiological inactivator of TH.

The DIO3 gene in humans located on chromosome 14q32, encodes the selenoenzyme DIO3 [142]. The DIO3 genomic structure contains a single exon and a unique RNA structure known as SECIS (selenocysteine insertion element), which is essential for the insertion of the selenocysteine residue and for maximizing enzymatic catalytic effectiveness and is present in the 3′-UTR of the DIO3 gene [143]. This gene belongs to a cluster of imprinted regions, at the delta like non-canonical Notch ligand 1 (DLK1)-DIO3 locus. The paternal allele preferentially expresses itself in the imprinted DIO3 gene [144].

In mice, DIO3 is critical for the maturation and function of the hypothalamus-pituitary-thyroid (HPT) axis, as mice lacking DIO3 activity develop perinatal thyrotoxicosis followed by mild central hypothyroidism in adulthood [145].

Genetic variation in DIO3 was not associated with TSH and/or TH levels in humans in candidate gene studies [146,147,148] or previous Genome-wide association studies (GWAS) [149–150].

Few previous studies on DIO3 was performed on European populations [151,152,153] indicated that there was no association between this gene and the TH levels. There is no genetic study in the Middle East searches the role of this gene on the thyroid gland function or TH levels.

This is the first study in Iraq and Middle East area investigates the impact of this gene on the TH levels. Some SNPs could be candidate to study the role of DIO3, rs945006 SNP is located in the 3′ untranslated regions (3′-UTR) of the mRNA, where it may exert a posttranscriptional influence on the gene expression through interaction with microRNAs and various translational regulatory proteins. Studies have established an association between several 3′-UTR SNPs and neurodegenerative disorders [154, 155]. Another study suggests that 3′-UTR SNPs can affect miRNA activity and consequently DIO3 protein expression [156]. Also, a GWAS identified an association between rs945006 and osteoarthritis.

Another genetic variant is rs1190716, it is located in the intron of the DIO3 opposite strand (DIO3OS) gene. The DIO3OS gene is a potential regulator of DIO3 expression, it is present at the DIO3 locus and encodes a spliced non-coding RNA that transcripts in the opposite direction. The genomic structure of this gene contains at least 6 exons. DIO3 and DIO3OS are overlapping genes transcribed in opposite directions [157],

Kester *et al.* demonstrated that DIO3 and DIO3OS are co-expressed in various human cell lines [158]. Whereas DIO3 is an imprinted gene, DIO3OS may be involved in maintaining the monoallelic expression of DIO3 [159, 160], which could affect the TH levels. Two other variants rs12323871; T>C and rs11626434; C>G within the DIO3OS gene have been associated with FT4 levels in the most recent GWAS on the thyroid function [161].

1.4 Aims of the Study

1. To detect the presence and the distribution of the genetic single nucleotide polymorphisms (rs945006 and rs1190716) of DIO3 in Iraqi female patients with primary hypothyroidism.
2. To investigate the impact of the DIO3 rs945006 and rs1190716 single nucleotide polymorphisms on the therapeutic response to levothyroxine.

Chapter two

Patients, Materials, and Methods

2. Patients, Materials, and Methods

2.1. Patients

This study was performed from November 2021 to July 2022. A total of 220 female patients were enrolled in this cross-sectional study when they visit a private clinic to get medication and advice about their cases. All the patients were already diagnosed with primary hypothyroidism (guideline of the diagnosis and management of primary hypothyroidism was American Thyroid Association (ATA)). The patients were receiving levothyroxine for at least 4 months.

2.1.1. Patient Criteria

A. Inclusion Criteria

The included patients were female patients with primary hypothyroidism who were aged 40 years or older and were receiving levothyroxine for at least 4 months and were receiving no medications that could interact with levothyroxine like proton pump inhibitors, cholestyramine, cimetidine, orlistat, sucralfate, or antacids.

B. Exclusion Criteria

1- Patients whose treatment period is less than 4 months.
2- The patient whom their age is less than 40 years.
3- Patients received any medication that interacts with levothyroxine or medications that affect DIO expression or activity.
4- Patients with any systemic disease.
5- The patient underwent a thyroidectomy.

2.1.2. Ethical and Scientific Approval

- The Scientific and Ethical Committee at the College of Pharmacy-at University of Kerbala discussed and approved the proposal of this 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 they were requested to fill out a specially designed questionnaire.

2.1.3. The Study Design

Two hundred twenty Iraqi female patients with primary hypothyroidism were included in this cross-sectional study. A blood sample was taken from overnight fasted participants who are already taken levothyroxine for the biochemical, hormonal, and genetic investigations.

2.2. Materials

2.2.1. Instruments and Equipment and their Suppliers.

All instruments used in this study accompanied by their manufacturing company in Table 2-1

Table 2-1 Instruments and the manufacturing companies

Equipment	Company	Country
Cobas e 411	Roch	Germany
Centrifuge	Hettich	Germany
Digital camera	Canon	England
Distillatory	GEL	Germany
Hood	Lab tech	Korea
Incubator	Binder	Germany

Micropipettes	Slammed	Japan
Nano pac 500 power supplier for electrophoresis	Cleaver	UK
PCR machine	verity	United state
Sensitive balance	AND	Taiwan
UV-trans illuminator	Syngene	England
Vortex mixer	Human twist	Germany

2.2.2. Chemicals, Kits, and their Suppliers.

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

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

Kits Type	Chemicals and kits	Company	Country
Chemical	Agarose	Bio basic	Canada
	Ethanol	SDI	Iraq
	Ethidium bromide	Intron	Korea
	Nuclease free water	Bioneer	Korea
	TBE buffer	Bioneer	Korea
Biochemical kits	Fasting serum glucose kit	Mindary	China
Hormonal kits	FT3 kit	Snibe Dignostic	China
	FT4 kit	Snibe Dignostic	China

	Insulin kit	Mindary	China
	rT3 kit	Snibe Dignostic	China
	T2 kit	Snibe Dignostic	China
	TSH kit	Snibe Dignostic	China
	TT3 kit	Snibe Dignostic	China
	TT4 kit	Snibe Dignostic	China
Kits of the genetic investigation	DNA extraction kit	Favogen	Taiwan
	DNA ladder marker (100bp)	Bioneer	Korea
	Pcr pre Mix kit	Bioneer	Korea
	Primers for detection of SNP	Alpha DNA	Canada

2.3 Methods

2.3.1 Sample collections

After overnight fasting, and by vein puncture, blood samples 5ml were collected from patients, these samples were divided into two parts, the first part 2ml was kept in an EDTA tube for DNA extraction and the second part 3ml was kept in a gel tube for sera extraction which were used to assess thyroid function tests and the other biochemical tests.

2.3.2 Assay of the Biochemical Parameters

2.3.2.1 Thyroid Function Tests.

2.3.2.1.1Estimation of Thyroid Stimulating Hormone

Quantitative determination of TSH in human serum was done by chemiluminescence immunoassay. This assay is a sandwich chemiluminescence immunoassay. The sample, aminobutyl-ethyl-isoluminol (ABEI) labeled with an anti-TSH monoclonal antibody, and magnetic microbeads coated with another anti-TSH monoclonal antibody are mixed thoroughly and incubated at 37 ^{0}C, forming a sandwich of the immune complex. After precipitation in a magnetic field, the supernatant is decanted and then a wash cycle is performed.

Subsequently, the starter1 + 2 are added to initiate a chemiluminescent reaction. The light signal is measured by a photomultiplier within 3 seconds as a relative light unit which is proportional to the concentration of TSH present in the sample.

2.3.2.1.2Estimation of Total Thyroxin (TT4)

Chemiluminescence immunoassay was used for the quantitative determination of T4 in human serum. The T4 assay is a competitive Chemiluminescence immunoassay. The sample, displacing solution, ABEI –labeled anti-T4 monoclonal antibody, buffer, and T4 antigen-coated magnetic microbeads are mixed thoroughly and incubated at 37c. T4 present in the serum sample competes with T4 antigen immobilized on the magnetic microbeads for a limited number of binding sites on the ABEI – labeled anti-T4 antibody, forming an immune complex. After precipitation in a magnetic field, the supernatant is decanted and then a wash cycle is performed.

Subsequently, the starter 1+2 are added to initiate a chemiluminescent reaction. The light signal is measured by a photomultiplier within 3 seconds as a relative light unit, which is inversely proportional to the concentration of T4 present in the sample.

2.3.2.1.3 Estimation of Free Thyroxine (FT4)

Chemiluminescence immunoassay was used for the quantitative determination of FT4 in human serum .The principle is the same of that mentioned in estimation of TT4.

2.3.2.1.4 Estimation of Total Triiodothyronine (TT3)

Chemiluminescence immunoassay was used for the quantitative determination of T3 in human serum. The principle is the same of that mentioned in estimation of TT4.

2.3.2.1.5Estimation of Free Triiodothyronine (FT3)

Chemiluminescence immunoassay used for the quantitative determination of Free Triiodothyronine (FT3) in human serum. The principle is the same of that mentioned in estimation of TT4.

2.3.2.1.6Estimation of Reverse Triiodothyronine (rT3)

Human rT3 was estimated using an enzyme-linked immunosorbent assay (ELISA) kit, which is a competitive assay method.

Assay Principle

Samples were added to the pre-coated plate. Then biotinylated antigen was added. The antigens in the samples compete with the biotinylated antigen to bind to the capture antibody and incubate. The unbound antigen was washed away during a washing step. An avidin-HRP was then added and then incubate. Unbound avidin-HRP was washed away during a washing step. TMB substrate is then added and color develops. The reaction is stopped by the addition of acidic stop solution and the color changes into yellow which can be measured at 450 nm. The intensity of the color developed is inversely proportional to the concentration of rT3 in the sample. The concentration of rT3 in the sample is then determined by comparing the optical density (O.D. value) of the samples to the standard curve.

2.3.2.1.7Estimation of Diiodothyronine (T2)

The assay was done is the same of that used to estimate rT3.

2.3.2.2 Determination of Glycemic Indices

2.3.2.2.1Estimation of Fasting Serum Glucose Level

By the catalysis of glucose oxidase (GOD), glucose is oxidated to yield H_2O_2, and then in the presence of peroxidase (POD), H_2O_2 oxidates 4-Aminoantipyrine (4-AAP) with p-hydroxybenzoic acid sodium to form a colored dye of quinonimine. The absorbency increase is directly proportional to the concentration of glucose.

$$\text{D-Glucose} + O_2 \xrightleftharpoons{\text{GOD}} \text{D-Gluconic acid} + H_2O_2$$

$$2H_2O_2 + \text{4-AAP} + \text{p-Hydroxy benzoic acid sodium} + H_3O^+ \xrightleftharpoons{\text{POD}} \text{Quinoneimine} + 5H_2O$$

2.3.2.2.2 Estimation of Fasting Serum Insulin Level

The chemiluminescence-series insulin assay is a two-site immunoenzymatically assay that can be used to assess insulin levels.

In the first step, paramagnetic microparticles coated with monoclonal anti-insulin antibody (mouse) and monoclonal anti-insulin antibody -alkaline phosphatase conjugate are added to the reaction cuvette. A sandwich complex is formed when insulin present in the sample binds to both anti-insulin antibody-coated microparticles and anti-insulin antibody alkaline phosphatase-labeled conjugate. Some unbound molecules are washed away when microparticles are magnetically captured.

In the second step, the substrate solution is added to the reaction cuvette. In the immunocomplex stored in the microparticles, the reaction is catalyzed by an anti-insulin antibody (mouse)-alkaline phosphatase conjugate. The photomultiplier built into the system measures the chemiluminescent reaction as relative light units. The relative light units produced during the reaction are proportional to the amount of insulin present in the sample.

2.3.2.2.3 Measurement of Homeostasis Model Assessment of Insulin Resistance.

Insulin resistance was assessed by using the homeostasis model assessment (HOMA IR), which is measured using the formula below from fasting serum insulin (FSI) and fasting serum glucose (FSG)[162].

HOMA-IR = [FSI (μU/ml) * FSG (mg/dl)]/405

2.3.3 Measurement of Body Mass Index

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

Weight / (Height) 2 =BMI

Normal weight is defined as a BMI of 18.5-24.9, overweight is defined as a BMI of 25-30, and obesity is defined as a BMI of greater than 30 [163].

2.3.4. Genetic Analysis.

2.3.4.1 Extraction of genomic DNA from a blood sample

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

Principle of extraction

Step 1-Sample Preparation

1. Up to 200µl blood was transferred to a 1.5ml microcentrifuge tube.

If the sample volume is less than 200µl, add the appropriate volume of PBS.

2. Proteinase K (30µl) was added to the sample and briefly mixed, then incubated for 15 minutes at 60ºC.

Step 2 –Cell Lysis

3. FABG Buffer (200µl) was added to the sample and mixed by the vortex.

4. The mixture was incubated in a 70ºC water bath for 15 minutes to lyse the sample.

During incubation, the sample was inverted every 3 minutes.

5. The required elution buffer (for Step 5 DNA elution) was preheated in a 70ºC water bath.

Step 3 – Binding

6. Ethanol (96~100%) with volume 200µl was added to the sample and vortexed for 10 seconds.

(Pipetting if there is any precipitate.)

7. A FABG column was placed in a 2ml collection tube. The sample mixture (including any precipitate) was transferred carefully to FABG column, centrifuged for 5 minutes at full speed (14,000 rpm or 10,000 x g) and the 2 ml collection tube was discarded. The FABG column was placed in a new 2ml collection tube.

Step 4 – Washing

8. FABG column was washed with 400µl W1 Buffer. Then was centrifuged for 1 min at full speed (14,000 rpm or 10,000 x g) and the flow-through was discarded

9. The FABG column was placed back in the 2ml Collection tube. The FABG column was washed with 600µl wash buffer (ethanol added), then was centrifuged for 1 min at full speed (14,000 rpm or 10,000 x g) and the flow-through was discarded

-it must be ensured that ethanol has been added into Wash Buffer when first opened.

10. The FABG column was placed back in the 2ml collection tube. Then was centrifuged for an additional 3 min at full speed (14,000 rpm or 10,000 x g) to dry the column.

Step 5 – Elution

11. The dry FABG column was replaced in a new 1.5ml microcentrifuge tube.

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

13. The FAGB column was incubated at 37ºC for 10 minutes in an incubator.

14. It was centrifuged for 1 minute at full speed (14,000 rpm or 10,000 x g) to elute the DNA.

Step Final - Pure DNA

15. The DNA was stored at -20ºC.

2.3.4.2 Amplification Refractory Mutation System Polymerase Chain Reaction (ARMS-PCR)

Primer Preparation

The primers were designed by Prof. Dr. Ahmed Abdul Jabbar Suleiman, and purchased as lyophilized products in various picomole concentrations from DNA Alpha, Canada. They were dissolved in specific amounts of nuclease-free water to prepare a stock solution with a concentration of 100 pmol/l. A diluted work solution was made by mixing 10 microliters of each stock solution of outer forward and outer reverse primer with 90 microliters of nuclease-free water and for inner forward and inner reverse primers by mixing 5 microliters of stock solution with 95 microliters of nuclease-free water. This work solution was stored at -20°C before it was required. The primers used to amplify the DIO3 gene

polymorphism rs945006; T>G and rs1119716; C>T SNPs are shown in Tables 2 – 3 and Table 2-4, respectively.

Table 2-3: Primers sequences for detected rs945006; T>G SNP

Primer	Sequence	Product size (bp)
Inner forward (IF)	TCCCTGGTAGGGGAAGTGATGTTGG	For G Allele 186
Inner reverse (IR)	GCCCACCCCTCCCCATTCA	For T Allele 143
Outer forward (OF)	TGGGTTCCAGGAGACTCTCAGCTCA	Of two outer 285
Outer reverse (OR)	GAGCACCCTCCCCCTAAGGTTTA	

Table2-4 primers sequences for detection rs1119716; C>T SNP

Primer	Sequence	Product size (bp)
Inner forward (IF)	CCACCCAGACCATACTTGCT	For T Allele 170
Inner reverse (IR)	CGCATGTGCATGTGTTTG	For C Allele 121
Outer forward (OF)	ATCCCTGGGGCTAGAAAGAG	Of two outer 253
Outer reverse (OR)	TTTTCCCACAGTTAGTTTCAGAAA	

Optimization of Polymerase Chain Reactions

For the amplification refractory mutation system –polymerase chain reaction (ARMS-PCR) reaction, the best annealing temperature and the number of amplification cycles was determined after several trials of PCR reaction optimization.

Running the Polymerase Chain Reaction

The PCR reaction was done by mixing DNA solution with PCR component using an optimized PCR program as shown in Tables 2-5 and 2-6 respectively.

Table 2-5: PCR reaction mixture to amplify the two DNA fragments encompasses rs945006; T>G and rs1190716; C>T SNPs

Component	Volume (µl)
Inner forward primer	1
Inner reverse primer	1
Outer forward primer	1
Outer reverse primer	1
DNA template	5
Deionized water	11
Premix	5
Total volume	25

Table 2-6: PCR program to detect rs945006; T>G and rs1190716; C>T SNPs

Steps	Temperature/ ^{o}C	Time	Cycle
Denature template	95	4 min	1
Initial denaturation	95	40 sec	35
Annealing	64/60 *	30 sec	
Extension	72	30 sec	
Final extension	72	5 min	1

*: 64 ^{o}C for rs945006; T>G SNP and 60 for rs1190716; C>T SNP

2.3.4.3 Agarose Gel Electrophoresis

Agarose gel electrophoresis is a method of gel electrophoresis used in biochemistry, molecular biology, genetics, and clinical chemistry to separate a mixed population of macromolecules such as DNA, RNA, or proteins in a matrix of agarose [164]:

1. To prepare gel, 1.5g agarose powder was mixed with electrophoresis buffer (100 ml of 1xTBE buffer) to the desired concentration, and heated in a microwave oven to melt it. Ethidium bromide was added to the gel (final concentration 0.5 µg/ml) to facilitate visualization of DNA after electrophoresis.
2. After cooling the solution to about 60 C^{0}, it was poured into a casting tray containing a sample comb and allowed to solidify at room temperature.
3. After the gel has solidified, the comb was removed with taking care not to rip the bottom of the wells.
4. The gel which was still in a plastic tray, was inserted horizontally into the electrophoresis chamber and is covered with buffer.

5. PCR products were directly loaded into the wells of the solidified agarose. The electrophoresis apparatus's voltage was set to ensure an electrical field of 5v.cm-1 for the distance between the cathode and anode.
6. For band detection at the end of the sprint, an ultraviolet trans-illuminator set to 320-336 nm was used. A digital camera was used to photograph the gel.

2.4 Statistical Analysis

The statistical program for the social sciences (SPSS) version 22 (SPSS Inc, Chicago, USA) was used to analyze the data. To compare the means of the three groups of study subjects, a one-way analysis of variances (one-way ANOVA) was conducted. A post hoc test after the ANOVA test was performed to achieve multiple comparisons between the groups. The Chi-square from the goodness of fit test was used to test the distribution of alleles and genotypes according to Hardy–Weinberg equilibrium. A significant difference between the means was regarded at $P < 0.05$.

Chapter Three

Result

3. Results

3.1 The Demographic Characteristics of the Hypothyroid Patients

The demographic characteristic of 220 hypothyroid female patients is demonstrated in Table 3-1.

Table 3-1: Demographic characteristics of the hypothyroid patients

Parameters	Mean ±SD (N= 220)	Minimum	Maximum
Age (year)	49.15±9.11	40.00	74.00
BMI (Kg/m^2)	30.98±5.83	17.78	55.63
Duration of the treatment (years)	4.47±4.03	0.33	21.00

N: Numbers of the study subjects, SD: Standard deviation, BMI: Body mass index.

3.2 The Genetic Analysis

3.2.1 Analysis of rs945006; T>G SNP

After performing tetra ARMS- PCR, the detection of rs945006; T>G SNP was done by performing horizontal agarose gel electrophoresis. The PCR amplicons sizes differ according to the presence of T or G alleles. In the case of the wild type (TT), two PCR bands with 285 bp and 143 bp appeared on the agarose gel. In the case of heterozygous mutant (TG), three PCR bands with 285 bp, 186 bp, and 143 bp appeared, while two PCR bands with 285 bp and 186 bp appeared in the case of homozygous mutant type (GG), this is demonstrated in Figure 3 -1.

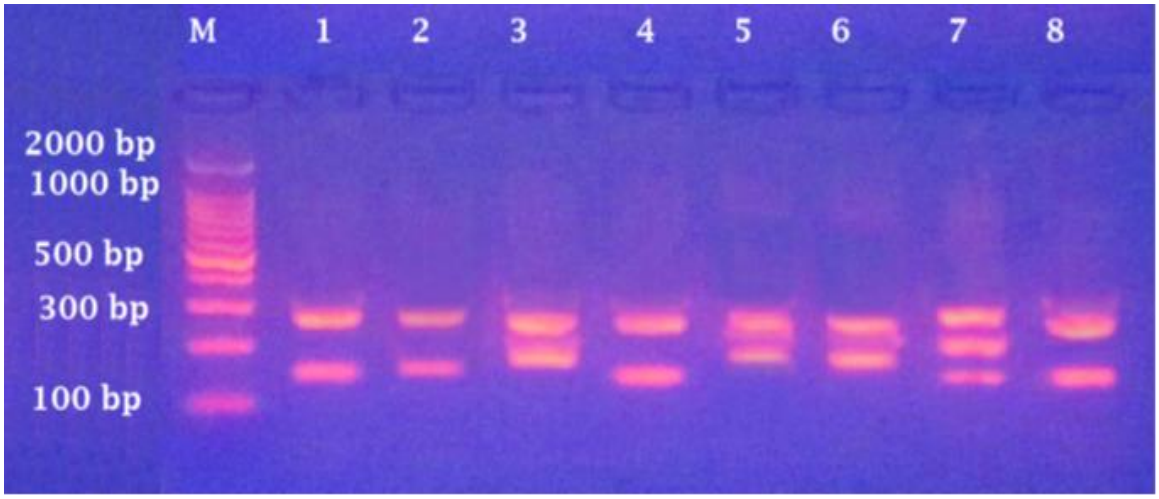

Figure 3-1: The horizontal agarose gel electrophorese (1.5% w/v) of amplification refractory mutation system-polymerase chain reaction detecting rs945006; T>G SNP. M: 100 bp DNA marker. Lanes 1, 2, 4, and 8 show the wild type (TT), lane 7 shows the heterozygous mutant type (TG) and lanes 3, 5, and 6 show the homozygous mutant type (GG).

The distribution of alleles and genotypes of the rs945006; T>G SNP is demonstrated in Table 3-2. The mutant allele (G) was more frequent among the study subjects with frequency of 0.77, while the wild allele (T) was less frequent with frequency of 0.23. The alleles and genotypes distribution was out of the Hardy-Weinberg equilibrium

Table 3-2: The distribution of alleles and genotypes of rs945006; T>G in the study subjects

Genotype (N=220)	Frequency (%)	Allele Frequency		Chi-square	P- value
		T	G		
TT (wild type)	25(11.4)	0.23	0.77	26.947	0.0001
TG (heterozygous mutant type)	50(22.7)				
GG (homozygous mutant type)	145(65.9)				

N: Numbers of the study subjects

3.2.2 Analysis of rs1190716; C>T SNP

After performing tetra ARMS- PCR, the detection of rs1190716; C>T SNP was done by performing horizontal agarose gel electrophoresis. The PCR amplicons sizes differ according to the presence of C or T alleles. In the case of the wild type (CC), two PCR bands with 253 bp and 121 bp appeared on the agarose gel. In the case of heterozygous mutant (CT), three PCR bands with 253 bp, 170 bp, and 121 bp appeared, while two PCR bands with 253 bp and 170 bp appeared in the case of homozygous mutant type (TT), this is demonstrated in Figure 3-2.

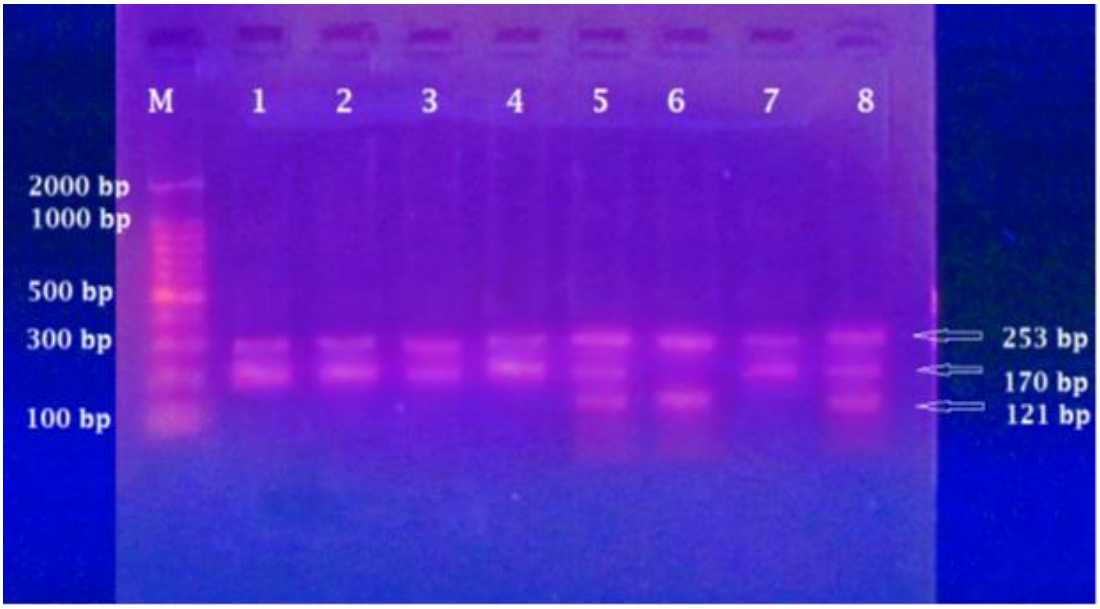

Figure3-2: The horizontal agarose gel electrophorese (1.5% w/v) of amplification refractory mutation system-polymerase chain reaction detecting rs1190716; C>T SNP. M: 100 bp DNA marker. Lane 6 shows the wild type (CC), lanes 5 and 8 show the heterozygous mutant type (CT) and lanes 1, 2, 3, 4, and 7 show the homozygous mutant type (TT).

The distribution of alleles and genotypes of the rs1190716; C>T is illustrated in Table 3-3. The mutant allele (T) was more frequent among the study subjects with frequency of 0.84, while the wild allele (C) was less

frequent with frequency of 0.16. The alleles and genotypes distribution was compatible with the Hardy-Weinberg equilibrium.

Table 3-3: The distribution of alleles and genotypes of rs1190716; C>T in the study subjects

Genotype (N=220)	Frequency (%)	Allele Frequency		Chi-square	P- value
		C	T		
CC (wild type)	10 (4.5)	0.16	0.84	4.94	0.084
CT (heterozygous mutant type)	50 (22.7)				
TT (homozygous mutant type)	160 (72.7)				

N: Numbers of the study subjects

3.3 The Association between the Deiodinase type-3 Gene Polymorphisms and the Demographic Characteristics

There were no significant differences in the age, the BMI, and the duration of treatment among all the three groups regarding the rs945006; T>G SNP and rs1190716; C>T SNP (Table 3-4) and (Table3-5)

Table 3-4: The demographic characteristic according to the genotypes of rs945006; T>G SNP among the hypothyroid patients

Demographic characteristic	Patients' genotypes (N= 220)			P-value
	TT (N=25)	TG (N=50)	GG (N=145)	
Age (year)	50.36±1.24	50.04±1.46	48.64±0.75	0.50
BMI (Kg/m^2)	29.08±0.96	30.61±0.93	31.44±0.47	0.15
Duration of the treatment (years)	4.63±0.87	4.33±0.47	4.50±0.35	0.94

The data is represented as mean± standard error, N: Numbers of the study subjects.

Table3-5: The demographic characteristic according to the genotypes of rs1190716; C>T SNP among the hypothyroid patients

Demographic characteristic	Patients' genotypes (N= 220)			P-value
	CC (N=10)	CT (N=50)	TT (N=160)	
Age (year)	51.20±2.96	47.98±1.17	49.39±0.73	0.48
BMI (Kg/m^2)	29.55±1.40	31.16±0.65	31.02±0.49	0.72
Duration of the treatment (years)	7.63±1.83	4.38±0.46	4.30±0.32	0.04

The data is represented as mean± standard error, N: Numbers of the study subjects.

3.4 The Association between the DIO3 Polymorphisms and the Thyroid Hormones

Regarding the rs945006; T>G SNP, Table 3-6 demonstrate that there were no significant differences in all the thyroid hormones levels, TSH levels, and in levothyroxine dose between wild type, the heterozygous and the homozygous mutant type groups.

Table3-6: The thyroid hormones analysis according to the genotypes of rs945006; T>G SNP among the hypothyroid patients

Parameter	Patients' genotypes (N= 220)			P-value
	TT (N=25)	TG (N=50)	GG (N=145)	
TT3 (nmol/L)	1.60±0.09	1.54±0.05	1.56±0.03	0.86
TT4 (nmol/L)	1.09.00±7.18	100.41±3.70	105.64±2.62	0.45
FT3 (pmol/L)	6.33±0.25	6.44±0.19	6.65±0.13	0.51
FT4 (pmol/L)	15.31±1.01	15.18±0.39	16.32±0.66	0.53
TSH (µlU/mL)	3.39±0.94	4.99±0.93	6.97±1.07	0.24
rT3 (nmol/L)	0.93±0.08	0.95±0.05	0.92±0.03	0.94
T2 (nmol/L)	2.00±0.16	1.96±0.15	2.06±0.09	0.86
TT3/ TT4	1.49±0.11	1.59±0.05	1.55±0.04	0.70
FT3/FT4	0.44±0.03	0.43±0.01	0.43±0.01	0.84
T2/TT3	1.26±0.12	1.30±0.09	1.38±0.06	0.70
rT3/T4	0.93±0.09	1.00±0.07	0.94±0.03	0.75
T4 dose (µg)	101.00±7.96	85.00±4.94	94.37±3.56	0.22

The data is represented as mean± standard error of the mean, N: Numbers of the study subjects, T3: 3, 3, 5-Triiodothyronine, T4: Thyroxin, T2: 3, 5- Diiodothyronine, rT3: reverse triiodothyronine. The reference ranges of TT4=64-3-185nmol/L, TT3=0.92-2.33nmol/L,FT4=9-20pmol/L,FT3=2-9pmol/L,and TSH=0.4-4.0µIU/L.

Regarding rs119716; C>T, the results in Table 3-7 show significant differences in TT3 between the wild type and the heterozygous mutant type. Total T4 was significantly higher in the wild type than in the homozygous mutant type. The T2 levels were significantly higher in heterozygous mutant type compared to homozygous mutant type. The levels of TSH, FT3, FT4 and rT3 and the ratio of TT3:tT4, FT3:fT4, T2:tT3 and rT3: T4) showed no significant differences among the three groups. The dose of levothyroxine also showed no significant differences among the groups.

Table 3-7: The thyroid hormones analysis according to the genotypes of rs1190716; C>T SNP among the hypothyroid patients

Parameter	Patients' genotypes (N= 220)			P-value
	CC (N=10)	CT (N=50)	TT (N=160)	
TT3 (nmol/L)	1.49±0.10	1.71±0.07	1.52±0.03	0.019[a]
TT4 (nmol/L)	125.74±9.52	108.81±5.31	102.29±2.22	0.039[b]
FT3 (pmol/L)	6.40±0.41	6.60±0.28	6.56±0.10	0.93
FT4 (pmol/L)	16.54±1.47	15.65±0.74	16.00±0.58	0.91
TSH (µlU/Ml)	3.03±0.90	7.29±2.20	5.94±0.76	0.51
rT3 (nmol/L)	0.86±0.10	1.01±0.05	0.91±0.02	0.21
T2 (nmol/L)	1.97±0.30	2.38±0.20	1.92±0.07	0.032[c]
T3/T4	1.22±0.09	1.61±0.07	1.56±0.03	0.06
FT3/FT4	0.40±0.03	0.44±0.01	0.43±0.009	0.57
T2/TT3	1.32±0.18	1.54±0.13	1.31±0.05	0.54
rT3/T4	0.72±0.10	1.01±0.06	0.95±0.03	0.19
T4 dose (µg)	67.50±9.16	97.00±5.23	93.34±3.35	0.11
Post hoc test: a: CC vs CT, b: CC vs TT, c: CT vs TT				

The data is represented as mean± standard error, N: Numbers of the study subjects, T3: 3,3,5-Triiodothyronine, T4: Thyroxin, T2: 3,5- Diiodothyronine, rT3: reverse triiodothyronine, *: significant at P<0.05. The reference ranges of TT4=64.3-185nmol/L, TT3=0.92-2.33nmol/L,FT4=9-20pmol/L,FT3=2-9pmol/L,and TSH=0.4-4.0µIU/L.

3.5 The Association between the DIO3 Polymorphisms and the Glycemic Profile

The results showed that there were no significant differences in all the glycemic parameters (fasting blood glucose, fasting insulin, and HOMA-IR) among the three groups of patients according to rs945006; T>G SNP and rs1190716; C>T SNP as demonstrated in Table 3-8 and Table 3-9, respectively.

Table3-8: The glycemic parameters according to the genotypes of rs945006; T>G SNP among the hypothyroid patients

Parameter	Patients' genotypes (N= 220)			P-value
	TT (N=25)	TG (N=50)	GG (N=145)	
FBS (mg/dL)	122.62±9.00	108.05±6.32	113.35±4.20	0.47
Fasting insulin (µu/ml)	12.39±1.77	21.20±3.43	16.63±1.11	0.06
HOMA-IR	3.94±0.62	5.54±0.85	4.93±0.47	0.49

The data is represented as mean± standard error, N: Numbers of the study subjects, FBS: Fasting blood glucose, HOMA-IR: Homeostasis model assessment-Insulin resistant

Table3-9: The glycemic parameters according to the genotypes of rs1190716; C>T SNP among the hypothyroid patients

Parameter	Patients' genotypes (N= 220)			P-value
	CC (N=10)	CT (N=50)	TT (N=160)	
FBS (mg/dL)	123.14±16.82	12149±9.50	109.99±3.23	0.27
Fasting insulin (µu/ml)	24.24±8.75	20.16±2.93	15.82±1.06	0.09
HOMA-IR	6.07±1.61	6.18±1.06	4.50±0.37	0.13

The data is represented as mean± standard error, N: Numbers of the study subjects, FBS: Fasting blood glucose, HOMA-IR: Homeostasis model assessment-Insulin resistant

3.6 The Association between DIO3 Polymorphisms and the Blood Pressure Parameters

The results indicated that there were no significant differences in systolic, diastolic blood pressure, and mean arterial pressure among the three

groups of patients regarding to rs945006 T>G SNP and rs1190716 C>T SNP as demonstrated in Table 3-10 and Table 3-11, respectively.

Table 3-10: The blood pressure parameters according to the genotypes of rs945006; T>G SNP among the hypothyroidism patients

Parameter	Patients' genotypes (N= 220)			P-value
	TT (N=25)	TG (N=50)	GG (N=145)	
Systolic BP (mmHg)	127.20±3.08	127.20±2.28	126.62±1.27	0.96
Diastolic BP (mmHg)	75.20±4.00	78.00±2.13	78.82±1.20	0.55
MAP	92.53±2.29	94.40±1.44	94.75±0.82	0.60

The data is represented as mean± standard error, N: Numbers of the study subjects, BP: Blood pressure, MAP: Mean arterial pressure.

Table 3-11: The blood pressure parameters according to the genotypes of rs1190716; C>T SNP among the hypothyroidism patients

Parameter	Patients' genotypes (N= 220)			P-value
	CC (N=10)	CT (N=50)	TT (N=160)	
Systolic BP (mmHg)	124.00±3.05	126.60±2.36	127.06±1.21	0.82
Diastolic BP (mmHg)	80.00±1.49	78.20±2.17	78.12±1.25	0.93
MAP	94.66±1.87	94.33±1.44	94.43±0.82	0.99

The data is represented as mean± standard error, N: Numbers of the study subjects, BP: Blood pressure, MAP: Mean arterial pressure.

3.7 The Correlation between the DIO3 Genetic Polymorphisms and the Significantly Different Parameters

Total T4, total T3, and T2 showed significant differences among the patients groups regarding the rs1190716 SNP. The estimated effect size represented by Eta and partial Eta squared was calculated to analyze the

effect of the rs1190716 SNP on these parameters as demonstrated in Table 3-12.

Table 3-12: The correlation between rs1190716; C>T SNP and some of the biochemical parameters.

Parameters	Eta	Partial Eta Squared	P-value
TT3	0.189	0.036	0.019
TT4	0.172	0.03	0.039
T2	0.176	0.32	0.030

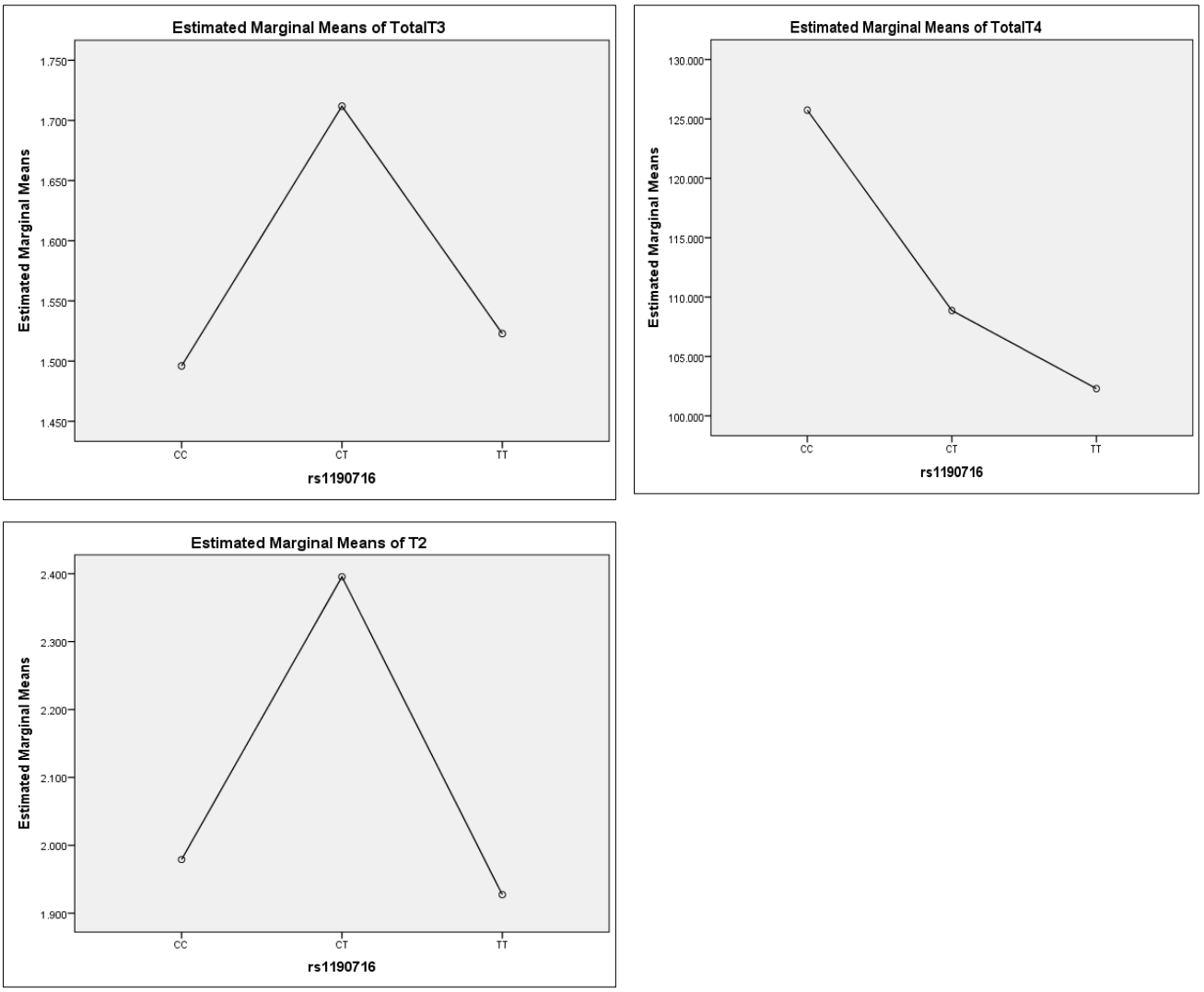

Figure 3-3: the estimated marginal means of TT4, TT3 and T2 among the three groups of patients regarding the genotypes of rs1190716 SNP.

Another statistical analysis was performed to investigate how the presence of both the SNPs in the patients could affect the level of thyroid hormones compared with the presence of one SNP. The results showed no significant differences in all studied parameters between patients have one SNP and patients have both SNPs (Table 3-13).

Table3-13: **The effect of the presence of both SNPs on the study parameters**

Parameter	**Patients' genotypes (N= 220)**			**P-value**
	No SNP (N=2)	**Either rs945006 SNP or rs1190716 SNP (N=31)**	**Both SNPs (N=187)**	
Age	52.50±2.50	50.35±1.34	48.91±0.68	0.42
BMI	28.46±0.91	29.31±0.88	31.29±0.43	0.08
Duration of treatment	13.50±3.50	4.45±0.71	4.47±0.28	0.92
TT3 (nmol/L)	1.70±0.20	1.55±0.08	1.56±0.03	0.89
TT4 (nmol/L)	161.15±41.15	107.67±5.10	103.76±2.24	0.50
FT3 (pmol/L)	7.10±0.60	6.26±0.23	6.61±0.11	0.24
FT4 (pmol/L)	21.15±7.15	14.96±0.72	16.06±0.52	0.41
TSH (µlU/ml)	1.91±1.89	3.47±0.79	6.60±0.87	0.14
rT3 (nmol/L)	1.20±0.58	0.88±0.06	0.94±0.02	0.41
T2 (nmol/L)	2.77±0.55	1.90±0.14	2.04±0.08	0.49
T3/T4	1.09±0.15	1.46±0.09	1.58±0.03	0.18
FT3/FT4	0.36±0.09	0.44±0.02	0.43±0.008	0.71
T2/TT3	1.69±0.52	1.22±0.10	1.36±0.05	0.34
rT3/T4	0.89±0.59	0.86±0.07	0.97±0.03	0.25
T4 dose (µg)	100.00±0.00	90.32±7.57	93.36±3.00	0.70
FSI µIU/ml	11.28±2.78	16.36±3.22	17.39±1.17	0.74
FBG mg/dl	202.50±17.50	112.48±6.89	112.37±3.62	0.99
HOMA-IR	5.52±0.90	4.42±0.72	5.03±0.42	0.57
Systolic BP mmHg	130.00±10.00	125.80±2.57	126.95±1.14	0.70
Diastolic BP mmHg	80.00±0.00	76.12±3.26	78.55±1.09	0.41
MAP	96.66±3.33	92.68±1.91	94.68±0.74	0.31

t-Test. The data is represented as mean± standard error, N: Numbers of the study subjects, T3: 3,3,5-Triiodothyronine, T4: Thyroxin, T2: 3,5- Diiodothyronine, rT3: reverse triiodothyronine, FBS: Fasting blood glucose, FSI: Fasting serum insulin, HOMA-IR: Homeostasis model assessment-Insulin resistant , BP: Blood pressure, MAP: Mean arterial pressure. The reference ranges of TT4=64-3-185nmol/L, TT3=0.92-2.33nmol/L,FT4=9-20pmol/L,FT3=2-9pmol/L ,and TSH=0.4-4.0µIU/L.

Chapter Four

Discussion

4. Discussion

This is the first genetic study in Iraq that investigates the effect of DIO3 gene polymorphism rs945006; T>G and rs1190716; C>Ton the levothyroxine therapeutic response in hypothyroid female patients.

Iodothyronine deiodinases may be the cause for the inability to normalize serum levels of T3 and TSH in patients receiving levothyroxine monotherapy as well as the inadequate symptomatic response experienced by a significant portion of patients with hypothyroidism. These enzymes were once hailed as the ultimate regulators of thyroid hormone availability and the key to levothyroxine treatment efficacy.

4.1 The Association between the Deiodinase type-3 Genetic Polymorphisms and the Demographic Characteristics.

In this study there were no significant differences in the BMI, the age, and the duration of treatment among the three patients' groups that were obtained regarding to the genotypes of rs945006; T>G SNP (Table 3-4). However, the results showed that the heterozygous mutant type and the homozygous mutant type carriers had higher BMI compared to the wild type carriers but not to a significant manner. This could be due to the higher levels of serum TSH in these two groups compared to the wild type group, yet this elevation does not reach a significant level.

It is worth mentioning that the TSH receptors that are located on the adipocytes membrane regulate adipose proliferation. This may partly explain the association between high TSH concentration and a high risk of overweight or obesity [(165)]. The heterozygous mutant type and the homozygous mutant type carriers also had marginally lower TT4 than that of the wild type carriers. These results are compatible with several previous studies that reported a strong association between higher TSH and

lower FT4 levels and increased BMI and subcutaneous fat deposition that are risk factors of metabolic syndrome [166].

Regarding rs1190716 SNP, the results also showed no significant association between this SNP and the BMI or the age (Table 3-5). The results showed higher BMI in the heterozygous mutant type and in the homozygous mutant type groups of patients compared to the wild type group but not to a significant manner. These two groups of patients had significantly lower TT4 and marginally higher TSH.

The duration of treatment regarding the groups of rs1190716 SNP was significantly higher in the wild type group of patients compared to the mutant types groups, this could due to the older age of this group of patients as demonstrated in Table 3-5.

4.2 The Association between Deiodinase type -3 Genetic Polymorphisms and Thyroid Parameters

Genetic association studies' findings showed that variations in the genes involved in the control of thyroid function could affect the TSH and the thyroid hormone levels. Deiodinase type 3 gene that encodes the D3 enzyme has an important role in thyroid hormone homeostasis. Accordingly, it is hypothesized that any variant in this gene could affect the regulation of thyroid hormones signaling.

The current study found that there was no association between rs945006; T>G SNP and TSH, the FT4 and the TT4, the FT3and the TT3, rT3, T2 and L-T4 dose (Table 3-6), this is consistent with several previous genome-wide associated studies [149,150] and candidate gene studies [146-148]. Despite the thyroid hormone levels in patients were not affected by this SNP, it is noteworthy that DIO3 gene is widely expressed in the brain [134].

Therefore the levels of THs could be changed in the local tissue rather than in the circulation.

The hypothyroidism patients who are treated with levothyroxine for not less than 4 months and yet continue to have disease symptoms could have genetic variants in other genes that regulate the metabolism of T4 and T3 hormones such as deiodinase type-1, deiodinase type-2, TSH receptor gene, or even thyroid transporters.

In addition, it is worth mentioning that DIO3 is one of the imprinted genes and the effects of DIO3 polymorphism on thyroid hormone homeostasis depend on the parental origin of the variant allele, therefore the effect of DIO3 variants on D3 enzyme activity might be hampered by the epigenetic process of genetic imprinting. This fact could explain why there is no significant association between rs945006; T>G SNP in the DIO3 gene and all the THs.

Regarding rs1190716 SNP the data in Table 3-7 indicated that there was no significant association between rs1190716; C>T SNP and TSH level, FT3, FT4, rT3, and LT4 dose. On the other hand, TT4, TT3, and T2 were found to be significantly different among the three groups of patients. The patients with mutant allele (T) had significantly lower TT4 and greater tT3 levels, at the same time they had slightly higher TSH levels and slightly higher LT4 doses. The patients' group who carried the wild-type allele (C) had significantly higher tT4 levels, slightly lower LT4 dose, and lower TSH levels. A part from the effect of D3, this could be due to the role of D2, which convert T4 to T3, and as an enzyme inhibited by its substrate, D2 activity can be inhibited by the high T4 concentration. This results in decreased T3 concentration, meanwhile, serum TSH concentration could be within the normal range because of the slightly elevated T4 concentration [(167)].

According to a study carried out on rats, the hypothalamus tanycytes and pituitary thyrotropes absorb plasma T4 and locally convert it to T3 by the D2, thus TSH secretion is decreased as a result of the D2 action at these two locations. [168].

Although the D3 enzyme is not implicated in the T4 to T3 conversion, it is worth mentioning that there are other candidate genes to be potential effectors. Deiodinase type I, deiodinase type II, and thyroid hormone transporters could notably influence the response to LT4. Previous studies suggested that common genetic variations in these loci might have an impact on the changing of the levels of TH metabolites and accordingly the response to LT4 treatment [169, 170].

While normalizing serum TSH is the aim of LT4 therapy, a gradual increase in LT4 dose raises the circulating T3 levels while concurrently lowering TSH secretion (predominantly through D2) [171].

The patients who had the mutant allele of rs1190716 SNP had higher LT4 doses but lower tT4 and higher TSH levels (Table 3-7). This indicates that LT4 treatment did not restore normal TSH levels. This could be explained by that the carriers of the mutant allele (TC and TT) had lower tT4 levels, thus their TSH levels were above the reference range, and therefore they needed a higher LT4 dose. This could be an indication of that rs1190716; C>T SNP increases D3 enzyme activity, which means increased conversion of T4 to rT3 making the levels of tT4 lower in the mutant allele carriers. This is confirmed by the increased rT3 levels in the mutant allele carriers compared to the wild allele carriers, but not to a significant level. As a result of this, the ratio of rT3/T4 was increased in the mutant types (CT and TT) groups but also in a non-significant manner (Table 3-7).

The TSH level is lower (within the normal range) in the wild-type group compared to both hetero and homo mutant type carriers, but not to a significant level (Table 3-7). This could due to the feedback inhibition of the elevated level of tT4 in this group. At the same time, this could indicate that the hypothyroidism patients who were not carriers for the rs1190716; C>T SNP might have a better response to LT4 therapy and that the D3 enzyme could have a role in THS regulation.

These findings could be novel regarding the rs1190716; C>T SNP and DIO3 gene because the previous studies demonstrated that this SNP and this gene had no impact on the THs and TSH levels [147, 161, 172].

4.3 The Association between the Genetic Polymorphisms of the Deiodinase Type-3 and Levothyroxine Dose.

There was no significant difference in LT4 dose between all the genotypes groups of rs945006 SNP (Table 3-6). These results come along with the absence of the association between rs945006 SNP and the levels of TSH and thyroid hormones. This SNP is located in the noncoding region, thus it cannot affect the structure of the enzyme, but it could affect the expression level of the gene [156]. Accordingly, it may contribute to disturbance pathways that control the healthy cell function, however the obtained results showed that it has no impact on the thyroid hormones levels and LT4 dose.

Regarding rs1190716 SNP, the results (Table 3-7) showed that there were no significant association between this genetic variant and the LT4 dose. However, the dose of the drug administered to the patients with the heterozygous mutant type and homozygous mutant type is higher than that administered to the wild type groups. This could be due to the effect of rs1190716 SNP on the level of tT4, which is significantly lower in the

mutant allele carriers. The LT4 dose could be higher in these two groups of patients in order to reach the desired level of T4 that achieves the therapeutic response, yet the response to the drug is not improved.

4.4 The Association between the Genetic Polymorphisms of Deiodinase type-3 and the Glycemic Parameters.

This study is the first one that investigates the impact of these polymorphisms on the glycemic parameters. Thyroid hormones represent a major regulator of glucose metabolism. They achieve their effects through the promotion or demotion of expression or activation of enzymes responsible for the regulation of different metabolic pathways [(173)].Accordingly any genetic variants in the genes involved in the regulation of thyroid function may be disturbed metabolic pathway and exacerbated or contributed to concomitant diseases such as diabetes [(174)].

According to rs945006 SNP**,** there was no significant difference in the glycemic parameters (FBS, insulin level, and HOMO-IR) among the three groups of patients (Table 3-8). No previous studies illustrated or investigate the effect of this polymorphism on glycemic parameters to compare these results with.

Regarding the rs1190716 SNP, there were no significant differences in glycemic parameters among the groups of patients. Although some patients who had this SNP had slightly raised HOMA-IR (Table 3-9). It is reported that the hypothyroidism association with obesity promotes the production of pro-inflammatory cytokines and contributes to the development of insulin resistance [(175)]. Hypothyroidism is associated with peripheral insulin resistance due to a reduction in glucose uptake. Rochon and co-workers demonstrated that hypothyroidism induced a decrease in

the insulin-mediated glucose disposal that reverted upon treatment, similar results were obtained by Stanicka and co-workers [176, 177].

4.5 The Association between the Deiodinase type-3 Genetic Polymorphisms and the Blood Pressure.

Numerous studies were performed in the last 2 years to reveal the genetic variants that could be association with thyroid impairment and altered its function. These encompass both linkage and candidate gene analyses targeting the hypothalamus-pituitary-thyroid pathway. A few polymorphisms were found to be associated with blood pressure; however, the results remain controversial [13].

Although the DIO3 gene is one of the genes involved in the regulation of thyroid hormone and any genetic variants may affect the metabolism of thyroid hormone and consequently affect its function such as blood pressure. The results of this study showed no association between rs945006 SNP rs1190716 SNP and blood pressure parameters (Table3-10 and Table 3-11, respectively), no previous studies are available to compare with regarding theses SNPs.

Additionally, this study found that the patients who had the both SNPs had serum TSH level out of the reference range (0.4-4.0 µlU/ml), while the patients who had one SNP had serum TSH level within the reference range (Table 3-13). The findings also indicated that the presence of one SNP or both SNPs had no effects on all thyroid hormones. These results indicate that there was no association between the presence of one SNP or both of them and studied parameters.

4.6 Conclusions

1. The rs945006 SNP was not associated with the thyroid hormones levels, while the rs1190716 SNP was significantly associated with altered levels of TT4, TT3, and T2. Accordingly, the first SNP might have no impact on the therapeutic response to levothyroxine, while the second could have an impact.
2. Both rs945006 and rs1190716 SNPs were not associated with the blood pressure parameters or the glycemic parameters in Iraqi hypothyroid female patients.
3. The wide distribution of the mutant allele of both rs945006 and rs1190716 SNPs in the hypothyroid patients make these SNPs cannot be excluded from being involved in the occurrence of the hypothyroidism disease.

4.7 Recommendations and Future Work

1. Investigation of other SNPs in DIO3 gene along with a large number of hypothyroidism patients and find their impact on the response to levothyroxine.
2. Investigation of the genetic variations in the levothyroxine transporters and receptors that may contribute to individual variations in the therapeutic response to levothyroxine.
3. A healthy control group could be enrolled to certainly determine if the genetic polymorphism has a role in developing hypothyroidism.

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Appendices

Questionnaire for Hypothyroidism Patients

Demographic characterization

الاسم: رقم الهاتف:

الوزن: الطول:

Parameters	variable	Notes
Age		
Gender	Female	
Occupation		
Smoking	Yes No	
Alcohol intake	Yes No	
Family history of hypothyroidism	Yes No	الاب الام
Hypothyroidism complications		
Duration of treatment of levothyroxine		
Drug side effects		
Other diseases		
Other medication		
Systolic blood pressure		
Diastolic blood pressure		
Mean B.P		
BMI		

Biochemical Parameters

Parameters	Results
TSH	
Total T3	
Free T3	
Total T4	
Free T4	
rT3	
T2	
Fasting blood glucose	
Fasting insulin level	
HOMA-IR	
Results of Genotyping	

University of Kerbala
Consent to be in Research

Study Title: The Impact of Genetic Polymorphism of Deiodinase Type-3 Enzyme on Therapeutic Response of Levothyroxine in Hypothyroidism Female Patients of Kerbala province

The Researcher Name: Alaa Hashim Mohammed

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

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

In this study, the researcher are collecting blood samples from you to learn more about the association of genetic polymorphism of deiodinase type-3 enzyme 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)

الملخص

الخلفية: هنالك الكثير من العوامل تؤثر على الحصول على الهدف العلاجي لليفوثايروكسين، لذلك فأن الكثير من المرضى يعانون من بقاء اعراض مرض قصور الغدة الدرقية على الرغم من استخدامهم علاج الليفوثايروكسين لمدة طويله. وان انزيم الديايودينيز من النوع الثالث هو الانزيم المسؤول عن تحويل هرمون الثايروكسين الى مضاد الشكل النشط الترايايودوثايرونين وايضا تحويل الشكل النشط لثلاثي ايودوثايرونين الى دايايودوثايرونين كلا الناتجين غير فعاله وبهذا يلعب دورا اساسيا في تنظيم توازن هرمونات الدرقية.

الهدف: هذه الدراسه تبحث تاثير تعدد اشكال النيكليوتيدات المفرده لجين الديايودينيز النوع الثالث على الاستجابه العلاجية لعلاج اليفوثايروكسين في مريضات قصور الغده الدرقيه في محافظة كربلاء المقدسة.

المنهجية: تم اخذ مئتين وعشرين مريضة مصابة بقصور الغده الدرقيه، يبلغ عمر المريضات من 40 سنه فما فوق وجميعهن كن ياخدن العلاج لمده لاتقل عن اربعة اشهر. تم تقدير هرمونات الغده الدرقيه لجميع المريضات.اما مايخص التحليل الجيني فقد تم باستخدام طريقه تفاعل التضخيم المتسلسل نظام تضخيم الطفرات رباعي البوادئ المقاوم للكشف عن الطفرتين rs1190716 وrs945006 .

النتائج: توزيع الانماط الجينيه للتغاير الجيني rs945006 كان 11.4% للنوع السائد (TT)و 22.7% للنوع المتغاير الزيجة(TG),65.9% للمتماثل الزيجة (GG) . لاتوجد هناك فروقات ذات دلائل احصائيه في مستويات هرمونات الغده الدرقيه بين المرضى. اما توزيع الانماط الجينيه للتغاير الجيني rs1190716 فقد كان4.5% للنوع السائد (CC)و22.7% للنوع المتغاير الزيجة (CT) و72.7% لنوع المتماثل الزيجة (TT) .وجدت فروق معنويه بين مجاميع الانماط الجينيه في مستوى الثايروكسين الكلي وفي مستوى ثلاثي الثايرونين الكلي وثنائي ايودوثايرونين p=0.039 و 0.019 و0.032 بالتتابع.

الاستنتاج: التغايرالجيني rs945006 لجين الديايودينيز النوع الثالث لايظهر اي علاقه مع مستويات هرمونات الغده الدرقيه لذلك ليس له تاثير على الاستجابه العلاجية لعقارالليفوثايروكسين في مريضات قصور الغدة الدرقية. اما التغاير الجيني rs1190716 يظهر تاثير واضح على مستويات هرمونات الغده الدرقيه لذلك من الممكن ان يؤثر على الاستجابه العلاجيه لليفوثايروكسين في مريضات قصور الغده الدرقيه.هذه الدراسه تعتبر اول دراسه توضح تاثير

التغايرات الجينية لجين الديايودينيز النوع الثالث على الاستجابه العلاجيه لعلاج الليفوثايروكسين في العراق لذلك نحتاج دراسات اخرى لاثبات هذا التاثير.

جامعة كِربلاء

كلية اِلصيدلة

فرع اِلادوية وِالسموم

تأثيرتعددالاشكال الجينيه لجين الديايوديناز- النوع الثالث على الأستجابة العلاجية لعقار الليفوثايروكسين عند مريضات قصور الغدة الدرقية في محافظة كربلاء

رساله مقدمه الى

كليه الصيدله في جامعه كربلاء كجزء من متطلبات درجه الماجستير في الادوية والسموم

من قبل

الاء هاشم محمد

(بكالوريوس صيدلة/جامعة الموصل 2004)

باشراف

ا.د.بان حوشي خلف

ا.م.د.سوزان جبير عباس

2023 م

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