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"Genetic Polymorphisms in TSH, Iodothyronine Deiodinase I, and Phosphodiesterase Beta: Association with Goiter Patients in the White Nile State

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قال تعالى:

"وَمَا تَوْفِيقِى إِلَّا بِاللَّهِ ۚ عَلَيْهِ تَوَكَّلْتُ وَإِلَيْهِ أُنِيبُ "

صدق الله العظيم

سورة هود (88)

Dedication

To all my family and all the people in my life who touch my heart, I dedicate this work

To my wife and my beloved kids whom I can't force myself to stop loving ,for his support and without the stability and security provided by their love and encouragement, this study would not have been possible.

To my beloved brothers and sister for their endless love, support and encouragement

To my homeland Sudan, the warmest womb

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This thesis is only a beginning of my journey.

Abstract Abstract

Introduction: The widespread occurrence of thyroid disorders in humans highlights the urgent need for effective treatment methods. The global prevalence of thyroid dysfunction ranges from 10 to 30%,

Objective: This study aimed to investigate the association between genetic variations in thyroid-related genes and thyroid function, as well as goiter prevalence, in the White Nile State of Sudan

Methodology: This cross-sectional study was conducted at Kosti and Rubik hospitals in Sudan from June 2019 to September 2024. The study included 153 participants with thyroid disorders, classified as simple difuse goiter, nodular goiter and , euthyroid goiter, and healthy controls. A questionnaire was used to collect information from participants for inclusion or exclusion in the study. Blood samples were collected from participants and analyzed using ELISA techniques to measure thyroid hormone levels .DNA was extracted from blood samples using phenol-chloroform extraction and PCR amplification was performed. SNP genotyping was done using RFLP analysis .

Results : Family history of thyroid goiter was assessed, with 45% of patients having a first-degree family history and33% have second degree family history 22% having no family history. There was no significant difference in mean age between patient and control groups, and patients were equally distributed among simple, nodular , and euthyroid gories. Significant differences in thyroid hormone levels (FT3, FT4, and TSH) were observed between patient groups and controls, with variations depending on the type of goiter. Genotype frequencies showed significant differences between all patient groups and the control group-based restriction analysis was used to identify DIO1a-C/T genotypes, with different band patterns observed for CC, TT, and CT genotypes

Conclusion : The study finds that goiter is prevalent across all groups, particularly among individuals aged 15-35 years in the Afro-Asiatic tribal population. It indicates a decrease in TSH levels in those with euthyroid goiter, while free T3 and free T4 levels remain unchanged. The DIO1a gene (rs11206244) is linked to euthyroid goiter, whereas the PDE8B gene (rs470439) shows an association with various types of goiter. Additionally, genetic polymorphisms in TSHRc and PDE8B are correlated with thyroid disorders, suggesting they could serve as prognostic markers**.**

المستخلص

المقدمة: إن الانتشار الواسع لاضطرابات الغدة الدرقية لدى البشر يبرز الحاجة الملحة لطرق علاج فعالة. تتراوح نسبة انتشار خلل الغدة الدرقية عالميًا بين 10 إلى 30٪.

الهدف: تهدف هذه الدراسة إلى التحقيق في العلاقة بين التغيرات الجينية في الجينات المرتبطة بالغدة الدرقية ووظيفة الغدة الدرقية، بالإضافة إلى انتشار الدراق في ولاية النيل الأبيض بالسودان.

المنهجية: تم إجراء هذه الدراسة المقطعية في مستشفيات كوستي وربك بالسودان من يونيو 2019 إلى سبتمبر 2024. شملت الدراسة 153 مشارك يعانون من اضطرابات الغدة الدرقية، تم تصنيفهم كحالات فرط نشاط الغدة الدرقية، قصور نشاط الغدة الدرقية، دراق يودوئي، وشهود أصحاء(فئة ضابطة). تم استخدام استبيان لجمع المعلومات من المشاركين للتضمين أو الاستبعاد من الدراسة. تم جمع عينات الدم من المشاركين وتحليلها باستخدام تقنيات ELISA لقياس مستويات هرمونات الغدة الدرقية. تم استخراج الحمض النووي من عينات الدم باستخدام استخراج الفينول - كلوروفورم وتم إجراء تضخيم ال DNAعبر تقنية ال PCR. تم إجراء تحليل تعدد الاشكال SNP باستخدام تحليل RFLP

النتائج: تم تقييم التاريخ العائلي للدراق، حيث كان 45٪ من المرضى لديهم تاريخ عائلي من الدرجة الأولى و33٪ لديهم تارخ مرضي من الاسرة الممتدة و22٪ ليس لديهم تاريخ عائلي. لم يكن هناك اختلافات ذات دلالة إحصائية في متوسط العمر بين مجموعتي المرضى والشهود، وكان المرضى موزعين بالتساوي بين فئات فرط نشاط الغدة الدرقية، قصور نشاط الغدة الدرقية،

والدراق اليودوي. لوحظت اختلافات كبيرة في مستويات هرمونات الغدة الدرقية (FT3، FT4، وTSH) بين مجموعات المرضى والشهود، مع اختلافات تعتمد على نوع الدراق. أظهرت ترددات الجينات اختلافات كبيرة بين جميع مجموعات المرضى ومجموعة الشهود، وتم استخدام تحليل القيود لتحديد الجينات DIO1a-C/T، مع أنماط مختلفة من الأشرطة لوحظت لجينات CC وTT وCT.

الخاتمة: تجد الدراسة أن الدراق منتشر بين جميع المجموعات، وخاصة بين الأفراد الذين تتراوح أعمارهم بين 15 -35 عامًا (الاعمار المبكرة). تشير إلى انخفاض في مستويات TSH لدى أولئك الذين يعانون من دراق يودوئي، بينما تظل مستويات FT3 وFT4 دون تغيير. يرتبط الجين DIO1a (rs11206244) بالدراق اليودوي، في حين يظهر الجين PDE8B (rs470439) ارتباطًا بأنواع مختلفة من الدراق. بالإضافة إلى ذلك، فإن التعدد الجيني في TSHRc وPDE8B مرتبط باضطرابات الغدة الدرقية، مما يشير إلى أنها قد تكون علامات تنبؤية.

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List of Abbreviations

Apreveation	Meaning
BAT	Brown Adipose Tissue
cAMP	Cyclic Adenosine Monophosphate
CAPZB	F-Actin-Capping Protein Subunit Beta
CDNA	Colony Deoxyribonucleic Acid
CH	Congenital Hypothyroidism
CTLA-4	Cytotoxic T Lymphocytes Antigen -4
D1	Deiodinase Types 1
D2	Deiodinase Types 2
D3	Deiodinase Types 3
DEHAL	Iodotyrosine Dehalogenase
DIO1	Iodothyronine Deiodinase 1
DIT	Diiodotyrosine
DNA	Deoxyribonucleic Acid
DUOX2	Dual Oxidase 2
ECM	Extracellular matrix
ELISA	Enzyme linked immunosorbent assay
FNAC	Fine Needle Aspiration Cytology
FNAB	Fine Needle Aspiration biopsy
FT3	Free Triiodothyronine
FT4	Free Thyroxine
GPCRS	G-Protein-Coupled Receptors
GWAS	Genome-Wide Association Studies
HAL	Major Histocompatibility Complex
HPT	Hypothalamus-Pituitary-Thyroid
IL6	Interleukins 6
Mcg	Micrograms
MCT	Monocarboxylate Transporter
MD	Major Depression
MIT	Mono Iodotyrosine
MRI	Magnatic resonance imaging
NIS	Sodium Iodide Symporter
OATP	Organic Anion Transporting Polypeptide
PCR	Polymerase chain reaction
PDE8B	Phosphodiesterase 8B
PTNP	Protein tyrosine phosphatase gene
RNA	Ribonucleic acid
RFLP	Restriction fragment length polymorphism

rT3	Reverse Triiodothyronine
SCH	Subclinical Hypothyroidism
SECIS	Sec Insertion Sequence
SNP	Single Nucleotide Polymorphism
T3	Tri iodo thyronine
T4	Tetra iodo thyronine
TARRs	Trace Amine Associated Receptors
TBG	Thyroxine-Binding Globulin
Tg	Thyroglobulin
THs	Thyroid Hormones
TMB	Tetra Methyl Benzidine
TMNG	Toxic Multi Nodular Goiter
TPO	Thyroid Peroxidase
TPOAbs	Thyroid Peroxidase Antibodies
TRH	Thyrotropin-Releasing Hormone
TSH	Thyroid-Stimulating Hormone
TSHR	Thyroid-Stimulating Hormone Receptor
TTR	Transthyretin
UCP	Uncoupling Proteins
UTR	Un Translated Regions
WHO	World Health Organization

Chapter one

- Introduction
- Justification
- Objectives

1. introduction

1.1 Background

The wide prevalence of thyroid pathology in the human population makes the problem of finding effective approaches to its correction extremely relevant. The worldwide incidence of thyroid dysfunctions varies from 10 to 30%, depending on dietary features at a given area, its remoteness from the sea, and the presence of adverse environmental impacts. (Vander pump2019) .

Thyroid hormone is the hormone that's mainly responsible for controlling the speed of the body's metabolism. In infants, thyroid hormone is critical for brain development, Thyroid gland is a small butterfly-shaped gland located at the front of the neck under skin, makes and releases thyroid hormone. It's a part of endocrine system, Hormones are chemicals that coordinate different functions in a body by carrying messages through blood stream to organs, muscles and other tissues. These signals tell the body what to do and when to do it, Metabolism is the complex process of how body transforms the food into energy, All of the cells in the body need energy to function, Thyroid hormone actually represents the combination of the two main hormones that thyroid gland releases: thyroxine (T4) and triiodothyronine (T3). They're often collectively referred to as "thyroid hormone" because T4 is largely inactive, meaning it doesn't impact cells, whereas T3 is active. Once the thyroid releases T4, certain organs in a body transform it into T3 so that it can impact cells and metabolism, Thyroid also releases a hormone called calcitonin to help regulate calcium levels in the blood by decreasing it. Calcitonin isn't grouped into the "thyroid hormone" name, and it doesn't impact body's metabolism like T3 and T4 do.(American Thyroid Association 2021)

1.1.2 Control of thyroid hormones :

The production and release of thyroid hormone — thyroxine (t4) and triiodothyronine (T3) — is controlled by a feedback loop system that involves Hypothalamus , Pituitary gland, Thyroid gland. And Multiple hormones. The hypothalamus is the part of your brain that controls functions like blood pressure, heart rate, body temperature and digestion.The pituitary gland is a small, pea-sized gland located at the base of your brain below your hypothalamus. It makes and releases eight hormones. pituitary gland is connected to your hypothalamus through a stalk of blood vessels and nerves. This is called the pituitary stalk. Through the stalk, your hypothalamus communicates with your pituitary gland and tells it to release certain hormones. (Peeters RP, Visser T 2017)To start the feedback loop, your hypothalamus releases thyroid-releasing hormone (TRH) which, in turn, stimulates pituitary gland to produce and release thyroid-stimulating hormone (TSH). TSH then triggers thyroid to produce T4 and T3, Of the total amount of hormones that TSH triggers thyroid to release, about 80% is T4 and 20% is T3, Thyroid also needs adequate amounts of iodine, a substance we get from the food , to create T4 and T3.This hormone chain reaction is regulated by a feedback loop so that when the levels of T3 and T4 increase, they prevent the release of TRH (and thus TSH). When T3 and T4 levels drop, the feedback loop starts again. This system allows the body to maintain a constant level of thyroid hormones in the body, If there are any issues with hypothalamus, pituitary gland or thyroid, it can result in an imbalance in the hormones involved in this system, including T3 and T4.(Boyce A, et al.2021)

1.1.3:Function of thyroid hormones:

Thyroid gland releases thyroxine (T4) into bloodstream, certain cells in the body transform it into triiodothyronine (T3) through a process called de-iodination. This is because cells that have receptors that receive the effect of thyroid hormone are better able to use T3 than T4. Therefore, T4 is generally considered to be the inactive form of thyroid hormone, and T3 is considered the active form of it. Liver , kidney ,Muscle , thyroid ,pituitary gland and brown adipose tissue these cells are responsible for conversion of T4 to T3:Thyroid hormone (T3 and T4) affects every cell and all the organs in your body by: Regulating the rate at which a body uses calories (energy) This affects weight loss or weight gain and is called the metabolic rate, Slowing down or speeding up your heart rate, Raising or lowering your body temperature, Influencing the speed at which food moves through your digestive tract, Affecting brain development ,Controlling the way our muscles contract and Managing skin and bone maintenance by controlling the rate at which our body replaces dying cells (a normal process).(Shahid MA, Ashraf MA, Sharma 2021)

1.1.4 Goiter:

Goiter is a condition where the thyroid gland grows larger. The entire thyroid can grow larger or it can develop one or more small lumps called thyroid nodules. thyroid gland is a small, butterfly-shaped endocrine gland located in your neck, below your Adam's apple. It produces the hormones thyroxine (also called T4) and triiodothyronine (also called T3). These hormones play a role in certain bodily functions, including metabolism , body temperature , mood and excitability , pulse and heart rate and digestion. Goiter may be associated with an irregular amount of

thyroid hormone in a body (hyperthyroidism or hypothyroidism) or with normal levels of thyroid hormone (euthyroid).Goiter has several possible causes. Depending on the cause, it may or may not require treatment.(American Thyroid Association2022)

1.2 Rationale:

The White Nile State in [Sudan] is a region with a reported prevalence of goiter, making it an appropriate setting to investigate the potential genetic underpinnings of this condition

Research Gap: Previous studies have primarily focused on the association between iodine deficiency and goiter, with limited exploration of the genetic factors involved. While some research has examined the role of specific genes in thyroid function, comprehensive studies investigating the interplay of multiple genes in goiter etiology, particularly in the context of a specific geographic region, are scarce.

The findings of this research will contribute to the following:

- Public Health: Identifying genetic risk factors for goiter can inform targeted prevention and early intervention strategies.
- Clinical Practice: Understanding the genetic basis of goiter can aid in personalized diagnosis and treatment approaches.
- Knowledge Advancement: The study will expand the knowledge base on the genetic etiology of goiter, particularly in populations with endemic goiter.

1.3 Objectives:

1.3.1 General objective:

Association of genetic polymorphism in thyroid-related genes and thyroid function, with goiter patients , in White Nile State of Sudan.

1.3.2 Specific objectives:

- To estimate and compare serum thyroid stimulation hormone (TSH), free thyroxin (T4), and free triiodothyronine (T3) levels between the test and control groups using the Enzyme linked immunosorbent assay Method (ELISA) method."
- To identify genetic polymorphisms in the Thyroid stimulating hormone receptor (TSHR) (rs1991517), iodothyronine deiodinase 1 (DIO1) (rs11206244), and Phosphodiesterase 8Beta (PDE8B) (rs4704397) genes within the test and control groups using Restriction fragment length polymorphism (RFLP) genotyping."
- To associate between single nucleotide polymorphisms (SNPs) in thyroid-related genes and thyroid dysfunction within the Sudanese population.
- To correlate the genotype and allele frequencies of TSHR (rs1991517), DIO1 (rs11206244), and PDE8B (rs4704397) genes in patients with thyroid diseases compared to healthy controls.
- To compare mean concentrations of thyroid hormones (FT3 and FT4) and thyroid-stimulating hormone (TSH) between individuals with normal and mutant alleles of each gene in patients with thyroid hormone disorders."

Chapter two

literature review

2. literature review

2.1 pathophysiology:

Hyperthyroidism: Disorders of the thyroid gland can result in excess T3 and T4 production along with the compensatory decrease of TSH. In addition, thyrotroph adenoma can produce unregulated TSH and can lead to increased T3 and T4 production. There is an ectopic production of thyroid hormone in some conditions, leading to increased thyroid hormones and compensatory TSH decrease, Hypothyroidism: In primary hypothyroidism, decreased production of thyroid hormones by the thyroid gland causes a compensatory increase of TSH. Secondary hypothyroidism is caused by pituitary disorders causing decreased TSH release and decreased T3/T4 levels. Tertiary hypothyroidism is caused by hypothalamic disorders, resulting in decreased TRH levels, decreased TSH, and T3/T4 levels.(Núñez A, Bedregal P, Becerra C, Grob L F 2017)

2.1.1 importance and synthesis of Thyroid hormones:

Thyroid hormone (TH) in adults is necessary for the regulation of multiple physiological events, such as cell growth, structure, and metabolism , The main thyroid hormones produced by the thyroid gland are thyroxine (T4),triiodothyronine (T3), and reverse triiodothyronine (rT3), which are controlled by thyroid-stimulating hormone (TSH). Under physiological conditions, both T4 and T3 are secreted into the bloodstream by the thyroid gland , THs circulating in the body exert metabolic activities on multiple organs, including heart, bone, brain, liver, thyroid, kidney and skeletal muscle , The actions of thyroid hormone are classified into two main mechanisms:

(1) A non-genomic activity : initiated at the plasma membrane that regulates downstream gene expression via integrin transcriptional activity: induced by interactions with nuclear thyroid hormone receptor proteins and further binding to thyroid hormone response elements of specific downstream genes. The isoform of integrin is a heterodimeric structure at the plasma membrane capable of interacting with a large number of extracellular matrix (ECM) proteins as ligands for activating downstream signal pathways , In addition, the protein structure of thyroid hormone receptor is similar to nucleus receptor superfamily and acts as a sequence-specific ligand-dependent transcription factor that mediates several downstream of THs on activation or repression of target genes , The above actions generated through either non-genomic or genomic overlap in the nucleus, Thyroid hormone activity is beneficial for normal cell development. However, when both the levels of THs and thyroid hormone receptors in the body are out of control, it causes multiple diseases, including cardiovascular disease, diabetes mellitus and chronic liver disease. Earlier studies by our group and other investigators conducted to clarify the significance of thyroid hormone in cells and tissues have revealed activity in regulation of proliferation of both tumour and non malignant cells (Yu-Chin Liu 2019).

synthesized THs induce leukocyte proliferation, migration, release of cytokines, and antibody production, triggering an immune response against either sterile or microbial insults. However, chronic patho-physiological alterations of the immune system, such as infection and inflammation, affect HPT axis and, as a direct consequence, THs mechanism of action. Herein, we revise the bidirectional crosstalk between THs and immune cells, required for the proper immune system feedback response among diverse circumstances. Available circulating

THs do traffic in two distinct ways depending on the metabolic condition. (Roberto De Luca1 2021).

Thyroid hormones are essential for optimal neurogenesis and neuronal differentiation, and play an important role in the maintenance of brain development and function throughout life , In a meta- analysis of five cohort studies, including 7,895individuals with subclinical hyperthyroidism [defined as decreased (low) serum thyrotropin(TSH) and normal levels of thyroid hormones] followed for a median of 34 months, the investigators found a decline in cognitive function based on a Mini- Mental State (Folkestad, Lars 2011).

2.1.2 Production of thyroid hormone:

The production of thyroid hormones is based on the organization of thyroid epithelial cells in functional units, the thyroid follicles. A single layer of polarized cells forms the envelope of a spherical structure with an internal compartment, the follicle lumen. Thyroid hormone synthesis is dependent on the cell polarity that conditions the targeting of specific membrane protein, either on the external side of the follicle (facing the blood capillaries) or on the internal side (at the cell-lumen boundary) and on the tightness of the follicle lumen that allows the gathering of substrates and the storage of products of the reactions, Thyroid hormone secretion relies on the existence of stores of pre-synthetized hormones in the follicle lumen and cell polarity-dependent transport and handling processes leading to the delivery of hormones into the blood stream (Françoise Miot, 2015).

D1 and D2 are predominantly activating enzymes; both convertT4 to T3 (and rT3 to T2)by outer ring deiodination. D1 is found in pituitary, kidney, thyroid, and liver in humans and D2 in Heart , central nervous system, pituitary, thyroid, skeletal muscle, and brown adipose tissue. Predictions based on isolated cell deiodinase activity and reported tissue

activities in humans suggest that both are responsible for maintaining serum levels of T3, although D2 predominates in hypothyroidism and D1 in hyperthyroidism. D3 inactivates thyroid hormones by inner ring deiodination, converting T3 to T2 and T4 to rT3. It has been found in the central nervous system and placenta in adults and in many additional tissues in the fetal state. A more detailed description of deiodinase action can be found in recent reviews (Panicker *et al.*, 2008)

The thyroid gland releases a combination of Tetra iodothyronine (T4) and triiodothyronine (T3) in a ratio of approximately17:1.3Conversion of T4 to T3 is catalyzed by type1 and type 2 iodothyronine deiodinases(D1 and D2, respectively), which influence the relative balance of these hormones in the circulation (Dayan and Panicker, 2009).

2.1.3 New aspects of thyroid hormone synthesis:

A number of molecular causes of thyroid dysenteric, accounting for approx. 80% of cases suffering from congenital hypothyroidism (CH), have been identified over the last few years and were shown to include mutations in transcription factors important for thyroid development such as Pax8, Nkx2.1, FoxE1, and HoxA3 (Gru *et al.*, 2004).

Mutations in these genes often also lead to developmental dysfunction in However, only about 5% of these genetic defects could account for the pathogenesis of thyroid dysgenesis (Krude, 2009).

Furthermore, the gene products of Dehal and Thox2 as well as inactivating and activating mutations of the Gs alpha gene have been added to the growing list of candidate proteins with important thyroid-specific functions (Moreno *et al*. 2008; Anon 2002; Spada *et al*.2016).

Recently, potassium channel subunits Kcnq1 and Kcne2 were found to be crucial for TSH-stimulated thyroid hormone biosynthesis.

A more profound understanding of thyroid hormone biosynthesis has also lead to a wide view on the molecular regulation of the thyroid gland and has increased our knowledge about the differentiated state of thyroid epithelial cells. In this context, novel players have been identified, which are important for the maintenance of thyroid physiology, i.e. molybdenum-dependent enzymes with undispensable functions in the oxidative system of thyrocytes and molecules that play a role in thyroglobulin processing for thyroid hormone liberation from the thyroid gland (megalin and cysteine cathepsins) (Havemeyer *et al*. 2006; Jordans *et al.* 2009)

2.1.4 Transport of Thyroid hormones:

All thyroid hormone is transported in the circulation bound to different proteins, largely, thyroxine-binding globulin (TBG), transthyretin (TTR), and albumin. However, itis the free fraction of T4 and T3 which is available for metabolism and action in the tissues, Based on the lipophilic structure of thyroid hormones, it was long thought that thyroid hormone enters the cell through passive diffusion. However, it has become increasingly clear that there are specific thyroid hormone transporters, and that the activity of these in part determines the intracellular thyroid hormone concentration (Van der Deure*et al.,*2007).

2.1.5 Action of Thyroid hormones :

Thyroid hormone (TH) action serves important regulatory functions throughout all phases of life. Disturbed TH action is linked with major health problems especially in critical life phases such as development, disease or ageing (Bauer *et al.*, 2008).

2.1.6 Novel concepts of thyroid hormones:

Although the fundamental pathogenesis of AITD remains unknown, it is generally acknowledged that genetic predispositions and environmental factors including exposure to cigarette smoke, high dietary iodine intake and stressful life events are implicated, Concordance studies in twins suggest that genetic factors confer 80% contribution to the etiology of AITD , In recent years, the genome-scanning and single nucleotide polymorphisms (SNP) studies have made great progress in identification of susceptibility genes. Currently, several candidate genes have been reported, which include human leukocyte antigen (HLA), cytotoxic T-lymphocyte-associated antigen- 4 (CTLA-4) [7-9], thyroid stimulating hormone receptor (TSHR) , thyroglobulin (TG) , protein tyrosine phosphatase (PTPN) gene ,CD40 gene and FCRL3 gene , Among these genes, TSHR is deemed to be an important autoantigen for thyroid and definitely plays a significant role in the pathogenesis of AITD (Lin Liu 2012).

These are decarboxylated thyroid hormones that exert effects with kinetics different from those of thyroid hormone mediated actions and principally counter-acting those (so called cool thyroid hormones). Most obvious effects of thyronamines are observed in decreased heart rates and in negative regulation of body temperature. Thyronamines display their effects via activating a new subfamily of G-protein-coupled receptors (GPCRs), the traceamine associated receptors (TAARs) (Staubert., *et al* 2010; Piehl*et al.*, 2011).

Because of their central roles in signaling resulting in a multitude of regulatory effects on almost all biological processes, GPCRs are interesting targets for pharmacological intervention (Piehl *et al.*, 2011).

The precise mechanisms and the physiological implications of non-classical TH actions however remain elusive. Besides the classic

hormones T4 and T3 new data demonstrate that the rare thyroid hormone metabolite 3,5-T2 is effective in the prevention of high fat diet induced adiposity and prevents hepatic steatosis, however, without exerting the severeside effects on the cardiac system that have been observed with T3-based treatments(Lanni *et al*., 2005).

The vital importance of thyroid hormones for regulation of thermogenesis and for maintenance of the homeostasis of the mitochondrial energy metabolism has long been established. However, the functional interactions between the activities of uncoupling proteins (UCP) which are triggered by T3 and catecholamine's affecting brown adipose tissue (BAT) as well as skeletal muscle of the adult, provide new possibilities for therapeutic intervention in obesity that have only recently become apparent (Ribeiro *etal.,* 2010).

2.2 Functions of Thyroid hormones :

The thyroid gland influences almost all of the metabolic processes in human body. Thyroid-related disorders can vary widely from a small, harmless goiter (enlarged thyroid gland) that needs no specific treatment to life threatening cancer which might need invasive treatment. Thyroid diseases rank as the most prevalent endocrine disorders after diabetes, The thyroid gland secretes hormones that help regulate the body's metabolism. According to a recent study, thyroid-related diseases affect 200 million people worldwide, of which about 60% are unaware of their disease. There are several factors involved in thyroid dysfunction, the most common of which is iodine deficiency that is estimated to be affecting 2 billion individuals worldwide and is thought to cause primary Hypothyroidism, The most common cause of hypothyroidism is chronic autoimmune thyroiditis, also known as Hashimoto's disease (HT). High concentrations of anti-thyroid antibodies (predominantly thyroid peroxidase (TPO) antibodies and anti-thyroglobulin antibodies) are

present in most patients with autoimmune thyroiditis, Histological and cytological features of HT constitute a dense thyroidal accumulation of lymphocytes, plasma cells and occasional multinuclear giant cells. HT can be seen with various severities from a life-time hypothyroid
state to a euthyroid. It was demonstrated that even having a parent with Hashimoto's disorder can put the child at the risk of thyroid disorders (Fahime Sadat Naghibi 2022) .

The major function of thyroid gland is makes hormones that regulate heart rate, body temperature, and the conversion of food into energy. Thyroid stimulating hormone (TSH) stimulates the production of thyroid hormones by the thyroid gland. Increased levels of TSH indicate that the thyroid is not functioning properly (Procopciu*c et al.*, 2011).

2.3 Regulation of Thyroid hormones:

Thyroid hormones (TH) are essential for normal growth and differentiation, regulation of energy metabolism, and physiological function of virtually all human tissues , The hypothalamic-pituitary-thyroid (HPT) axis plays a predominant role in maintaining normal circulating TH concentrations. Hypothalamus derived thyroid-releasing hormone (TRH) stimulates the pituitary to release thyrotropin [thyroid stimulating hormone (TSH)], which stimulates the thyroid to produce thyroxine (T4) and, to a lesser extent, triiodothyronine (T3). TH availability and action on a peripheral level is regulated by TH transporters, deiodinases, nuclear receptors, and receptor cofactors. TH degradation is primarily regulated by successive deiodination. Glucuronidation and sulfation result in elimination of TH via urine or feces or recycling in the enterohepatic circulation. All these biological processes influence the variation in TH concentrations, In the last decade, epidemiological studies have demonstrated that subclinical thyroid disease, defined as TSH concentrations outside the reference range and

free T4 (FT4) concentrations within the reference range, is associated with several adverse health outcomes, such as coronary heart disease, stroke, and atrial fibrillation, among others (Rosalie B. T. M. Sterenborg 2022).

About 80% of circulating T3 is produced enzymatically by the deiodination of T4 in peripheral tissues mainly the liver and kidneys. T4 is converted to T3 in most tissues by two related enzymes, deiodinase types 1 and 2, while another enzyme, deiodinase type 3,converts T4 to an inactive form of T3 (called reverse T3 or rT3). Thus, replacement of thyroid hormone with T4 alone provides a long-lasting store of thyroid hormone that is gradually converted to T3, resulting in stable plasma levels of both T4 and T3 (Daniels &Dayan, 2006).

The TSH receptor and its role in the thyroid: The established biological function of the TSH receptor (TSHR) in the thyroid gland is to regulate synthesis and secretion of thyroid hormones from follicular thyroid cells; it also plays an important role in controlling the growth and development of the thyroid gland, Thyroid stimulating hormone (TSH) is the most important regulator in the hypothalamus–pituitary–thyroid(HPT) axis, via its receptor (TSHR) which located in the surface of the basal membranes of thyroid follicular cells, increases thyroid hormone levels by upregulating expression of the sodium iodide symporter (NIS), thyroid peroxidase (TPO), and TG genes. They all play vital role in the synthesis of thyroid hormone (Guo *et al*., 2005).

2.4 Disorders of thyroid gland:

Thyroid hormones are essential for energy metabolism and act on almost all cells. Thyroid dysfunction is associated with secondary cardiovascular, mental health, ophthalmic and other disease, Hypothyroidism has a high prevalence(2) and is most commonly due to autoimmune (Hashimoto) thyroiditis, in areas where iodine intake is

sufficient, Hyperthyroidism, prevalence 0.2%-1.3%, is most commonly due to autoimmune (Graves) disease or toxic nodular goiter, Ageing , diet (including iodine deficiency), smoking status, genetic susceptibility, ethnicity, and endocrine disruptors are risk factors for thyroid diseases; defining genetic variants, genes, proteins and pathways associated with hypothyroidism and hyperthyroidism will inform a deeper understanding of the mechanisms of thyroid disease and inform prevention and treatment strategies(Alexander T Williams 2023).

With a population of around 1.25 billion, an estimated 42 million people would be suffering from thyroid disorders it is a spectrum of disorders manifesting either as hypo function or hyper functioning of the thyroid gland reflected in the circulating levels of Triiodothyronine(T3), Thyroxin (T4) and Thyroid stimulating hormone (TSH). The disorders of thyroid hormone can be due to diseases of the thyroid gland itself (primary), secondary to pituitary disorder (secondary) or due to hypothalamic diseases (tertiary) (Singh *et al.*,2016).

2.4.1 Hyperthyroidism:

Hyperthyroidism disorder also known as "thyrotoxicosis" occurs when the thyroid gland produces excessive amount of thyroid hormone in the blood more than the body needs. Hyperthyroidism causes many of the body's functions to speed up(1). Hyperthyroidism linked with a variety of signs as nervousness, fatigue, heat intolerance, trouble sleeping, hand tremors,

irregular or rapid heartbeat, and diarrhea. (Yasimin Hassan Juma'a1 2021)

Thyrotoxicosis is a disorder of excess thyroid hormone, whereas the term hyperthyroidism specifically describes increased thyroid hormone synthesis and secretion. The tissue effects of high concentrations of thyroid hormones have many clinical manifestations (Franklyn and

2.4.2 Graves' disease:

Autoimmune hyperthyroidism, or Graves' disease, is considered as one of the most common thyroid disorders. There is no consensus on the factors that certainly lead to its development, but the most important among them are genetic predisposition and female sex (Vander pump MPJ (2019) .

The pathogenesis of Graves' disease lies in the generation of stimulatory autoantibodies to the TSHR extracellular domain, which is often accompanied by a decrease in the immune tolerance to other components of the system of thyroid hormone synthesis, such as thyroperoxidase and thyroglobulin. TSHR hyper activation by stimulatory autoantibodies leads to an uncontrolled increase in the blood level of thyroid hormones, followed by the implementation of their systemic metabolic effects on target tissues, which leads to the clinical manifestations of hyperthyroidism. Treatment of Graves' disease is mainly associated with the eradication of excess hormones, either by blocking their synthesis or by reducing the volume of thyroid tissue. All the currently used approaches are associated with several complications. Although thionamide derivatives, which are the most widely used group of antithyroid drugs, have a favorable safety profile, their application entails a number of undesirable side effects, such as agranulocytosis, vasculitis, liver damage, and skin reactions (Wémeau J, Klein M2018) Moreover, treatment with antithyroid drugs does not avoid a high risk of disease recurrence within two years after treatment discontinuation [Burch HB, Cooper DS (2018).

Radioiodine therapy, which is the next step in the therapeutic strategy for Graves' disease, is characterized by a delayed effect, which necessitates simultaneously prescription of antithyroid drugs , Surgical treatment is associated with risks of such complications as bleeding, infection,

aesthetic defects, laryngeal nerve palsy, and hypoparathyroidism ,In addition, patients who have undergone total thyroidectomy have to take L-thyroxine preparations for life due to a significantly pronounced postoperative hypothyroidism. (5 Burch HB, Cooper DS (2018). Such a variety of side effects and sometimes quite complicated ways of their correction create an urgent need to search for qualitatively new pharmacological approaches to treating hyperthyroid conditions. One of the most interesting of them is targeted therapy, which allows affecting a specific pathogenetic link, such as the TSHR, the target of TSHR-stimulatory autoantibodies. Among such approaches under consideration is the use of autoantibodies able to block the TSHR, as well as its low-molecular allosteric antagonists. Currently, anti-TSHR autoantibodies are in different phases of clinical trials and considered as a very promising group of antithyroid drugs (7 Burch HB, Cooper DS (2018).

Graves' disease (GD) is the most important factor of hyperthyroidism and is a typical autoimmune thyroid disease, Approximately 3% of women and 0.5% of men all over the world suffer from this disease. GD is characterized by a high level of thyroid hormone, a diffuse goitre, a positive test for thyroid stimulating hormone receptor antibody (TRAb), Graves ophthalmopathy and anterior tibia mucous oedema. Approximately 25–50% of GD patients have a clinical manifestation of Graves ophthalmopathy (GO), which is also called thyroid-associated ophthalmopathy (TAO), As the most common clinical manifestation of GD, GO is characterized by the retraction of the upper eyelids, chemosis, palpebral oedema, exophthalmos and extra ocular muscle hypertrophy (Haibo Xiong 2016).

2.4.1.1 Causes of hyperthyroidism:

Common causes of hyperthyroidism include: Grave's' disease; toxic multinodular goiter; solitary toxic adenoma; thyroiditis (autoimmune / post-viral); exogenous TH (excess intake, either iatrogenic or factitious).Un common causes of hyperthyroidism include:

TSH-secreting pituitary adenoma; drug-induced (iodine, iodine-containing drugs such as amiodarone, contrast agents); inflammatory (excess release of TH due to damage to thyroid gland cells) (Franklyn and Boelaert 2012).

2.4.2 Hypothyroidism:

Hypothyroidism is known as inability of the thyroid gland to make adequate thyroid hormone to obtain the metabolic requirements of the body, Also hypothyroidism linked with a variety of signs as Cold intolerance, Dry skin Fatigue, Difficulty concentrating, Constipation, Depression, Weight gain, Hair thinning/hair loss, Memory impairment, decreased sweating, slowed heart rate, fertility difficulties or menstrual Changes (Yasimin Hassan Juma'a1 2021).

The factors that predispose to the development of hypothyroidism, including its autoimmune form, are burdened family history and female sex, but the greatest contribution to the development of this pathology is made by an alimentary iodine deficiency. A combination of factors leads to the production of autoantibodies against various components of the system of thyroid hormone synthesis, including the TSHR, The classical approach to the treatment of hypothyroidism of any etiology is thyroid hormone replacement therapy, primarily by L-thyroxine. Replacement therapy is relatively safe, and side effects are typically due to overdose and iatrogenic thyroxine excess , However, it should be noted that some patients with hypothyroidism have individual intolerance to thyroxin

preparations, which hampers their treatment because of the lack of alternative approaches ,The development of selective agonists of thyroid hormone receptors and allosteric agonists of the TSHR are promising approaches that can become an alternative to the existing therapeutic standard, The mechanism of action of thyroid hormone receptor agonists consists in their specific binding to the β-isoform of these receptors. (Bakhtyukov 2022)

which results in the potentiation of physiological effects of the thyroid hormones thyroxine and triiodothyronine. However, for some thyromimetics, clearly pronounced toxic effects have already been found at early stages of clinical studies, as manifested in the destruction of cartilaginous and bone tissues, In addition the use of thyroid hormone receptor agonists is associated with a significant risk of cardiovascular complications. Selective allosteric TSHR agonists refer to a poorly studied, though very interesting group of drugs. The range of their clinical application is not confined to hypothyroidism and can be extended to a variety of metabolic diseases accompanied by a decreased thyroid function, such as obesity and diabetes mellitus of various etiologies. They will be discussed below in an appropriate section, Hypothyroidism is one of the most common endocrine disorders. It is characterized by underproduction of thyroid hormone which plays an essential physiologic role in the development of the individual and body metabolism (Sayer *et al*., 2014).

2.4.2.1 Signs and symptoms of hypothyroidism:

Hypothyroidism is associated with a wide spectrum of signs, symptoms and long-term complications such as skin manifestations, obesity, hyperlipidemia, bradycardia, fatigue and depression ,The common

clinical features associated with hypothyroidism are tiredness, weight gain, dry skin, cold intolerance, and constipation, and muscle weakness, puffiness around the eyes, hoarse voice, and poor memory (Al-Azzam *et al.,* 2014).

Although the likelihood of hypothyroidism increases with increasing numbers of symptoms, absence of symptoms does not exclude the diagnosis. Furthermore, these symptoms are non specific and common in the euthyroid population with around 20% of euthyroid subjects having four or more hypothyroid symptoms (Rodondi*et al*., 2008).

2.4.2.2 Management of Hypothyroidism:

T4 is the replacement therapy of choice because of its long half-life, allowing once daily administration, ease of administration and low cost. Response to T4 therapy should be monitored by TSH levels (taking into account the time lag in TSH response) and the dosage increased at 25-50mcg increments, up to a maximum daily dose of 200 mcg, until clinical evidence of normal function is present and TSH levels are restored to within the normal range (Woeber, 2005; Daniels and Dayan, 2006)

2.4.2.3 Subclinical hypothyroidism (SCH):

Subclinical hypothyroidism (SCH) is defined as a serum thyroid-stimulating hormone(TSH) level above the upper limit of normal despite normal levels of serum free thyroxin. Serum TSH has a log-linear relationship with circulating thyroid hormone levels (a 2-fold change in free thyroxin will produce a 100-fold change in TSH). Thus, serum TSH measurement is the necessary test for diagnosis of mild thyroid failure when the peripheral thyroid hormone levels are within normal laboratory range, Subclinical Hypothyroidism (SCH) also is described as absence of hypothyroidism symptoms with normal FT4 And elevated TSH Values

Although there's no consensus over upper normal limit of TSH, Currently some authors suggest 2.5mlU/L (Karaca andAkpak, 2015).

Subclinical hypothyroidism or mild thyroid failure is a common problem, with a prevalence of 3% to 8% in the population without known thyroid disease, The prevalence increases with age and is higher in women. After the sixth decade of life, the prevalence in men approaches that of women, with a combined prevalence of 10% (Bennett *et al.*, 2005)In congenital neonatal hypothyroidism hypothermia, bradycardia, jaundice, feeding unwillingness, apathy, voice hoarseness, constipation and are mainly observed. However, in early stages there may be few symptoms. Thus, measurement of thyroid hormones is considered necessary. In children growth retardation, mental retardation, voice hoarseness, constipation and either retarded or premature sexual maturation are mainly observed. Diagnosis and management of congenital hypothyroidism should be performed with caution ((Karmisholt *etal.*, 2008;)

2.5 Goiter:

A goiter, or goiter, is a swelling in the neck resulting from an enlarged thyroid gland.[1]"Thyroid Nodules and Swellings".*(British Thyroid Foundation.2019)*] A goiter can be associated with a thyroid that is not functioning properly. Worldwide, over 90% of goiter cases are caused by iodine deficiency The term is from the Latin gutturia, meaning throat. Most goiters are not cancerous (benign), though they may be potentially harmful. (NHS Choices. 2017).

The thyroid is the main site of iodine uptake in the body, which is then incorporated into TH. The highest iodine content is found in fish, with smaller amounts in milk, eggs and meat (Vanderpump, 2011).

Iodine deficiency is typically due to the consumption of local produce in areas where the soil has low iodine content (such as high mountainous

areas and lowlands situated far from the oceans). The recommended daily intake of iodine for adults is 150-300micrograms (mcg). Reduced iodine intake is the principal cause of thyroid disease worldwide (Hermus and Huysmans, 2005). intake of <50mcg/day of iodine is associated with reduced thyroid function (either hypothyroidism in adults or cretinism in the presence of inadequate intake from birth) or goiter (diffuse or nodular enlargement of the gland) whereby the gland size increases to compensate for the lower iodine in an effort to maintain normal TH levels (Vanderpump,2011; Franklyn and Boelaert, 2012).

2.5.1: Signs and symptoms of goiter:

A goiter can present as a palpable or visible enlargement of the thyroid gland at the base of the neck. A goiter, if associated with hypothyroidism or hyperthyroidism, may be present with symptoms of the underlying disorder. For hyperthyroidism, the most common symptoms are associated with adrenergic stimulation: tachycardia (increased heart rate), palpitations, nervousness, tremor, increased blood pressure and heat intolerance. Clinical manifestations are often related to hyper metabolism (increased metabolism), excessive thyroid hormone, an increase in oxygen consumption, metabolic changes in protein metabolism, immunologic stimulation of diffuse goiter, and ocular changes (exophthalmos), Hypothyroid people commonly have poor appetite, cold intolerance, constipation, lethargy and may undergo weight gain. However, these symptoms are often non-specific and make diagnosis difficult, According to the WHO classification of goiter by palpation, the severity of goiter is currently graded as grade 0, grade 1, grade 2palpable, however, not visible in normal position of the neck; the thickened mass moves upwards during swallowing. Grade 1 includes also nodular goiter if thyroid enlargement remains invisible. Grade 2 – neck swelling, visible

when the neck is in normal position, corresponding to enlarged thyroid – found in palpation. (World Health Organization. 2014.)

2.5.2 Causes of goiter:

Worldwide, the most common cause for goiter is iodine deficiency, commonly seen in countries that scarcely use iodized salt. Selenium deficiency is also considered a contributing factor. In countries that use iodized salt, Hashimoto's thyroiditis is the most common cause. Goiter can also result from cyanide poisoning, which is particularly common in tropical countries where people eat the cyanide-rich cassava root as the staple food. (Toxicological Profile For Cyanide 2017)"

Causes of goiter include:

Iodine deficiency: Thyroid gland needs iodine to produce thyroid hormone. If you don't get enough iodine in your diet, your thyroid makes more cells (and grows) to try to make more thyroid hormone. While this is the most common cause of goiter worldwide, it's not common in the United States. You can get the recommended amount of iodine in your diet by including sea food, dairy products and iodized salt in your diet. Supplementation with iodine is not recommended for most people and may have unintended negative effects on your health, Graves' disease: Graves' disease is an autoimmune disease in which your immune system attacks your thyroid, causing it to grow larger. Graves' disease also causes hyperthyroidism, which requires treatment.

Hashimoto's disease: This is an autoimmune disease that causes inflammation of thyroid gland. Some people with Hashimoto's disease develop a compensatory increase in the thyroid gland's size. This type of goiter usually gets better on its own over time. Some cases of

Hashimoto's disease require treatment with thyroid hormone, Thyroid cancer: Cancer of the thyroid gland often enlarges thyroid gland, Pregnancy: Human chorionic gonadotropin, a hormone that a person produces during pregnancy, can cause their thyroid to grow,

Thyroiditis: Inflammation of the thyroid gland itself can cause your thyroid gland to grow, This can happen for several reasons, Sporadic goiters, in most cases, have no known cause. In some cases, certain drugs can cause this type of goiter. For example, the drug lithium, which is used to treat certain mental health conditions, as well as other medical conditions, can cause this type of goiter. (Women's health.gov. Thyroid disease 2022)

2.5.3 Diagnosis of goiter:

Your healthcare provider usually diagnoses goiter when they perform a physical examination and feel that you have an enlarged thyroid. However, the presence of a goiter indicates that there's an issue with your thyroid gland. They'll need to figure out what the issue is your provider can use several tests to diagnose and evaluate goiter, including the following:

Physical exam: Your provider may be able to tell if your thyroid gland is enlarged by feeling your neck area for nodules and signs of tenderness.

Thyroid blood test: This blood test measures thyroid hormone levels, which reveal if your thyroid is working properly.

Antibody test: This blood test looks for certain antibodies that are produced in some forms of goiter. An antibody is a protein made by white blood cells. Antibodies help defend against invaders (for example, viruses) that cause disease or infection in the body,

Thyroid ultrasound: Ultrasound is a procedure that sends high-frequency sound waves through body tissues. The echoes are recorded and transformed into video or photos. Your provider can "see" your thyroid to check its size and if it has nodules.

Biopsy: A biopsy is the removal of a sample of tissue or cells to be studied in a laboratory. You may need a thyroid biopsy if there are large nodules in your thyroid gland. A biopsy is taken to rule out cancer. Thyroid uptake and scan: This imaging test provides information on the size and function of the thyroid. In this test, a small amount of radioactive material is injected into a vein to produce an image of the thyroid on a computer screen. Providers don't order this test very often, since it's only useful in certain circumstances,

CT scan or MRI (magnetic resonance imaging) of your thyroid: If the goiter is very large or spreads into your chest, a CT scan or MRI is used to measure the size and spread of the goiter.*(*https://medlineplus.gov/ency/article/001178.htm 2022*)*

2.5.3 Management and Treatment of goiter:

A simple goiter may happen for only a short time and may go away on its own without treatment. Many goiters, such as multinodular goiter, are associated with normal levels of thyroid hormone. These goiters usually don't require any specific treatment after your healthcare provider has diagnosed it. However, you may be at risk of developing hypothyroidism or hyperthyroidism in the future. If you have an enlarged thyroid gland, it's still important to see your healthcare provider since goiters have multiple possible causes — some of which require treatment. (Can AS, Rehman 2022).

Treatment for goiter depends on how large your thyroid has grown, symptoms and what caused it. Treatments include:

No treatment/"watchful waiting": If the goiter is small and isn't bothering you, your healthcare provider may decide that it doesn't need to be treated. However, they'll carefully monitor your thyroid for any changes.

Medications : Levothyroxine (Levothroid, Synthroid) is a thyroid hormone replacement therapy. Your provider will likely prescribe it if the cause of the goiter is an underactive thyroid (hypothyroidism). Other medications are prescribed if the cause of the goiter is an overactive thyroid (hyperthyroidism). These drugs include methimazole (Tapazole) and propyl thiouracil. Your provider might prescribe aspirin or a corticosteroid medication if the goiter is caused by inflammation.

Radioactive iodine treatment: This treatment used in cases of an overactive thyroid gland, involves taking radioactive iodine orally. The iodine goes to your thyroid gland and kills thyroid cells, which shrinks the gland. After radioactive iodine treatment, you'll likely need to take thyroid hormone replacement therapy for the rest of your life.

Surgery: Your provider may recommend surgery to remove all or part of your thyroid gland (thyroidectomy). You may need surgery if the goiter is large and causes problems with breathing and swallowing. Surgery is also sometimes used to remove nodules. Surgery must be done if cancer is present. Depending on the amount of thyroid gland removed, you may need to take thyroid hormone replacement therapy for the rest of your life. (Can AS, Rehman 2022)

2.5.4 Prevention from goiter:

A goiter caused by iodine deficiency (simple goiter) is generally the only type of goiter you can prevent. Consuming a diet that includes fish, dairy and a healthy amount of iodized table salt prevents these types of goiters. Iodine supplements and other supplements are generally not recommended and may do more harm than good. The prognosis (outlook) for goiter depends on its type and what caused it. Simple goiter has a good prognosis. If your thyroid continues to enlarge, it may compress the surrounding structures and may cause difficulty in breathing and swallowing and hoarseness. If the goiter is a sign of another thyroid disease like Graves' disease or Hashimoto's disease, the prognosis depends on the underlying cause of thyroid enlargement. (American Thyroid Association2022)

2.6 Thyroid nodule:

A thyroid nodule is a palpable swelling in a thyroid gland with an other wise normal appearance. Thyroid nodules are common and may be caused by a variety of thyroid disorders. While most are benign, about 5 percent of all palpable nodules are malignant, Thyroid nodules are four times more common in women than in men and occur more often in people who live in geographic areas with iodine deficiency. After exposure to ionizing radiation, thyroid nodules develop at a rate of 2 percent annually, Most patients presenting with a solitary thyroid nodule are euthyroid, Thyroid status is confirmed by thyroid function tests, which include measurement of free thyroxine (T4),thyroid-stimulating hormone (TSH), and free triiodothyronine (T3) in appropriate case, The causes of thyroid nodules are iodine deficiency, thyroid adenomas (autonomous or hyper functional thyroid nodules) and thyroid cyst. Thyroid cancer and nodules are common and can occur in up to 60% of

the population. Fine needle aspiration cytology (FNAC) in thyroid nodules has higher sensitivity and is a rapid, cost-effective, and very useful method for classifying thyroid nodules as either benign nodules, reducing unnecessary surgery, or malignant nodules requiring surgery. There have been several guidelines or indications on when to perform FNAC in thyroid Nodules. (Daniel Asmelash.2019)

2.7 Thyroid cancer:

Thyroid cancer (TC) is the most common endocrine malignancy in thyroid tissues, which is considered to be related to hormone levels and genetic predisposition factors , Due to the characteristics of abnormal cell proliferation and the possibility of spreading to other parts of the body, the incidence of TC has increased rapidly in recent decades. According to the surveillance of National Cancer Institute, the epidemiology survey indicated that TC patients accounted for 3.4% of all annual diagnoses . As of 2015, 3.2 million people worldwide suffered from TC. In differentiated thyroid cancer, about 4% of patients have distant metastasis at diagnosis, while another 7% to 23% of patients develop metastatic disease within five years. The lymph nodes, lungs, bones, and brain are common metastatic sites for TC cancer cells (Zhenguo Sun 2021).

Thyroid-stimulating hormone receptor (TSHR) is a G-protein-coupled receptor for thyrostimulin and thyroid-stimulating hormone TSHR activation leads to the stimulation of adenylyl cyclase that produces the second messenger cyclic adenosine monophosphate (cAMP), which activates protein kinase A. *TSHR* transcripts are abundant in thyroid follicular cells , *TSHR* is also expressed in some extra-thyroid tissues and in some cancers (Valentina Taglietti,2023)

Thyroid cancer accounts for about 0.5-1% of all malignant tumors in the human body, which is a lowly malignant tumor with a relatively good prognosis , The number of female patients with thyroid cancer is about 3

times that of male patients, and the risk of onset increases in postmenopausal women which may be related to the body's disorders of sex hormone levels and the endocrine system, The onset of thyroid cancer may be related to radiation, familial inheritance and genetic mutation, In addition, immune factors, such as cytokine levels also have an important impact on the development of the disease ,Gene polymorphism is an important factor regulating susceptibility to various diseases. The differences in the same allele of different individuals may be the driving factor for changing various pathological processes in the body , Gene polymorphism can also affect the growth and development of malignant tumor cells in different individuals. Studies have demonstrated that the prognosis of thyroid cancer is related to MALAT1 rs 619586 ,The TERT rs10069690 , and IL-6 gene polymorphism are related to the thyroid cancer risk. The cytotoxic T lymphocyte associate protein-4 (CTLA-4) gene (a regulatory molecule for immune checkpoint) polymorphism may have a strong association with susceptibility and development of thyroid cancer, immune-related cancer (Liping Sun1*, Tianfeng Niu2, Yi Zhang 2023).

The incidence of thyroid tumours has been sharply growing around the world, identification and characterization the risk factors for thyroid cancer and nodules have become a scientific hot topic in recent years. Thyroid tumours are divided into benign thyroid adenoma and malignant thyroid cancer (TC), and thyroid nodules (TNs) are a common benign proliferative disease of the thyroid gland with the detection rate in the general population as high as 65%(Huirong Wang1,2022)

2.8 Thyroid Function Testing:

Thyroid hormones are essential for energy metabolism and act on almost all cells. Thyroid dysfunction is associated with secondary cardio vascular, mental health, ophthalmic and other disease. Hypothyroidism has a high prevalence and is most commonly due to autoimmune (Hashimoto) thyroiditis, in areas where iodine intake is sufficient. Hyperthyroidism, prevalence 0.2–1.3%, is most commonly due to autoimmune (Graves) disease or toxic nodular goiter. Ageing, diet (including iodine deficiency), smoking status, genetic susceptibility, ethnicity, and endocrine disruptors are risk factors for thyroid diseases; defining genetic variants, genes, proteins and pathways associated with hypothyroidism and hyperthyroidism will inform a deeper understanding of the mechanisms of thyroid disease and inform prevention and treatment strategies (Alexander T.Williams2023).

In patients with overt hypothyroidism, the lack of T4 feedback leads to TSH levels >20mIU/L, whereas in milder or subclinical hypothyroidism the TSH levels are between 3and 20 mIU/L with normal T4 and T3 levels, In contrast, all forms of hyperthyroidism are accompanied by TSH levels that are suppressed to <0.1 mIU/L. Thus the TSH test is the appropriate initial test to screen for thyroid dysfunction in a variety of clinical situations known to be affected by thyroid disease as well as to confirm a suspected diagnosis and follow the response to treatment. Various authors have suggested that the reference range for TSH be narrowed especially with regard to the upper limit at which hypothyroidism may be present (Paschke and Ludgate, 2007).

Fine needle aspiration biopsy (FNAB) is the most important step in the workup of the thyroid nodule, as cytology is the primary determinant in whether thyroidectomy is indicated. FNAB is widely available and well tolerated, with a low risk of complications, Its use has dramatically

decreased the number of thyroidectomies performed, and improved the yield of malignancy in glands that have been extirpated FNAB can be performed with or without ultrasound guidance, but diagnostic accuracy is improved using sonographic needle localization due to a decreased number of inadequate specimens and false negative results (Danese *et al*., 2008).

Ultrasonography is the imaging study of choice for thyroid nodules. It can identify nodules too small to be palpated, the presence of multiple nodules, central, or lateral neck lymphadenopathy, and provides accurate measurements of nodule diameter for interval monitoring. Additionally, it allows characterization of nodules by sonographic features which suggest malignancy. Solid appearance (or hypo echogenicity), increased vascularity, micro calcifications, irregular margins, and the absence of a halo are features that have been consistently associated with malignancy (Papini *et al.*, 2016; Fish *et al*.,2008).

2.8.1 Diagnosis of hyperthyroidism:

Measurement of serum TSH has the highest sensitivity for diagnosis and is the most appropriate screening test to exclude thyrotoxicosis. In a sensitive assay, serum TSH will be undetectable (commonly reported as <0.01 mIU/L) because of negative feedback of thyroid hormones on the anterior pituitary. Diagnostic accuracy is improved if free T4serum concentration is measured at the same time. Free T4 concentrations are raised in nearly all cases of over hyperthyroidism, although if free T4 is normal and TSH is low, free or total T3 concentration should also be measured to identify potential T3 toxicosis (Franklyn &Boelaert, 2012).

In some circumstances, such as during pregnancy, physiological increases in thyroxine binding globulin result in inaccurate free T4 and free T3 measurements, so calculation of the free T4 index could be helpful (Lee *et al*., 2009). Once the thyrotoxic state is established, measurement of the

uptake of a 123I tracer is recommended to allow the stratification of patients (Iagaru& McDougall, 2007).

A diagnosis of Grave's' hyperthyroidism can be confirmed by measurement of TSHR antibodies (new technologies can provide sensitive and specific results), but this test is not widely used. Thyroid peroxidase antibodies are present in about 75% of cases of Grave's' hyperthyroidism and could help to differentiate autoimmune disease from toxic nodular hyperthyroidism (Franklyn &Boelaert, 2012).

2.8.2 Diagnosis of hypothyroidism:

TSH and FT4 measurement are the laboratory examinations necessary for the diagnosis of hypothyroidism and the differential diagnosis between primary (clinical or subclinical) and secondary one, When TSH is increased and FT4 is decreased or normal hypothyroidism is primary. In this case increased anti-TPO or anti-Tg antibodies point to the cause of hypothyroidism, which is autoimmune thyroiditis. Primary hypothyroidism is divided in clinical when TSH is increased and FT4 is decreased and in subclinical when TSH is increased and FT4 is normal. When TSH is normal or decreased and FT4 is low hypothyroidism is secondary (central). In order to discriminate whether the cause is in the pituitary or the hypothalamus a test with the TSH releasing factor is performed (TRH test). In the first case the response is normal, while in the second it is abnormal. In central hypothyroidism imaging studies of the brain and the pituitary are performed aiming at finding its cause. Usually the reported normal limits of TSH are between 0.4-4.0 mU/l.

When TSH is found in the upper normal limits it may show mild hypothyroidism which may progress to hypothyroidism, especially if antibodies are increased (Kostoglou-Athanassiou&Ntalles, 2010).

2.9 Genetic Aspects of thyroid function:

Genetics play a prominent role in both determinations of thyroid hormone and thyrotropin(TSH) concentrations. Heritability studies have suggested that up to 67% of circulating thyroid hormone and TSH concentrations are genetically determined, suggesting a genetic basis for narrow intra-individual variation in levels, perhaps a genetic 'set point'.

The search for the genes responsible has revealed several candidates, including the genes for phosphodiesterase 8B(*PDE8B*), iodothyronine deiodinase 1(*DIO1*), F-actin-capping protein subunit beta (*CAPZB*) and the *TSH* receptor; however, each of these only contributes a small amount to the variability of hormone concentrations, suggesting that further genes and mechanisms of genetic influence are yet to be discovered (Panicker,2011).

2.9.1 Type 1iodothyroinine gene and protein:

Iodine is the essential micronutrient for thyroid hormone biosynthesis in humans. The impact of this biosynthesis of thyroid hormones is to ensure the availability of iodine in the thyroid gland homeostasis. Biosynthesis of thyroid hormones consists of two steps. The first step is the activation of membrane transporters by the Sodium/Natrium Iodine Symporter (*NIS*) gene to accumulate iodine in the thyroid cells. The second is the recycling of iodine through monoiodotyrosine (T1) and diiodotyrosine (T2) deiodination by iodothyronine deiodinase. This second step is the main product of thyroid hormone synthesis . Iodothyronine deiodinase is an enzyme that regulates thyroid hormone activity and plays a role in activating thyroid hormone precursor in the form of thyroxine (T4) into the active form of triiodothyronine (T3), by reducing the iodine-specific group of T4 precursor molecules. It also plays a role in thyroid hormone inactivation by converting T3 hormone into a reverse form of T3 (rT3),

an inactive form, when an excess level of T3 occurs. The availability of T3 is primarily regulated by three different seleno deiodinases, which are deiodinase 1 (D1), 2 (D2) and 3 (D3), and each of them plays a different physiological role (Agus Wibowo 2022).

(*DIO1*), product of the *DIO1* gene catalyzes two types of deiodination reaction, an outer ring(5′-deiodination - 5′D) and an inner-ring (5-deiodination - 5D). These processes result, respectively, in the activation and inactivation of thyroid hormones (Bianco *et al.*,2002).

The *DIO1* gene polymorphism, located in the 3′untranslated region of the *DIO1* gene (chr1p33-p32), is a substitution of C with T at position 785 of the gene (C785T-GI 4557521;rs11206244. This punctiform mutation is associated with a decreased activity of the type1 iodothyronine deiodinase enzyme and influences serum thyroid hormone levels, FT3 and FT4 (Procopciu*et al.,* 2012).

The three iodothyronine deiodinases play an important role in thyroid hormone action and are likely to influence serum and local tissue concentrations of T4 and T3. A candidate gene study of the genes using Hap Map to identify SNPs representing most of the variation across genes discovered a SNP, rs2235544, in the gene coding for D1(*DIO1*) associated with circulating free T3/free T4 ratio, free T4 and rT3 concentrations(Panicker *et al.,* 2008).

Roef *et al* found significant associations were observed between different single nucleotide polymorphisms (SNPs) in the thyroid pathway and TSH, FT4, ratio FT3:FT4,and rT3. Nevertheless, these SNPs only explain a limited part of the heredity .A total of nine SNPs were determined. (Log)TSH is highly, significantly, positively associated with the presence of rs4704397 in *PDE8B* (explaining 1.5% of variation in an unadjusted model). The other SNP in *TSHR*, rs1991517, does not show associations with TH concentrations. Significant associations with FT4 concentrations

are observed for two SNPs in *DIO1*; a positive association for rs11206244 and a negative association forrs2235544 (both explaining 0.5% of variation) (Roef *et al.*, 2013).De Jong *et al* Studied the association of polymorphisms in the *DIO1* (D1a-C/T, D1b-A/G) and *DIO2* (D2-ORFa-Gly3Asp, D2-Thr92Ala) genes with circulating thyroid parameters and early neuroimaging markers of Alzheimer disease. Carriers of the *D1a*-Tallele had higher serum free T4 and reverse rT3, lower T3, and lower T3/rT3. The *D1b*-Gallele was associated with higher serum T3 and T3/rT3 (De Jong *et al*., 2007).

Procopciu*cet al* investigated the biochemical and genetic thyroid status in women with preeclampsia by the determination of serum FT3 and FT4 levels in association with *D1-C785T* genotypes. FT3 levels were low, and FT4 levels were high in women with preeclampsia compared to normal pregnant women. The association with severe preeclampsia was stronger for the homozygous T/T genotype. Women with preeclampsia with the *D1-T785* mutated allele had lower FT3 levels, higher FT4 levels than women with preeclampsia with the *D1-C/C* genotype. Significant decrease in serum FT3 levels in positive women with severe preeclampsia compared to women negative for this genetic was observed (Procopciu*cet al.*, 2012).

Philibert *et al* genotyped 12 single nucleotide polymorphisms identified in previous genome wide association analyses of thyroid function in DNA contributed by 1555subjects from three longitudinal ethnically diverse studies that are well characterized for lifetime major depression and thyroid function. We then examined associations between genetic variants and key outcomes of thyroid stimulating hormone (TSH), free thyroxine(FT4) and depression. We confirmed prior findings that two variants in deiodinase1(*DIO1*), including a variant in the 3' UTR of

DIO1 (rs11206244), were associated with altered free thyroxine (FT4) levels in both White and African American subjects. We also found that rs11206244 genotype was associated with lifetime Major depression (MD) in White female subjects, in particular those from high-risk cohorts. However, we found no association of current FT4 levels with lifetime MD in either ethnic group. We conclude that genetic variation influencing thyroid function is a risk factor for MD. Given the evidence from prior studies, further investigations of role of HPT variation in etiology and treatment of MD are indicated (Philibert *et al.,* 2011).

Wibowo *et al* their results showed polymorphism of *D1-C/T* were found at one subject that diagnosed with hypothyroid and two subjects with subclinical hypothyroid. But there was no polymorphism at *D1 A/G* and D3. Two types of polymorphisms were found inD2. Ratio of fT3/fT4 in hypothyroid subject was higher than others (Wibowo *et al.*,2015).

2.9.2 Phosphodiesterase 8B (PDE8B) gene and protein:

Different cohort studies reported phosphodiesterase 8B (*PDE8B*) as a genetic modulator of TSH levels. PDE8B gene encodes a cyclic adenosine monophosphate (cAMP) specific phosphodiesterase (PDE) enzyme, *PDE8B* affects cAMP levels in the thyroid gland resulting in changes in the levels of thyroid hormones, which in turn affects the release of TSH from the pituitary gland. *PDE8B* is mainly expressed in thyroid and brain , Several single nucleotide polymorphisms (SNPs) for *PDE8B* have been demonstrated to associate with increased levels of serum TSH. More than 360,000 SNPs were tested for their associations with serum TSH levels with an additive model. The obtained results revealed three SNPs (i.e. rs4704397, rs6885099 and rs2046045) with genome-wide significance (P<10-10). These three SNPs were reported to be in strong linkage disequilibrium, Of the three SNPs, rs4704397 showed strongest association and it could explain 2.3% of the variations

in TSH levels, *PDE8B* rs4704397 polymorphism has been found to associate with myocardial infarction, height, pregnancy, recurrent miscarriage and obesity in children, apart from thyroid function. Another *PDE8B* polymorphism, rs6885099 has also been shown to increase TSH levels, but to a lesser extent, in different

populations, The relevance of human reproduction to PDE has been well-documented, While the underlying mechanism regulating oocyte maturation is not clearly known yet, the second messenger cyclic adenosine monophosphate (cAMP) role in oocyte maturation is well known, and thus research investigating the role of rs4704397 in the oocyte maturation might give an insight to primary infertility caused by hypothyroidism (Tabassum Mansuri, 2020)

The human phosphodiesterase type 8B (*PDE8B*) gene is located at human chromosome5q14.1 in intron 1 and encodes a high affinity cyclic adenosine monophosphate (cAMP)specific nucleotide phosphodiesterase The *PDE8B* gene is abundantly expressed in the thyroid but has also been detected in human placenta and ovaries . Based on a recent genome-wide association study, six different single nucleotide polymorphisms (SNP) in the *PDE8B* gene were associated with increased serum concentrations of thyroid stimulating hormone (TSH) (Granfors *et al.*, 2012).

(*PDE8B*) is found on chromosome 5 encodes a protein which catalysis the hydrolysis and inactivation of cyclic AMP (cAMP). performed a (genome-wide association studies)GWAS and discovered an A>G SNP (rs4704397) within this gene to be associated with circulating TSH concentrations, each copy of the rarer A allele conferring a mean increase of 0.13 mU/L TSH. The strongest association with increased TSH levels (although in the normal range) was reported for one specific SNP in *PDE8B*, rs4704397,which is found in the promoter region of the gene (Arnaud-Lopez *et al.*, 2008; Horvath *et al*., 2010) resulting in a difference

between the major and minor homozygote subjects of 0.25 mIU/L TSH. This SNP has been associated with subclinical hypothyroidism (Shields *et al*., 2009). In SNP rs 4704397 of *PDE8B* an adenine (A) nucleotide is replaced by a guanine (G).The association between the polymorphism and high levels of TSH and low free T4levels, indicating relative hypothyroidism, is found in homozygous carriers of A/A(Taylor *et al.*, 2011). Based on previous results it has been proposed that the SNP rs 4704397 in PDE8B and in particular the presence of A alleles might induce increased phosphodiesterase activity inPDE8B, thereby reducing the ability of the thyroid gland to generate free T4 when stimulated by TSH (Arnaud-Lopez *et al*., 2008).

JORDE *et al* From the Tromso Study, 8938 subjects without thyroid disease or thyroid medication were successfully genotyped for rs4704397. Among these, 2098 were registered with MI, 1025 with T2DM, 2748 with cancer, and 3592 had died. The minor homozygote genotype (A:A) had a median serum TSH level that was 0.29 mIU/L higher than in the major homozygote genotype (G:G) There was a trend for a reciprocal association with FT3 and Ft4 with the genotype A:A having the lowest level. However, this was not statistically significant (JORDE *et al.*, 2013).

Arnaud-Lopez *et al* genotyping 362,129 SNPs in 4,300 Sardinians, we identified a strong association (p=1.3×10-11) between alleles of rs4704397 and circulating TSH levels; each additional copy of the minor A allele was associated with an increase of 0.13 mIU/ml in TSH (Arnaud-Lopez *et al.*, 2008).

Shields *et al* Found TSH, but not FT4, FT3, or TPO Abs, varied with genotype and was highest in those with the AA genotype (median, 2.16, 1.84, and 1.73 mIU/liter for AA,AG, and GG genotypes, respectively; *P* _0.0004). A greater proportion of women with the AA genotype had TSH

concentrations above 4.21 mIU/liter, the upper limit of the reference range, compared with the AG and GG genotypes (9.6 *vs*. 3.5%, respectively; *P*_ 0.004) (Shields*et al.,* 2009).

Grandone *et al* Found *PDE8B* A/A homozygous subjects showed higher TSH (PZ0.0005)compared with A/G or G/G. No differences were found for peripheral thyroid hormones, Among A/A children, 22% had hyperthyrotropinaemia, compared with 11.6% of heterozygotes and 10.8% of G/G (Grandone *et al.,* 2012).

Groussin, *et al* Measured allelic frequencies at the 4 loci Four snps at the *PDE8B* gene(rs4704397, rs6453293, rs4361497 and rs13158164) - and confirmed a higher frequency of the alleles associated with higher TSH levels in patients with non toxic multinodular goiter (p=0,04) as well as in patients with papillary cancer (p=0,022). Interestingly, the small group of patients with hypersecreting thyroid tumors had a significantly higher frequency of the alleles associated with lower TSH plasma levels in 3 out of the 4 SNPs (Groussin, *et al.*, 2012).

Taylor *et al* Confirmed that genetic variation in *PDE8B* was associated with TSH, However, the additional power available to them in this meta-analysis enabled them to detect that this SNP is also reciprocally associated with free T4 levels. For each additional minor A allele at this SNP, there was an increase in TSH levels and a reduction in free T4 levels, indicating relative hypothyroidism (Taylor *et al.,* 2011).

2.9.3 *TSH* receptor gene and protein:

The *TSHR* is a G protein-coupled receptor and shares the classic structure of the serpentine receptor family (i.e. seven membrane spanning segments, three extracellular loops, three intracellular loops, an amino terminal ectodomain and an intracellular carboxy terminal), The hormonal binding specificity of the receptor is determined by the ectodomain or a subunit (Kleinau and Krause, 2009).

Whilst coupling to the G protein is via the serpentine portion. The *TSHR* is encoded by ten exons located on chromosome 14 and is coupled mainly to the subunit of the stimulatory guanine-nucleotide-binding protein. In the thyroid, ligand binding predominantly activates adenylate cyclase with a resultant increase in the intracellular concentration of cAMP. Stimulation of the *TSHR* via this cAMP second messenger system regulates the transcription of genes central to thyroid hormone synthesis. Recent studies have illustrated the potential heterogeneity of signaling via the *TSHR* either as the consequence of coupling to other G proteins (G q/G11) or as a result of cascades stimulated by the liberated G protein b/g subunits. Thus TSHR activation can up-regulate kinases such as phosphoinositide-3 kinase and P70S6K and increase concentrations of the second messenger's inositol-phosphate (IP) and diacylglycerol. Chronic stimulation of the *TSHR* leads to over activation of the cAMP pathway that in turn causes thyroid hyperplasia and hyperthyroidism (Kero *et al.*, 2007; Zaballos*et al*., 2008;)

These mutations are located predominantly in exon 10, which encodes the serpentine portion of the *TSHR*. All activating mutations induce an increase in cAMP levels in the absence of TSH but retain TSH responsiveness. The phenotype can vary according to the specific germ line mutation but also between individuals harboring the same point mutation (for example the age of onset of hyperthyroidism). This variation is likely to reflect epigenetic and environmental factors in addition to the inherent biological activity of the particular mutant form (e.g. a minority of mutations will increase both cAMP and IP3 concentrations (Fuhrer *et al*., 2003).

A number of polymorphisms are detected within the *TSHR* gene including those that affect the coding region. There are three germ-line polymorphisms resulting in amino acid substitutions. Two of them are

found in the extracellular domain of the receptor molecule (D36Hand P52T), and the third (D727E) is located within the intracellular tail of the receptor. The D727E dimorphism shows an association with a significantly higher cyclic adenosine 3´, 5´-monophosphate (cAMP) response to TSH stimulation in vitro than the wild-type receptor (Chistiakov, 2003).

These families have been found to exhibit varying degrees of TSH resistance (as is reflected in the thyroid function test results; extent of the TSH increase and/or thyroid hormone deficiency), which correlates with the clinical phenotype. Individuals with partial resistance to TSH usually retain some *TSHR* function (Alberti *et al*., 2002; Jordan *et al*., 2003).

Muhlberg *et al* They found no significant differences in codon 727 polymorphism frequencies between patients with autonomously functioning thyroid disorders (13.3%)and the healthy control group (16.2%; $P = 0.57$). Moreover, the subtypes of toxic non autoimmune thyroid disease (toxic adenoma, 13.2%; multinodular goiter, 9.6%; disseminated autonomy, 21.4%) were not related to significant differences in codon 727polymorphism frequencies compared with the healthy control group ($P = 0.67$, $P = 0.40$, and $P = 0.70$, respectively) (Muhlberg *et al.*, 2000).

Procopciuc *et al* found all women with severe preeclampsia had the Asp/Asp genotype. The risk for preeclampsia in association with TSH44 mU/ml and Asp/Asp genotype is20.8 (p50.01). Preeclamptic women with TSH levels44 mU/ml and the Asp/Asp genotype delivered earlier neonates with lower birth weight than preeclamptic women with TSH levels54 mU/ml and the Asp/Glu genotype (Procopciuc*et al*., 2011).

Tug *et al* they found the CC and CG genotype incidence for the patient group to be 0.71and 0.29, respectively, and for the control group to be 0.8 and 0.2, respectively. No statistically significant difference was found

between the genotype and allele distribution of both groups (p = 0.417 and p = 0.449, respectively). However, the polymorphism is significantly correlated with the low serum level of the TSH (p = 0.047) (Tug *et al*., 2012). Ma *et al* they made Sequencing of *TSHR* gene revealed a homozygous mutation (CGC--> CAC, Arg450His) and a polymorphism (GAC --> GAG, Asp727Glu). The controls revealed no variants. The 12 relatives of the prob and were enrolled and investigated. Six relatives, including his mother and father, were heterozygous for R450H mutation andD727E polymorphism of the *TSHR* gene. Thyroid hormone levels were normal except for circulating TSH (5.96-6.92 mU/L) level slightly elevated in six heterozygous family members (Ma *et al.*, 2010).

Chapter three

Methodology

3. Materials and Methods

3.1 Study design:

Analytical cross-sectional non-interventional hospital-based approach

3.2 Study area and period:

The study was conducted at Rabak and Kosti hospital in White Nile state in Sudan during the period from June 2019 to September 2024

3.3 Study population:

One hundred and fifty three patients with goiter were enrolled in this study which classified as Seventy simple diffuse goiter, thirty three nodular goiter, fifty euthyroid goiter and fifty subjects as healthy control**.**

3.3.1 Inclusion criteria:

Patients diagnosed with goiter (simple diffuse goiter, nodular goiter and euthyroid goiter)

3.3.2 Exclusion criteria:

Patients with goiter under treatment and exclusion criteria for patients and controls with hypertension, alcoholism, smoking, diabetes, cardiovascular disease, liver disease and taking of any vitamin and minerals

3.4 Sample size:

Sample size was calculated according to formula: $N= Z^2*P*(1-P)/D^2$

N= Number of samples

Z= Confidence interval which = 1.96

P= Prevalence of the disease

Q= (1-P)

D= Percentage of error which equal 0.05%

Due to financial consideration the sample size of current study was 168 samples

3.5 Ethical consideration:

Permission of this study obtained from the local authorities in the area of the study. The objectives of the study were explained to the local

authorities in the area of the study and to all individual in the study. A written consent obtained from each participates in this study. (Appendix)

Methods:

3.6 Data collection:

Data were collected through a structured questionnaire (see appendix 1) was specifically designed to obtain information which helps in either including or excluding criteria.

3.7 Laboratory Methods:

3.7.1. Specimen collection:

Sample were collected during the period of 2019 to 2020. Blood sample obtained by using local anti septic for skin (70% ethanol) 3ml of venous blood collected from cases and controls using a disposable sterile plastic syringe. The blood was collected from cubical vein or the back of the hand. Serum was separated from blood cells after centrifugation for 10 minutes at 5000 r.p.m (round per minute) at room temperature and serum was obtained. The serum collected and kept at 80◦c for 6 months.

Serum sample obtained was subjected to microplate competitive enzyme immunoassay for free T3 and free T4 assay, TSH was measured by microplate immunoenzymatic assay. Also 3 ml was collected in container with EDTA anti coagulant for DNA extraction for PCR (polymerase chain reaction) technique.

Serum levels of thyroid hormones (freeT3 and freeT4) and TSH were measured in each participant using ELISA (Enzyme Linked Immuno sorbent Assay) technique by (Rayto Microplate Reader- RT-2100C-Germany)

3.7.2 Laboratory tests: ELISA Technique and Molecular Techniques (DNA extraction, PCR, RFLP analysis)

3.7.2.1 Equipments:

ELISA machine, Autoclave, Microcentrifuge, Microwave oven, pH

meter, weighin balance. water path vortex, Agarose gel Electrophoresis unit, Law voltage electrophoresis power supply Translaminator, Incubator, PCR Thermocycler, Vortex mixer, Raks, eppendorf tubes, droppers, Micropipettes (P10, 20, 50, 100, 1000µl), Steril DNase free pipette tips, Laboratory Parafilm.

3.7.3 ELISA Technique:

Serum levels of thyroid hormones (free T3, free T4& TSH) were measured in each participant using ELISA (Enzyme Linked Immune Sorbent Assay) technique (Omega Diagnostic).

All samples were divided into 50 controls, 150 patients (70: simple goiter, 30: nodulr goiter & 30: euthyroid goiter)

3.7.3.1 Measurement of free T3 using ELISA technique:

Procedure

1. All the kit components and the test serum had been brought to room temperature (20oC to 25oC) prior to the start of the assy.
2. One set of Standards had been run with each batch of test serum.
3. 50µl of standard, control or test serum had been dispensed into the assigned well.
4. 100µl of Triiodothyronine Conjugate Solution had been dispensed, to all wells. The microplate had been swirled gently for 20 to 30 seconds to mix and cover.
5. Incubated for 60 minutes at room temperature (20oC to 25oC).
6. Hand Washing: At the ended of the incubation period, the contents of the wells had been discarded by flicking plate contents into a Biohazard container. Then had been stroked the wells sharply against absorbent paper.
7. The wells had been Filled with a minimum of 300µl of washing solution per well. The empty wells had been washed 5 times.

8. The wells had been stroked sharply on to absorbent paper or paper towel to remove all residual water droplets.

9. After washing excess fluid had been removed by striking the wells sharply onto absorbent paper or paper towel to remove all residual water droplets.

10. 100µl of Substrate Solution had been dispensed to all wells.

11. Incubated in the dark for 20 minutes at room temperature (20oC to 25oC).

12. The reaction had been stopped by adding 100µl stop solution to each well.

13. Gently mixed for 30 seconds. It was important to make sure that all the blue color had been changed completely to a yellow color.

14. Absorbance had been read at 450 nm with a microtitre well reader within 10 minutes.

3.7.3.2 Measurement of free T4 using ELISA technique:

Procedure

1. All the kit components and the test serum had been brought to room temperature (20oC to 25oC) prior to the start of the assy.

2. One set of Standards had been run with each batch of test serum.

3. 50µl of standard, control or test serum had been dispensed into the assigned well.

4. 100µl of Thyroxine Enzyme Conjugate Solution had been dispensed, to all wells. The microplate had been swirled gently for 30 seconds to mix.

5. Incubated for 60 minutes at room temperature (20oC to 25oC).

6. Hand Washing: At the ended of the incubation period, the contents of the wells had been discarded by flicking plate contents into a Biohazard container. Then had been stroked the wells sharply against absorbent paper.

7. The wells had been Filled with a minimum of 300µl of distilled water per well. The empty wells had been washed 5 times.

8. The wells had been stroked sharply onto absorbent paper or paper towel to remove all residual water droplets.

9. After washing excess fluid had been removed by striking the wells sharply onto absorbent paper or paper towel to remove all residual water droplets.

10. 100µl of Substrate Solution had been dispensed to all wells.

11. Incubated in the dark for 20 minutes at room temperature (20oC to 25oC).

12. The reaction had been stopped by adding 100µl stop solution to each well.

13. Gently mixed for 30 seconds. It was important to make sure that all the blue color had been changed completely to a yellow color.

14. Absorbance had been read at 450 nm with a microtitre well reader within 10 minutes.

3.7.3.3 Measurement of TSH using ELISA technique:

Procedure

1. All the kit components and the test serum had been brought to room temperature (20oC to 25oC) prior to the start of the assy.

2. One set of Standards had been run with each batch of test serum.

3. 50µl of standard, control or test serum had been dispensed into the assigned well.

4. 100µl of Anti-TSH Conjugate Solution had been dispensed, to all wells. The microplate had been swirled gently for 30 seconds to mix.

5. Incubated for 60 minutes at room temperature (20oC to 25oC).

6. Hand Washing: At the ended of the incubation period, the contents of the wells had been discarded by flicking plate contents into a Biohazard

container. Then had been stroked the wells sharply against absorbent paper.

7. The wells had been Filled with a minimum of 300µl of distilled water per well. The empty wells had been washed 5 times.

8. The wells had been stroked sharply onto absorbent paper or paper towel to remove all residual water droplets.

9. After washing excess fluid had been removed by striking the wells sharply onto absorbent paper or paper towel to remove all residual water droplets.

10. 100µl of Substrate Solution had been dispensed to all wells and mixed gently for 5seconds.

11. Incubated in the dark for 20 minutes at room temperature (20oC to 25oC).

12. The reaction had been stopped by adding 100µl stop solution to each well.

13. Gently mixed for 30 seconds. It was important to make sure that all the blue color had been changed completely to a yellow color.

14. Absorbance had been read at 450 nm with a microtitre well reader within 10 minutes.

3.8 Molecular biology techniques:

After DNA extracted PCR amplification was performed using Peltier thermal cycler (CONVERGYSR td peltier thermal cycle, Germany) then SNPs were genotyped using RFLP analysis .

3.8.1 Phenol-chloroform DNA extraction:

DNA extraction with phenol/chloroform/ isoamyl alcohol is an easy way to remove proteins from nucleic acid samples and can be carried out in a manner that is very close to quantitative. During This organic extraction, protein contaminants are denatured and partition either with the organic

phase or at the interface between organic and aqueous phases, while nucleic acids remain in the aqueous phase, aqueous top phase contains the majority of DNA, interphase mostly proteins, and lower organic phase most of the RNA and lipids .

3.8.1.1 Conventional Ethanol Precipitation:

During the ethanol precipitation, salts and other solutes such as residual phenol and chloroform remain in solution while nucleic acids form a white precipitate that can, easily be collected by centrifugation.

Procedure:

A). Preparation of WBCS

1. 2.5ml blood was collected in EDTA as anticoagulant
2. After centrifugation at 3000 rpm for 5 minutes, the plasma removed and leaved RBCs and buffy coat behind.
3. Washed cells twice with 5ml normal saline (0.9%)
4. 5ml of RBCs Lysis Buffer was added, Mixed. RBCs was lysed but WBCs remained intact.
5. At 4000 rpm Centrifuged for 10min, supernatant carefully removed, leaving WBC soft sediment.

B). Lysis of WBCs

1. To the WBCs pellet added 4ml of WBCs lysis Buffer, Mixed thoroughly and incubated with 20µl proteinase K (10 µg /ml) overnight at 37oC.

C). DNA extraction

1. 4ml of phenol was added to the mixture: chloroform: isoamyl alcohol reagent, vortex vigorously to mix the phases. Then it was centrifuged at 4000 rpm for 10 minutes. The aqueous phase was removed to a new tube, being careful not to transfer any of the protein at the phase interface. The supernatant (aqueous layer) was collected with a wide mouthed Pasteur pipette leaving inter phase behind.

2. Extracted the sample with an equal volume of chloroform: isoamyl alcohol to remove any trace phenol. To the aqueous phase collected added 4ml chloroform: isoamyl reagent Centrifuged and collected aquous phase into anew tube.

3. 6ml of cold absolute ethanol was added (the DNA appears at this step). The DNA was collected.

4. Washed the DNA with 70% ethanol (2ml).

5. Dissolved the DNA in 200µl water or TE buffer (labeled and stored the sample).

A). Electrophoresis of the extracted DNA in agarose gel

DNA was detected by electrophoresis (MPSU-125/200-UK) on gels and stained with ethidium bromide, which has an intense fluorescence excited by ultra-violet radiation when it complexes with nucleic acids.

1. Gel Preparation (2 % agarose gel)

The gel was prepared by mixing 2 gm agarose, 100 ml 1X TBE buffer and 4 ml of ethidium bromide (10 mg/ml).

2. Loading of the samples

- 2 ml of the extracted DNA mixed with 2 ml of loading buffer.
- 4ml DNA was loaded on the gel.
- A Molecular weight DNA marker (Ladder) is run on every gel.

The gel was run in 1X TBE running buffer and electrophoresis was carried out at 120 volts for 15 min then the gel was viewed under U.V trans illuminator (UGENIUSSYUG–UK).

B). Spectrophotometric determination of DNA concentration and purity

- The DNA yield was determined spectrophotometrically by measuring the absorbance at 260 and 280nm.
- The DNA was diluted 1: 50 with distilled water (10 m DNA+ 490 ml H2O).

- The reading of DNA concentration was performed at 260 and 280nm using spectrophotometer,
- Distilled water was used as a blank.

1A260 double-stranded DNA = 50 mg/mL

1A260 single stranded DNA = 37 mg/mL

DNA concentration = A260 X dilution factor X conversion factor

DNA Purity A260/A280

An A260/A280 ratio greater than 1.8 indicates highly purified preparations of DNA and RNA respectively. Contaminants that absorb at 280 nm (e.g a protein) will lower the ratio.

3.9 Molecular Techniques:

3.9.1 PCR Analysis:

a- Primer design:

Three pairs of primers were designed using the published data for human thyroid stimulation hormone (TSH) receptor gene and iodothyronine deiodenase 1 (DIO1a) gene and phosphodesterse 8B (PDE8B) gene to amplify different DNA fragments Table (3.1)

3.9.2. Reagents:

- dNTPs
- Primers
- Gotaq polymerase (5u/µl, Promega/MADISON WI, Promega USA).
- 100 bp DNA ladder (Cat. No. G2101, Promega Product Information, Promega)
- Agarose
- Boric Acid
- Tris/base
- EDTA
- Ethidium promide dye 10 g\dl

3.9.3. Standard PCR reaction:

- The experiment consists of the experimental DNA, a negative control. In a 0.1 µl PCR tube the following solutions were placed in a total volume of 50 µl:
- 10X Taq buffer (final concentration 1X)
- 2.5 mM 4dNTP stock (final concentration 200 µmol)
- 10 pmol/µl primer F
- 10 pmol/µl primer R
- 100 ng of genomic DNA template.
- MgCl2 (final concentration 1.5µm)
- H2O (up to the total volume 0f 50µl)
- 1u Taq. Polymerase (Gotaq polymerase 5u/µl, Promega/MADISON WI USA).

3.9.4. Checking of the PCR Products:

To confirm the presence of amplifiable DNA in the samples, The specificity of PCR was typically analyzed by evaluating the production of the target fragment by gel electrophoresis of 5µl PCR products on 2% agarose gel stained with ethidium bromide.

Procedure:

1. Preparation of the 2% Agarose gel:
   - 2 gram of agarose
   - 100 ml 1X TBE buffer
   - Heated the mixture until the agarose completely dissolved, cooled to about 60°C and added 4µl of ethidium bromide. Poured the gel on the gel tank and leaved it to solidify.

2- Loaded 5µl PCR products on the gel

3- Run the gel at l00 volts for 15 min,

4- Viewed on the UV system and photograph.

3.9.5 RFLP analysis: Restriction Enzyme Digest Analysis

a- TSHR gene:

This would be done after completion of the PCR amplification.

For restriction enzyme analysis, 10 μL of the PCR product will be used.

A standard restriction enzyme analysis consists of the following components to a final volume of 25 μL:

- 10 μL of PCR product, 0,1 μL (1 U) of restriction enzyme
- 2.5 μL of 10X buffer
- 12.4 μL of H2O. This mixture was incubated at the 63.1ºc optimum temperature of the enzyme for 15 minutes and inactivated the enzyme at 65ºc for 20 minutes, after incubation was completed, the restriction analysis was carried out in an agarose gel (2%)electrophoresis with 1X TBE buffer.(Figure: 3.5)

b- DIO1a gene:

This would be done after completion of the PCR amplification.

For restriction enzyme analysis, 10 μL of the PCR product would be used.

A standard restriction enzyme analysis consisted of the following components to a final volume of 25 μL:

- 10 μL of PCR product, 0,5 μL (5 U) of restriction enzyme
- 2.5 μL of 10X buffer
- 12 μL of H2O. This mixture was incubated at the 67ºc optimum temperature of the enzyme for 10 minutes, after the incubation completed, the restriction analysis was carried out in an agarose gel (2%) electrophoresis with 1X TBE buffer.(Figure: 3.6)

c- PDE8B gene :

This would be done after completion of the PCR amplification.

For restriction enzyme analysis, 10 µL of the PCR product would be used.

A standard restriction enzyme analysis consisted of the following components to a final volume of 25 µL:

- 10 µL of PCR product, 0,5 µL (5 U) of restriction enzyme
- 2.5 µL of 10X buffer
- 12 µL of H2O. This mixture was incubated at the 50ºc optimum temperature of the enzyme for 10 minutes, after the incubation completed, the restriction analysis was carried out in an agarose gel (2%) electrophoresis with 1X TBE buffer.(Figure: 3.7)

3.9.6 SNP genotyping using RFLP analysis:

Restriction fragment length polymorphism or RFLP analysis was used to identify a change in the genetic sequence that occurs at a site where a restriction enzyme cuts.

After DNA was extracted by using phenol/chloroform/ isoamyl alcohol method as described by Chomczynski and Sacchi (Chomczynski and Sacchi, 1987), the PCR was carried out using thermal cycler (CONVERGYSR td peltier thermal cycle, Germany), by using the following primers (Macrogen, Korea) and restriction enzymes(New England Biolab) as seen in table (3.1) for each gene. In a total reaction volume of 25 µl (5µl Master mix of Maxime RT premix kit (*iNtRON BIOTECHNOLOGY*, Seongnam, Korea), 0.6 µl of forward primer, 0.6 µl of reverse primer, 2µl of DNA and 16.8 µl deionized sterile water). All samples were genotyped using polymerase chain reaction of known interest variant, methods described by Peeters (2003) with minor modifications (Peeters RP *et al*, 2003). PCR products were analyzed by electrophoresis in a 2% agarose gel in TBE 1X, that contain 2.5 µl of

(20mg/ml) ethidium bromide at 100 V for 10 min. Bands were visualized under U.V transilluminater (UGENIUS-SYUG/1304 –UK).

3.10 Quality control:

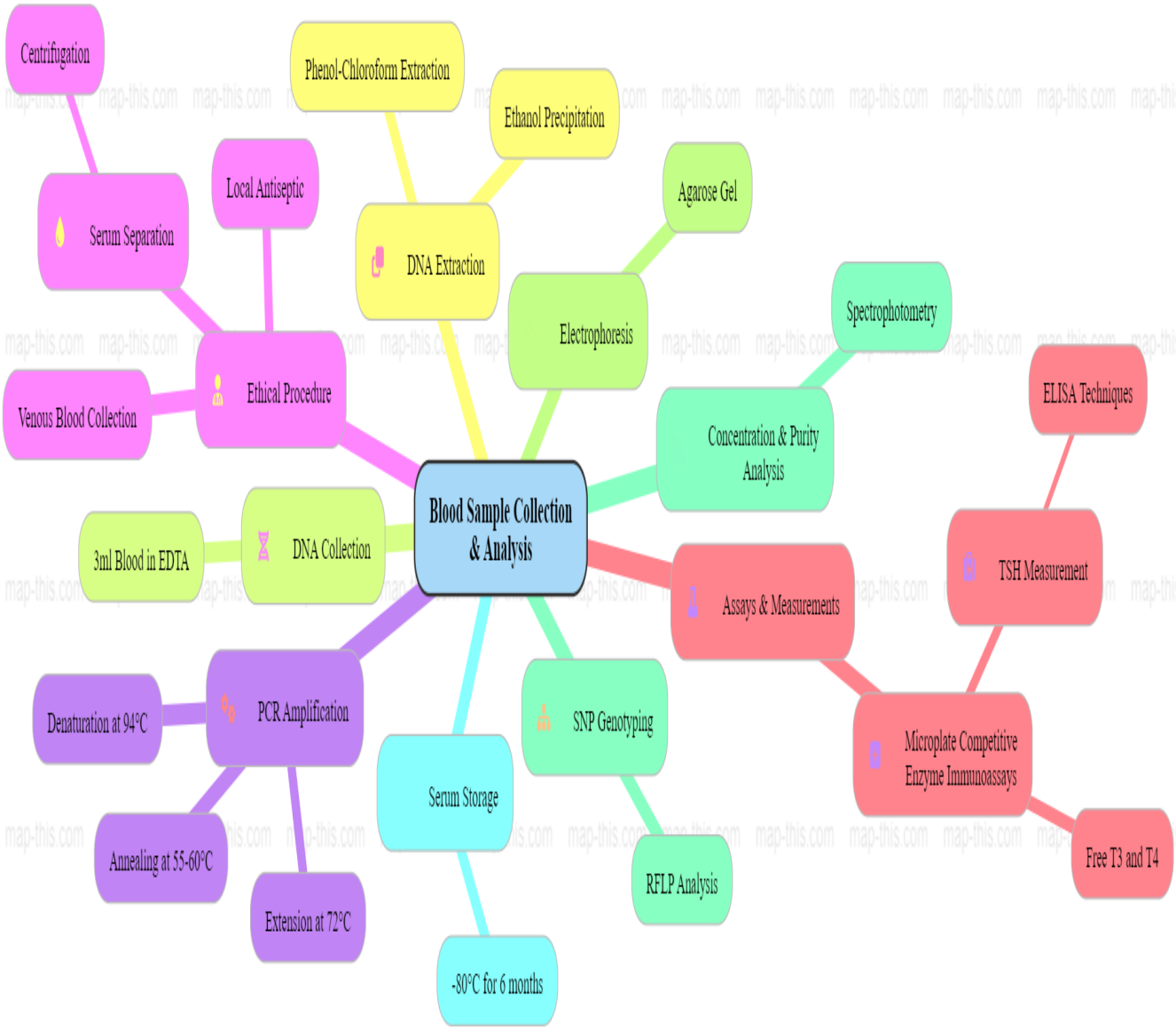
The precision and accuracy of all methods used in this study were checked each time a batch was analyzed by including calibrators and commercial prepared control sera.

3.11 Statistical analysis:

Data entered into the computer and was analyzed using SPSS program version 20. The results presented in tables and figures. The analysis of variance and the difference among the means for significant less than 0.05 levels using *t*- test. The relationship between variables was determined using Chi-square ($\chi 2$) test, Also using person correlation coefficients [r] value equal 1 refer as complete correlation .

Table (3.1): Primers and restriction enzymes used in this study:

Gene	Primers (´5 - 3')	Restriction Enzymes	Product size, bp	Anealing tempreture	References
TSHR	F: 5-AACGCCAGGCTCAGGCATAC-3 R: 5-AAGTTCCCCTACCATTGTGA-3	*Nla*III	196	63.1ºc	Peeters *et al*., 2003
DIO1a	F:5GAACTTGATGTGAAGGCTGGA-3 R: 5TAACCTCAGCTGGGAGTTGTT-3	*Bc11*	565	67ºc	Peeters *et al*., 2003
PDE8B	F: 5-GGCGCTACTCTAGGTTTGGA-3 R: 5-GTCTGCTCCTTGGCTTTTCC-3	*Bs11*	519	50ºc	Grandone *et al*.,2012



(Figure 3-1): show the mind map of procedure

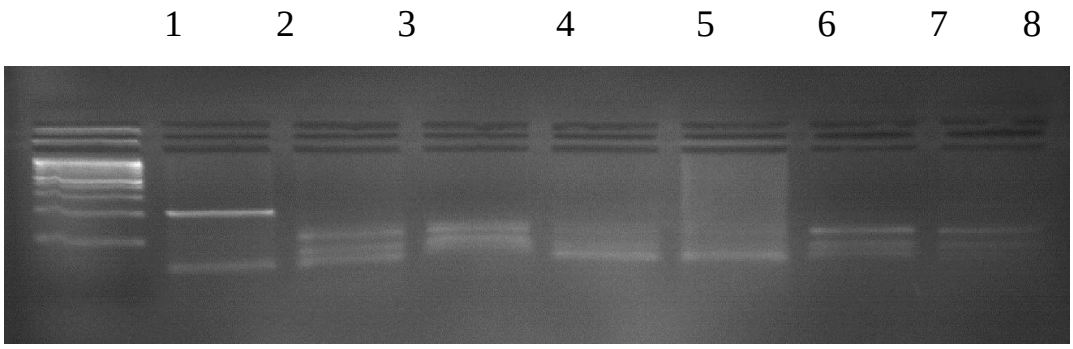


Figure (3.2): The DNA examination shown on 2% agarose gell electrophoresis

.Lane l was loaded with 50 bp ladder ; lane 2 is negative control 0 bp; lane 3, 4, 5, 6, 7 and 8 are positive DNA

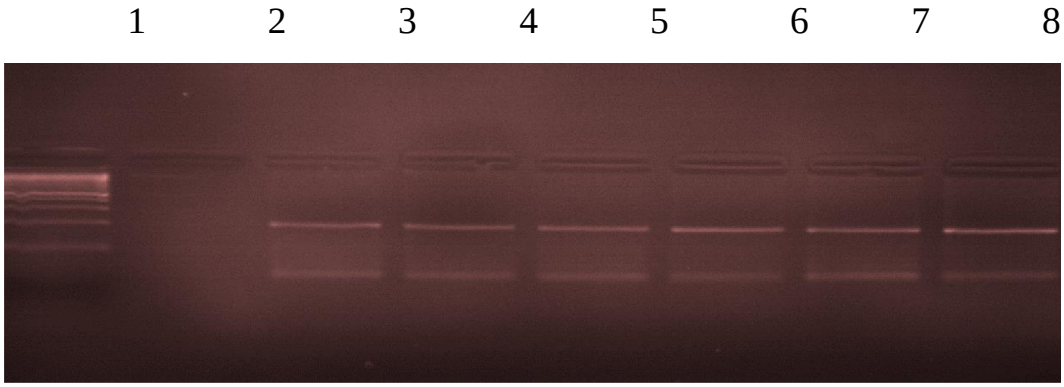


Figure (3.3): The PCR-product of the p.D727E polymorphisms of the *TSHR* gene shown on 2 % agarose electrophoresis. Lane l was loaded with 50 bp ladder; lane 2 is negative control 0 bp ; lane 3, 4, 5, 6, 7 and 8 are positive PCR-product 196 bp

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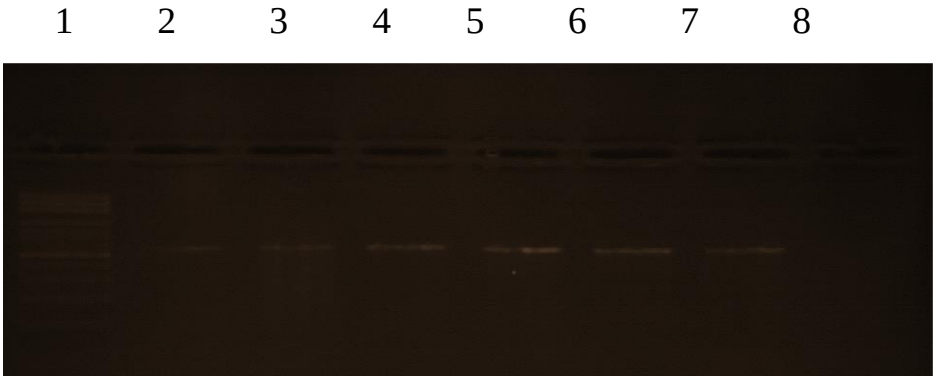

Figure (3.4): The PCR-product of the DIO1a gene shown on 2 % agarose electrophoresis .Lane l was loaded with 50 bp ladder; lane 8 is negative control 0 bp ; lane 2, 3, 4, 5, 6 and 7 are positive PCR-product 565 bp.

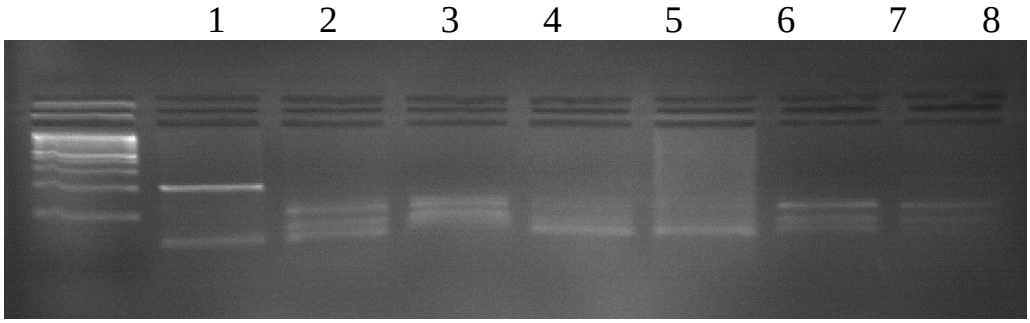

Figure:(3.5) The PCR-based restriction analysis of the p.D727E polymorphisms of the *TSHR* gene shown on 2 % agarose electrophoresis. The *Nla*III (*Hin*1II) restriction enzyme cuts in the CATG/G region. In the presence of 129 and 67 bp, the fragments were shown to be GG (polymorphic homozygote) genotype; in the presence of 108, 67 and 21bp, the fragments were shown to be CC (wild type) genotype; in the presence of 129, 108, 67 and 21 bp, the fragments were shown to be CG (polymorphic heterozygote) genotype. Lane l was loaded with 50 bp ladder ; lane 2 is PCR product 196 bp; lanes 3, 4 and 7are heterozygous CG subjects; lanes 5 and 6are homozygous CC subjects; lanes 8 is homozygous GG subjects. A 21 bp fragment was not seen in either CC, CG or GG genotypes.

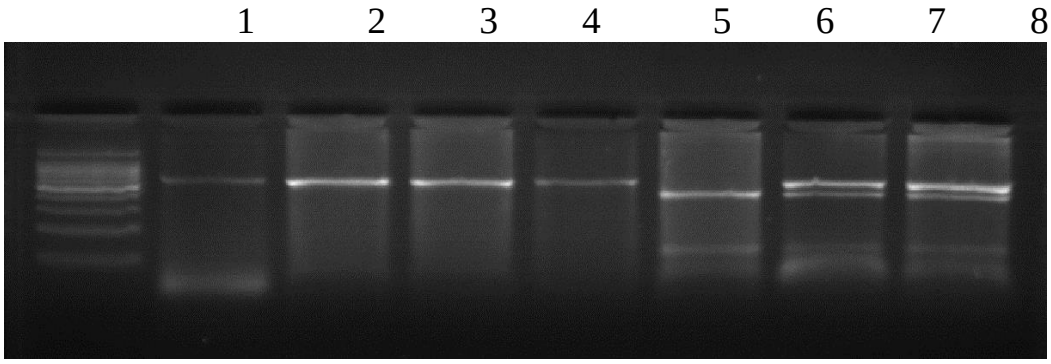

Figure: (3.6) The PCR-based restriction analysis of the DIO1a-C/T shown on 2% agarose electrophoresis. A PCR fragment was generated of 565 bp. TheBcl1 restriction enzyme cuts in the C/T region generates two fragments of 434 and 131 bp only in the presence of the D1a-T allele (polymorphic homozygote) genotype; in the presence of the 565- and 434-bp, the fragments were shown to be CT (polymorphic heterozygote) genotype. Lane l was loaded with 50 bp ladder ; lane 2 is PCR product 565 bp; lanes 3, 4 and 5 are homozygous CC subjects; lanes 6 is homozygous TT subjects; lanes 7 and 8 are heterozygous CT subjects.

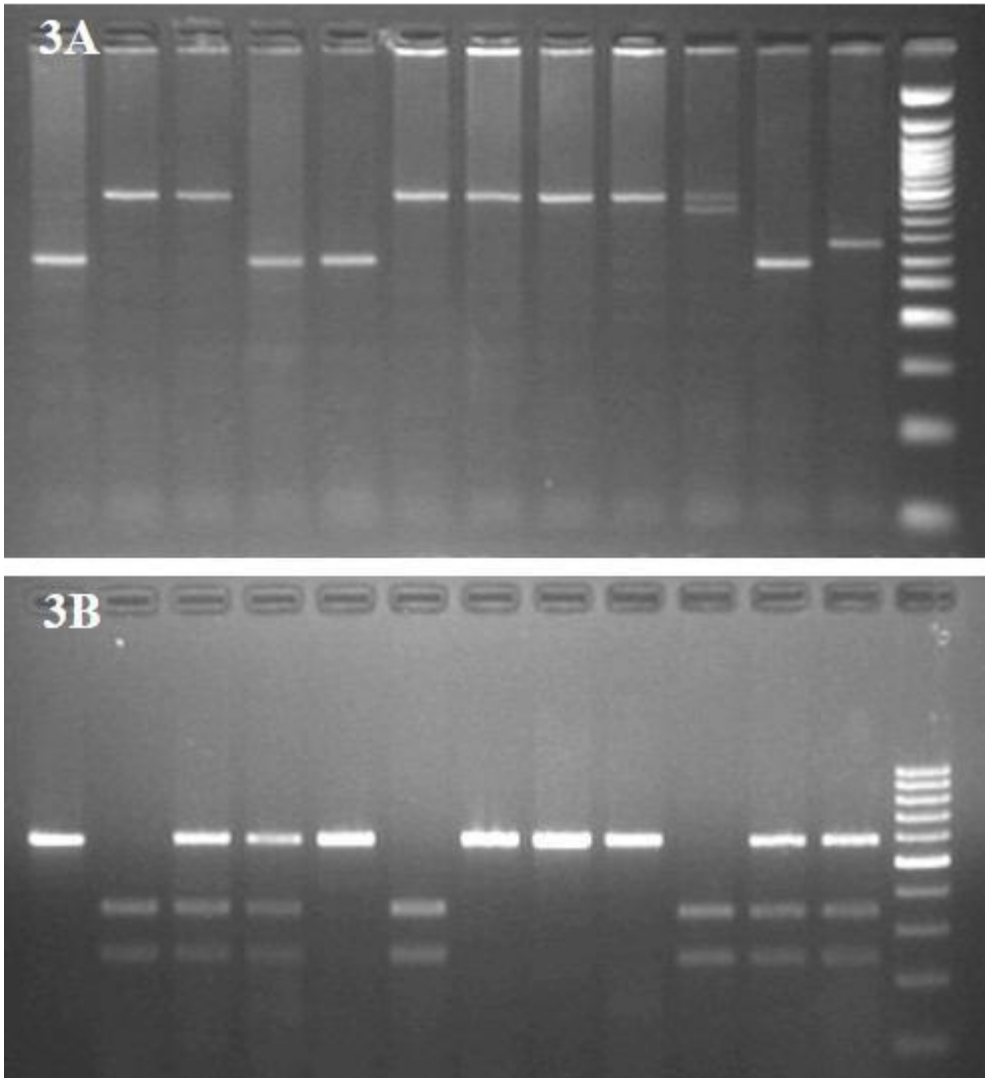

Figure (3.7): The PCR-based restriction analysis of the *PDE8B* (rs4704397) gene shown on 2% agarose electrophoresis. The *Bsl*I restriction enzyme cuts in the CCNNNNN/NNGG region. In the presence two fragments of 318 and 201bp only in the presence of the *PDE8B*-G allele were shown to be AA (wild type) genotype; in the presence of 519 only the fragment were shown to be GG (polymorphic homozygote) genotype; in the presence of 519, 318 and 201bp, the fragments were shown to be AG (polymorphic heterozygote) genotype. Lane l was loaded with 100 bp ladder , lane 6 is homozygous CC subjects; lanes 5, 7, 8 and 9 are heterozygous AG subjects, homozygous GG
subjects are seen in lanes 2, 3 and 4.

Chapter four

Results

4. Result

This cross-sectional study was conducted between June 2019 and June 2024, encompassing 153 participants. The study population included 70 patients with simple (diffuse) goiter, 33 with nodular goiter, and 50 euthyroid individuals. A control group of 50 apparently healthy individuals without a family history of thyroid goiter was age- and sex-matched to the patient group. Both male and female are enrolled in the study (37% Male (n= 56) and 63% female (n= 97), were assessed for serum levels of thyroid hormones (free T3, free T4, and TSH).

Family history of thyroid goiter was evaluated among patients. Of the 153 patients, 45% (n=69) had a first-degree family history (grade 1), 33% (n=51) had a second degree family history (grade 2), and 22% (n=33) had no family history. None of the control participants reported a family history of thyroid goiter.

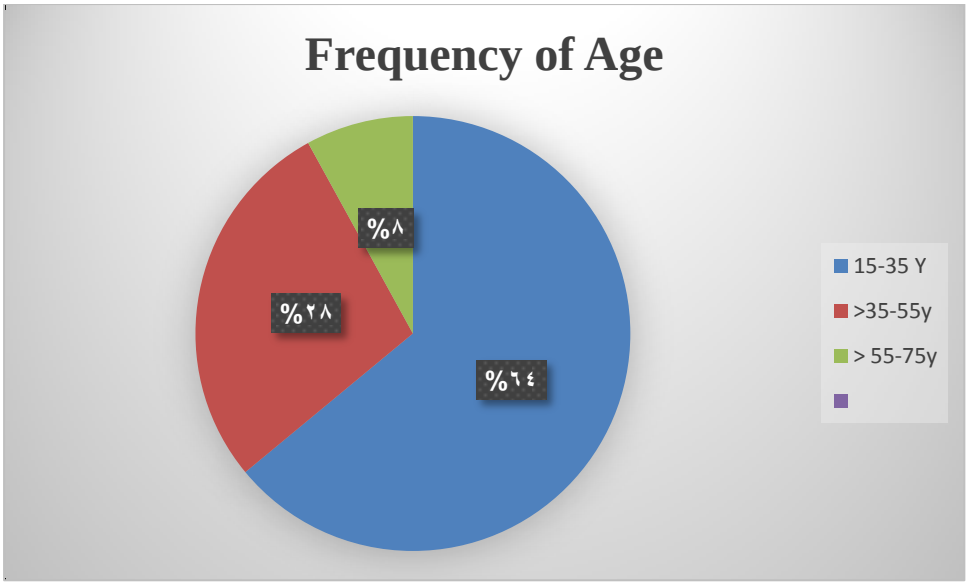

Figure (4-1) show comparison the percentages between age group which presented the age group 15-35 years (64%) had high percentage when compared with other age groups.

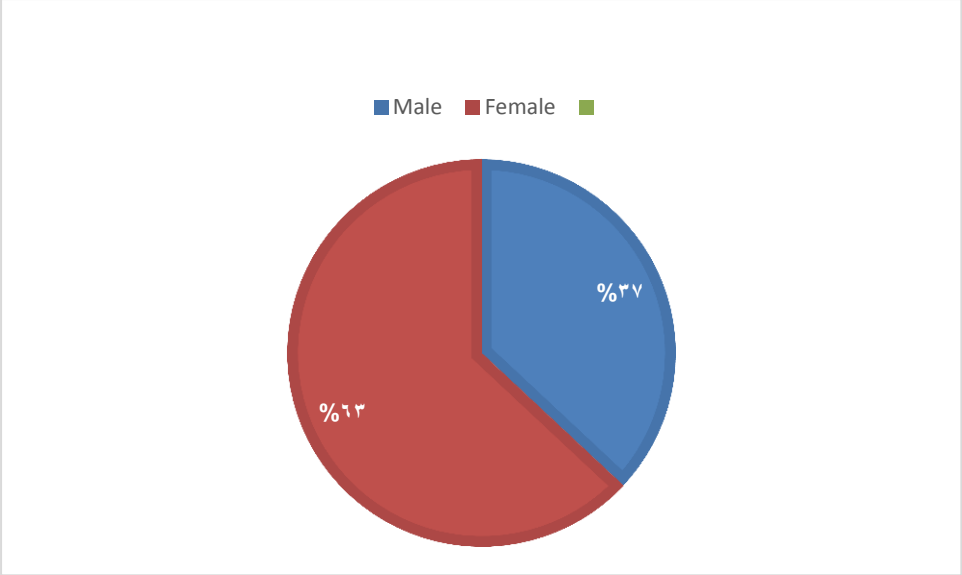

Figure (4.2) shows frequencies of gender within patients

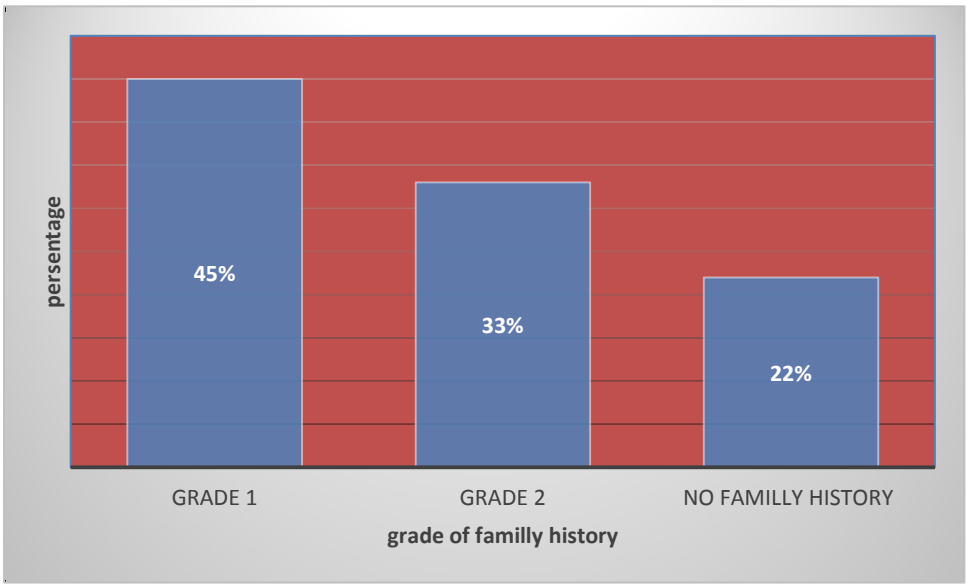

Figure (4.3): Shows frequencies of family history in patients

Key:

Grade 1: First degree relatives

Grade 2: Second degree relatives

Table (4-1): The demographic characteristics of the patients and control:

Age		**Patients(n=153)**	**Controls(n=50)**
		(mean ± SD)	
Age (Years)	15-35	(21.9±8.6)	(18.5±10)
	>35-355	(36.6±13.9)	(37.5±13.1)
	>55-75	(60 ±16)	(58 ±11)

This table Shows the demographic characteristics of the patients and controls which presented previous results and there is in significant difference (P= (0.51.0.69 and 0.64) respectively between the mean of the age (years)

Table (4-2): Thyroid hormone level among grad1, grade 2 and no family

Hormone	Mean ± SD		P value
	Grade 1 family history	No family history	
FT3	(1.5±0.3pg/ml)	(2.4±o.7pg/ml)	0.04
FT4	(0.7±1.1pg/ml)	(1.5±2.3pg/ml	0.06
TSH	(6-5±3.9pg/ml)	(1.9 ± 1pg/ml)	0.03
Hormone	Grade 2 family history	No family history	P value
FT3	(1.7±0.4pg/ml)	(2.1±1.2 pg/ml)	0.08
FT4	(1.1±1pg/ml)	(0.9±1.6pg/ml	0.02
TSH	(5.4±3.1pg/ml)	(0.7±2.4pg/ml)	0.05
Hormone	Grade 1 family history	Grade 2 family history	P value
FT3	(1.5±0.3pg/ml)	(1.7±0.4pg/ml)	0.57
FT4	(0.7±1.1pg/ml)	(1.1±1pg/ml)	0.069
TSH	(6-5±3.9pg/ml)	(5.4±3.1pg/ml)	0.08

This table presents the average concentrations of thyroid hormones (TSH, FT4, and FT3) in individuals with (grade 1, grade 2 and family no family history), Significant difference was found in FT3 and TSH levels (p=0.04 . 0.03) respectively. Also, there was a significant difference in FT4 levels (p=0.06), between grade 1 and no family history. However, a significant difference was observed in FT4 levels (p=0.02), and insignificant difference in TSH and FT3 P- Value (0.05,0.08)respectively regarding grade 2 and no family history. Finally the study were observed no significant difference in TSH,FT3 andFT4 p-values (0.08,0.57 and 0.069) respectively between grad1 and grade 2 .

Table (4-3): Comparison between simple defused goiter patients& control group

Hormone	Mean ± SD in		P value
	Simple goiter	Controls	
FT3	(12.4±6.8pg/ml)	(2.3±0.7pg/ml)	0.001
FT4	(13.4±6.9pg/ml)	(1.4±0.7pg/ml)	0.025
TSH	(0.05±0.06pg/ml)	(0.9±2.6pg/ml)	0.004

This table presents a comparison of mean thyroid hormone levels between patient groups and the control group. Statistically significant differences were found in FT3 levels between the simple goiter group (12.4 ± 6.8 pg/ml) and the control group (2.3 ± 0.7 pg/ml) (p=0.001). Similarly, FT4 levels were significantly higher in the simple goiter group (13.4 ± 6.9 pg/ml) compared to the control group (1.4 ± 0.7 pg/ml) (p=0.025). Finally, TSH levels were significantly lower in the simple goiter group (0.05 ± 0.06 pg/ml) compared to the control group (0.9 ± 2.6 pg/ml) (p=0.004).

Table (4-4): comparison between nodular goiter patients &control group .

Hormone	Mean ± SD in		P value
	Nodular goiter	Control	
FT3	0.9±0.3pg/ml)	(2.3±0.7)pg/ml	0.000
FT4	(6.1±1.8pg/ml)	(11±1.8pg/ml)	0.004
TSH	(13.4±6.9pg/ml)	(1.4±0.7pg/ml)	0.002

This table demonstrates significant differences in thyroid hormone levels between study groups. FT3 levels were significantly lower in the nodular goiter group (0.9 ± 0.3 pg/ml) compared to the control group (2.3 ± 0.7 pg/ml) (p=0.000). Similarly, FT4 levels were significantly lower in the nodular goiter group (6.1 ± 1.8 pg/ml) compared to the control group (11 ± 1.8 pg/ml) (p=0.004). Finally, TSH levels were significantly higher in the nodular goiter group (13.4 ± 6.9 pg/ml) compared to the control group (1.4 ± 0.7 pg/ml) (p=0.002).

Table (4-5): comparison between euthyroid patients &control group

Hormone	Mean ± SD		P value
	Euthyroid	Control	
FT3	(2.5±1.1pg/ml)	(1.9±0.5pg/ml)	0.54
FT4	(10.1±1.8pg/ml)	(11±8.1pg/ml	0.07
TSH	(12.3±3.7 pg/ml)	(0.8±1.7pg/ml)	0.036

This table presents a comparison of mean serum thyroid hormone levels between the euthyroid and control groups. No significant difference was found in FT3 levels (p=0.54) between the two groups (2.5 ± 1.1 pg/ml vs. 1.9 ± 0.5 pg/ml, respectively). While there was a trend towards a difference in FT4 levels (p=0.07), it did not reach statistical significance (10.1 ± 1.8 pg/ml vs. 11 ± 8.1 pg/ml). However, a significant difference was observed in TSH levels (p=0.036), with higher levels in the euthyroid group (12.3 ± 6.9 pg/ml) compared to the control group (0.8 ± 1.7 pg/ml).

Table (4.6): The association of the *TSHRc*-C/G (Asp727Glu) genotypes and allelic frequencies of the *TSHR* gene in patients and controls

Case study	Genotype Frequency			Allele Frequency		P value
	C/C	G/G	C/G	C	G	
Controls (n=50)	62%(n=31)	(n=0)	38%(n=19)	82 (85%)	18 (15%)	
Simple goiter (n=70)	47.2%(n=33)	22.8%(n=16)	30%(n=21)	98 (70%)	42 (30.0%)	0.03
Nodular goiter (n=33)	42.5%(n=14)	3%(n=1)	54.5%(n=18)	44 (68.3%)	20 (31.7%)	0.02
Euthyroid (n=50)	48%(n=24)	6 %(n=3)	46%(n=23)	68 (68.3%)	32 (31.7%)	0.03

This table presents genotype frequencies. Significant differences in genotype frequencies were observed between all patient groups (simple goiter, nodular goiter, and euthyroid) and the control group. Specifically, p-values were 0.003, 0.02, and 0.03, simple goiter patients, nodular goiter patients, and euthyroid patients, respectively, compared to controls

Table (4.7): The association of the *DIO1a* genotypes and allelic frequencies of the *DIO1a* gene in patients and controls

Case study	Genotype Frequency			Allele Frequency		P value
	C/C	T/T	C/T	C	T	
Controls (n=50)	62%(n=31)	(n=0)	38 %(n=19)	93 (93%)	7 (7%)	
Simple goiter (n=70)	75.7%(n=53)	7.14%(n=5)	17.16%(n=12)	98 (70%)	42 (30.0%)	0.153
Nodular goiter (n=33)	66.6(n=22)	3 %(n=1)	30.4%(n=10)	54 (82%)	12 (18%)	0.06
Euthyroid (n=50)	52 %(n=26)	6%(n=3)	42 %(n=21)	73 (73%)	27 (27%)	0.002

This table show genotype frequencies. No significant differences in genotype frequencies were observed in (simple goiter, nodular goiter, P-Values were (0.153 and 0.06) while significant differences were observed in euthyroid goiter p-value 0.02) and the control group. Specifically, p-values were 0.153, 0.06, and 0.02), simple goiter patients, nodular goiter patients, and euthyroid patients, respectively, compared to control.

Table (4-8) :The association of the PDE8B genotypes and allelic frequencies of PDE8B gene in patients and controls.

Case study	Genotype Frequency			Allele Frequency		P value
	G/G	A/A	G/A	G	A	
Controls (n=50)	60% (n=30)	-(n=0)	40% (n=20)	76 (85%)	24(15%)	
Simple goiter (n=70)	11.4% (n=8)	68.6%(n=48)	20% (n=14)	31 (22%)	109 (78 %)	0.008
Nodular goiter (n=33)	12.2%(n=4)	54.5 %(n=18	33.3%(n=11)	18 (26.7%)	48 (73.3%)	0.010
Euthyroid (n=50)	14%(n=7)	66%(n=33)	20%(n=10)	30 (15.3%)	70 (84.7%)	0.04

This table show presents genotype frequencies. Significant differences in genotype frequencies were observed between (simple goiter, nodular goiter, and euthyroid) and the control group. Specifically, p-values were 0.008, 0.010, and 0.04) , simple goiter patients, nodular goiter patients, and euthyroid patients, respectively, compared to control.

Table (4-9):This table presents the average concentrations of thyroid hormones (TSH, FT4, and FT3) in individuals with mutant genotypes (DIO1a, , PDE8B, and TSHR) compared to those with normal genotypes in patients with simple diffuse goiter.

Parameter	**Alleles of *DIO1a* gene (C/T)**		***p*-value**
	Mean ± SD	**Mean ± SD**	
	C Allele	T Allele	
FT3	13.6±6.8	8.3±**6.0**	0.070
FT4	51.1±**24.7**	31.1±**10.4**	0..040
TSH	0.04±**0.04**	0.09±**0.08**	0.190
Alleles of *PDE8B* gene (G/A)			
	Mean ± SD	**Mean ± SD**	***p*-value**
	G Allele	A Allele	
FT3	6.4±**1.5**	13.1±**6.9**	0.001
FT4	30.7±**5.9**	48.2±**24.3**	0.001
TSH	0.13±**0.06**	0.04±**0.05**	0.010
Alleles of *TSHR* gene (C/G)			
	Mean ± SD	**Mean ± SD**	***p*-value**
	C Allele	G Allele	
FT3	12±**7.3**	12.7±**6.6**	0.800
FT4	51±**30**	42.9±**17.3**	0.400
TSH	0.06±**0.06**	0.05±**0.05**	0.600

Table (4-10): This table presents the average concentrations of thyroid hormones (TSH, FT4, and FT3) in individuals with mutant genotypes (DIO1a, PDE8B, and TSHR) compared to those with normal genotypes in patients diagnosed with nodular goiter.

Parameter	**Alleles of *DIO1a* gene (C/T)**		**p-value**
	Mean ± SD	**Mean ± SD**	
	C Allele	T Allele	
FT3	0.9±0.3	0.9±**0.3**	0.600
FT4	6.2±**1.7**	5.9±**2.0**	0.700
TSH	12.4±**7.2**	15.4±**6.3**	0.300
Alleles of *PDE8B* gene (G/A)			
	Mean ± SD	**Mean ± SD**	***p*-value**
	G Allele	A Allele	
FT3	1.1±**0.2**	0.9±**0.3**	0.200
FT4	6.1±**2.2**	6.1±**1.8**	0.900
TSH	7.0±**2.3**	14.1±**6.9**	0.100
Alleles of *TSHR* gene (C/G)			
	Mean ± SD	**Mean ± SD**	***p*-value**
	C Allele	G Allele	
FT3	0.9±**0.3**	0.9±**0.3**	0.700
FT4	5.8±**1.8**	6.3±**1.8**	0.400
TSH	14.3±**8.0**	12.8±**6.2**	0.600

Table (4-11) This table presents the average concentrations of thyroid hormones (TSH, FT4, and FT3) in individuals with mutant genotypes (DIO1a, PDE8B, and TSHR) compared to those with normal genotypes, both within the context of euthyroid goiter.

Parameter	**Alleles of *DIO1a* gene (C/T)**		***p*-value**
	Mean ± SD	**Mean ± SD**	
	C Allele	**T** Allele	
FT3	2.4±0.8	2.3±0.4	0.600
FT4	11.1±2.4	9.6±0.9	0.020
TSH	1.1±0.8	0.6±0.4	0.200
Alleles of *PDE8B* gene (G/A)			
	Mean ± SD	**Mean ± SD**	***p*-value**
	G Allele	A Allele	
FT3	2.4±**0.8**	2.4±**0.7**	0.900
FT4	11.1±**2.7**	9.9±**1.6**	0.200
TSH	1.1±**1.2**	1.0±**0.6**	0.700
Alleles of *TSHR* gene (C/G)			
	Mean ± SD	**Mean ± SD**	***p*-value**
	C Allele	G Allele	
FT3	2.3±**0.8**	2.4±**0.7**	0.700
FT4	11.0±**2.5**	10.7±**2.1**	0.700
TSH	1.2±**1.0**	0.8±**0.5**	0.100

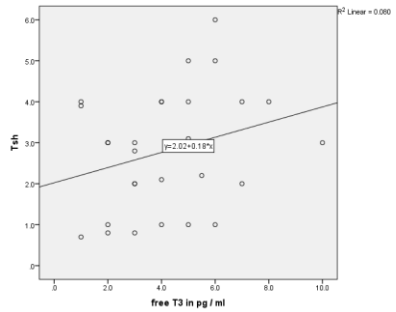

Figure (4-4): A scattered plot shows the relationship between serum levels of free T3 in pg/ml and serum levels of TSH in pg/ml in nodular goiter patients (r=-0.282 , P= 0.001)

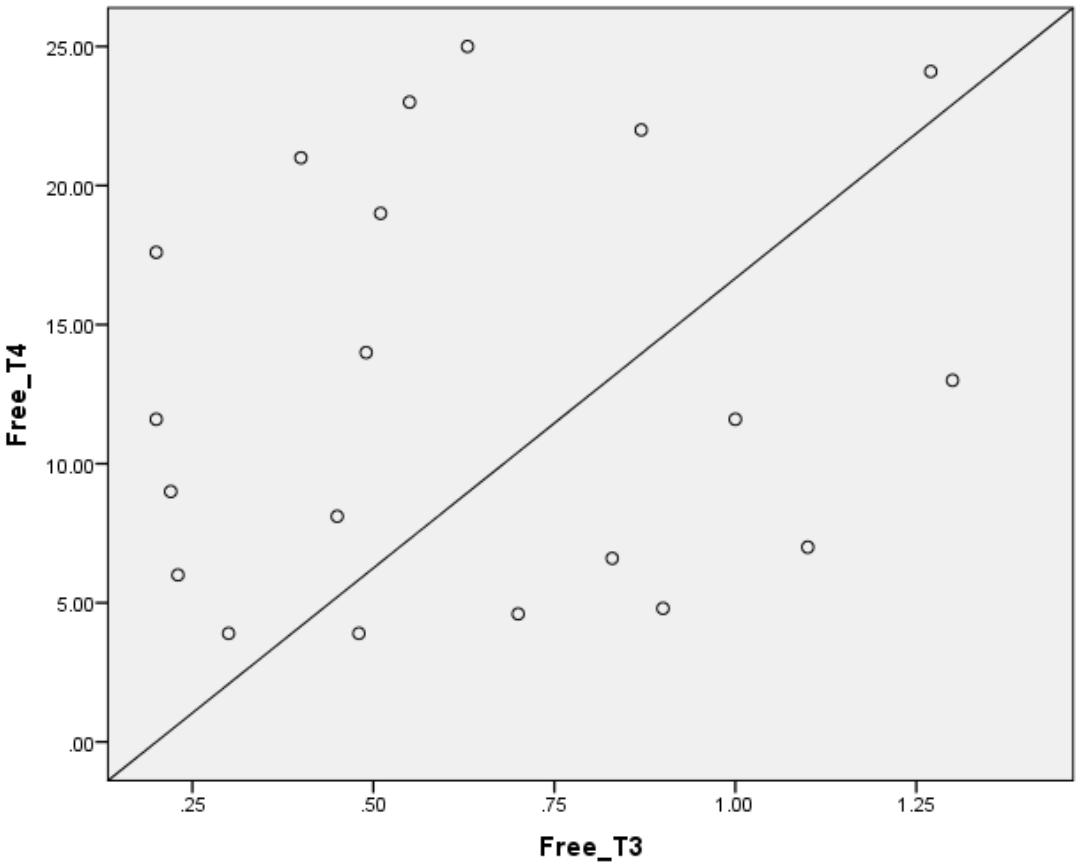

Figure (4-5) A scattered plot shows the relationship between serum levels of free T3 in pg/ml and serum levels of free T4 in pg/ml in simple goiter patients (r=0.757 , P= 0.002)

Chapter five

- Discussion
- Conclusion
- Recommendation

5.1 Discussion

Thyroid diseases are the most common endocrine disorder (Karaca & Akpak 2015).With a population of around 1.25 billion, an estimated 42 million people would be suffering from thyroid disorders (Singh *et al.*, 2016). Iodine deficiency is one of the commonest environmental factors responsible for thyroid diseases, more than one billion persons are at risk of iodine deficiency worldwide and 200 million have goiter. In Sudan, iodine deficiency and endemic goiter and variable thyroid dysfunctions are persistent health problems, with the prevalence of goiter reaching up to 22% in some areas, (with arange of 13% to 87%) (Medani*et al.*, 2011) Interactions between individual genetic and environmental factors determine the onset of goiter disease, as for the genetic factors there are only a few studies. The present study aimed to screen the *DIO1a,PDE8B* and *TSHR* genes polymorphism and its relation to goiter diseases (simple defuse goiter, nodular goiter and euthyroid goiter).In fact that the afro asciatic tribal group are most population of White Nile State which clearly high prevalence of goiter diseases , Which classified by Begona in this previos study(Begona *et al.,* 2015).

The current study revealed that a significant proportion of patients, 78%, had a family history of goiter-related diseases, including simple goiter, nodular goiter, and euthyroid goiter. This finding aligns with the results of Tug et al. (2012), who reported that 69% of their patients had a familial history of goiter.

The study revealed that thyroid disorders, including simple goiter, nodular goiter, and euthyroid goiter, were more prevalent in younger age groups, with 64% of cases occurring in these individuals. These findings are partially consistent with the results reported by (Biassoni et al. in 2014).

The current study demonstrated a significant decrease in mean serum TSH levels among individuals with euthyroid goiter compared to the control group (p-value = 0.030). However, the mean levels of free T3 and free T4 did not show significant differences (p-values = 0.063 and 0.070, respectively). These findings align with the results reported by Tug et al. in their study of the Turkish goiter population (Tug et al., 2012)

The present investigation demonstrated that. a significant association between the DIO1a gene (rs11206244) allelic frequency and euthyroid goiter (p-value = 0.002). However, no significant associations were observed with simple goiter or nodular goiter (p-values = 0.153 and 0.063, respectively) when compared to the control group. This study is the first to investigate the association of the DIO1a gene (rs11206244) polymorphism with simple goiter, nodular goiter, and euthyroid goiter. Previous studies in healthy and euthyroid individuals, such as Peeters et al., have reported similar allele frequencies for D1a-C/T (C = 66%, T = 34%) in a normal population, which is close to the percentages observed in our healthy control group (D1a-C/T: C = 93%, T = 7) (Peeters et al., 2011). The D1a deficiency caused by this polymorphism can lead to lower FT3 serum levels. The higher serum FT4 levels and lower serum FT3 levels observed in patients with nodular goiter who carry the T allele of the DIO1a gene may be explained by the fact that the conversion of FT4 to FT3 by deiodinase D1 occurs in the liver. This suggests that nodular goiter may be associated with impaired hepatic function, leading to decreased D1 activity. Additionally, the present study demonstrated significant associations between the PDE8B gene (rs470439) allelic frequency and simple gaiter, nodular goitre, and euthyroid goiter when compared to the control group (p-values = 0.008, 0.010, and 0.004, respectively). These findings are consistent with those reported by Sana (2014),

The current results unequivocally demonstrated a significant association between the TSHRc-C/G (Asp727Glu) gene allelic frequency and thyroid disorders, including simple goiter, nodular goiter, and euthyroid goiter, when compared to the control group (p-values = 0.03, 0.02, and 0.03, respectively). Previous studies conducted in healthy individuals, such as Peeters et al., have reported similar allele frequencies for TSHRc-C/G (C = 90.7%, G = 9.3%) in a normal population, which is close to the percentages observed in our healthy control group (C = 85%, G = 15%) (Peeters et al., 2003). However, the results of our study contradict those of MUHLBERG et al., who found no significant association between codon 727 polymorphism frequencies and autonomously functioning thyroid disorders (13.3%) compared to the healthy control group (16.2%) (p-value = 0.570) (MUHLBERG et al., 2000). Other studies have assumed that the CC and CG genotype incidences for the patient group are 0.71 and 0.29, respectively, while for the control group they are 0.8 and 0.2, respectively. No statistically significant difference was found between the genotype and allele distribution of both groups (p-values = 0.417 and 0.449, respectively) (Tug et al., 2012). These discrepancies may be attributed to differences in the populations studied.

This current study demonstrated a significant decrease in serum FT4 levels when comparing individuals with the mutant allele (T) of the DIO1a gene (rs11206244) to those with the normal allele (C) (p-values = 0.040 and 0.010, respectively). In contrast, the serum levels of FT3 (p-values = 0.070 and 0.010) and TSH (p-values = 0.190 and 0.01) remained unchanged in nodular and euthyroid goiter, respectively. However, no significant differences were observed in the serum levels of FT3, FT4, or TSH in patients with simple goiter (p-values = 0.800, 0.400, and 0.600,

respectively). These findings align with the results of Roef et al., who identified significant associations between FT4 concentrations and the SNP rs11206244 in DIO1. However, they contradict the findings of De Jong et al., who reported that carriers of the D1a-T allele had higher serum free T4 and reverse T3, lower T3, and a lower T3/rT3 ratio. Additionally, Procopciuc et al. found that women with the D1-T785 mutated allele had lower FT3 levels and higher FT4 levels compared to women with the D1-C/C genotype (Roef et al., 2013; De Jong et al., 2007; Procopciuc et al., 2012). Therefore, while the SNP rs11206244 in the DIO1 gene does not appear to be associated with hyperthyroidism or hypothyroidism, it may contribute to the development of goiter in euthyroid goiter patients by decreasing FT4 levels.

The present study revealed that, there was significant increase of serum levels of FT3 and FT4p-value (0.001and 0.000) respectively also there was significant decrease in serum level of TSH p-value (0.030)in simple diffuse goiter when compare normal allele with mutant one of PDE8B gene (G/A). In contrast there was significant difference of serum levels of FT3, FT4 and TSH in simple diffuse goiter p-value (0.008, 0.010 and 0.04) and euthyroid goiter patients p-value (0.620,0.070 and 0.03) respectively. These results agree with results of Groussin et al. (2012) whom found a small group of patients with hyper secreting thyroid tumors had a significantly higher frequency of the alleles associated with lower TSH plasma levels. However disagree with results of Arnaud-Lopez et al whom identified a strong association p-value (0.004) between alleles of rs4704397 and circulating TSH levels; each additional copy of the minor A allele was associated with an increase of 0.13 mIU/ml in TSH. Also JORDE et al found the minor homozygote genotype (A:A) had a median serum TSH level that was 0.29 mIU/L higher than in the

major homozygote genotype (G:G) but FT3 and Ft4 were not statistically significant this result assumed with Shields et al. Grand one et al. and Taylor et al (Groussin et al., 2012;Arnaud-Lopez et al., 2008; Shields et al., 2009; Grand one et al.,2012; Taylor et al., 2011; JORDE et al., 2013)In this study found, there was insignificant differences when compare normal allele with mutant allele of TSHRc gene (C/G) in serum levels of FT3, FT4 and TSH in nodular goiter p-value (0.00, 0.040and 0.002), simple goiter P-value (0.001,0.000 and 0.003) and in euthyroid goiter p-value (0.62, 0.070 and 0.03) respectively .These results agree with results of Roef et al whom found the SNP in TSHR, rs1991517, does not show associations with the thyroid hormones pathway and TSH, FT4, ratio FT3:FT4, and rT3. (Roef et al., 2013). However this SNP in TSHR, rs1991517 strongly associated with goiter (simple , nodular and euthyroid) . Nevertheless, there was no association between normal and mutant allele of TSHRc gene(C/G) in serum levels of FT3, FT4 and TSH this means this SNP was contributed to thyroid disorders but the mutant allele effect was benign to thyroid hormones levels. Mutant genotypes are more frequent in thyroid disorders compared with control subjects. This finding supports the view that thyroid disorders are complex polygenic disease and that more combined genes are needed to predict the risk of thyroid disorders. Based on study findings ,each candidate gene might modulate an association with one or more of thyroid disorders.

5.2 Conclusion

the study concludes that goiter is prevalent among all study groups, with three types of thyroid disorders being more common in the (15-35 years) early age group and the Afro-Asiatic tribal group. Additionally, TSH levels in euthyroid goiter are decreased, while free T3 and free T4 levels remain unchanged. The DIO1a gene (rs11206244) is associated with euthyroid goiter, but no association was observed with simple or nodular goiter. The detected polymorphism in the DIO1a gene may contribute to the increased prevalence of goiter in White Nile State. Furthermore, the PDE8B gene (rs470439) allelic frequency is associated with simple goiter, nodular goiter, and euthyroid goiter. Additionally, a relationship has been reported between the TSHRc-C/G (Asp727Glu) gene and all types of thyroid disorders. Finally, FT4 levels are decreased in nodular and euthyroid goiter, while FT3 and TSH levels remain unchanged when comparing the normal allele to the mutant allele of the DIO1a gene rs11206244 (C/T). Conversely, the mutant allele of the PDE8B gene reveals increased FT3 and FT4 levels but decreased TSH levels. These genes may contribute to the pathogenesis of related disorders. Moreover, genetic polymorphisms in the TSHRc and PDE8B genes are linked to simple goiter, nodulr goiter, and euthyroid goiter, suggesting their potential as prognostic markers for goiter.

5.3 Recommendations:

Based on the findings of this study, the following recommendations are proposed:

1. Genetic testing for the DIO1a gene (rs11206244) should be considered in individuals diagnosed with goiter, particularly those aged 15-35.
2. Early detection of the PDE8B gene (rs470439) in symptomatic simple goiter may help prevent the development of nodular or toxic nodular goiter.
3. Further studies with larger sample sizes are needed to elucidate the relationship between the DIO1a gene SNP and thyroid pathogenesis.
4. Additional studies should be conducted in various regions of Sudan to evaluate other genes associated with thyroid disorders and their potential impact on thyroid health.
5. Patients with euthyroid goiter should undergo regular monitoring of thyroid-stimulating hormone levels.
6. Future research should focus on identifying potential mutations through sequencing techniques.

Appendices

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Appendices

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Appendix "1"
Questionnaire
Shandi University of Science and Technology
Genetic Polymorphisms in TSH, T3 and T4 genes in Sudanese with goiter

-Patient No: …-Name ……-Age: ……

- Duration of disease: ……..

-How many years stayed in White Nile State : ……

-Gender: Male …….. Female ……..

-If female are you pregnant: Yes ……No……..

-Is thyroid disease in family history: Yes ……..No ……..

-If yes what are your degree: First …..Second…….. Third……

- On nutritional supply any source of iodine: Yes…….. No…….

-On medical supply any source of iodine: Yes…… No…….

-If yes what is it? .....

-Enlargement of thyroid gland (goiter): Yes No

- Cigarette smoking: Yes…… No……..

-Other chronic disease:

……

-Drugs have been used:

……..

Appendix "2"

Informed consent

اعلام موافقة

هذه دعوة منى :الباحث / **احمد الامين فضل الله** طالب دكتوراه - مختبرات طبية – كيمياء سريرية – بجامعة شندي لمشاركتكم في برنامج بحث هدفه تقييم الجينات المرتبطة بمستوي هرومونات وظائف الغدة الدرقية لدي السودانيين المصابين بأمراض الغدة الدرقية في الدم.

إذا رغبتم في إنجاح هذا البرنامج فإني وفريق البحث سنقوم-:بأخذ عينة من الدم لقياس مستوي هرومونات وظائف تضخم الغدة الدرقية و لتقييم الجينات المرتبطة بها.

بملء إستمارة بمعلومات تخصكم لها علاقة بموضوع البحث.

أي معلومة تخصكم في الاستمارة سوف تكون سرية.

مشاركتكم في البرنامج تسعدنا وتساعد في إنجاح هدف البحث.

لكم كامل الحرية في إختيار عدم المشاركة ,المشاركة أو النسحاب من برنامج البحث في أي وقت تشاءون.

يمكنكم الحصول علي إجابة لي سؤال عن برنامج البحث.

التاريخ .....

توقيع المتبرع .....

توقيع الباحث : .....

(Rayto Microplate Reader- RT-2100C- Germany

1- Touch panel: display program

2- Plastic cover

3- Plate carrier: Microplate in plate carrier

CONVERGYS® td peltier thermal cycle, Germany

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UGENIUS-SYUG/1304 –UK

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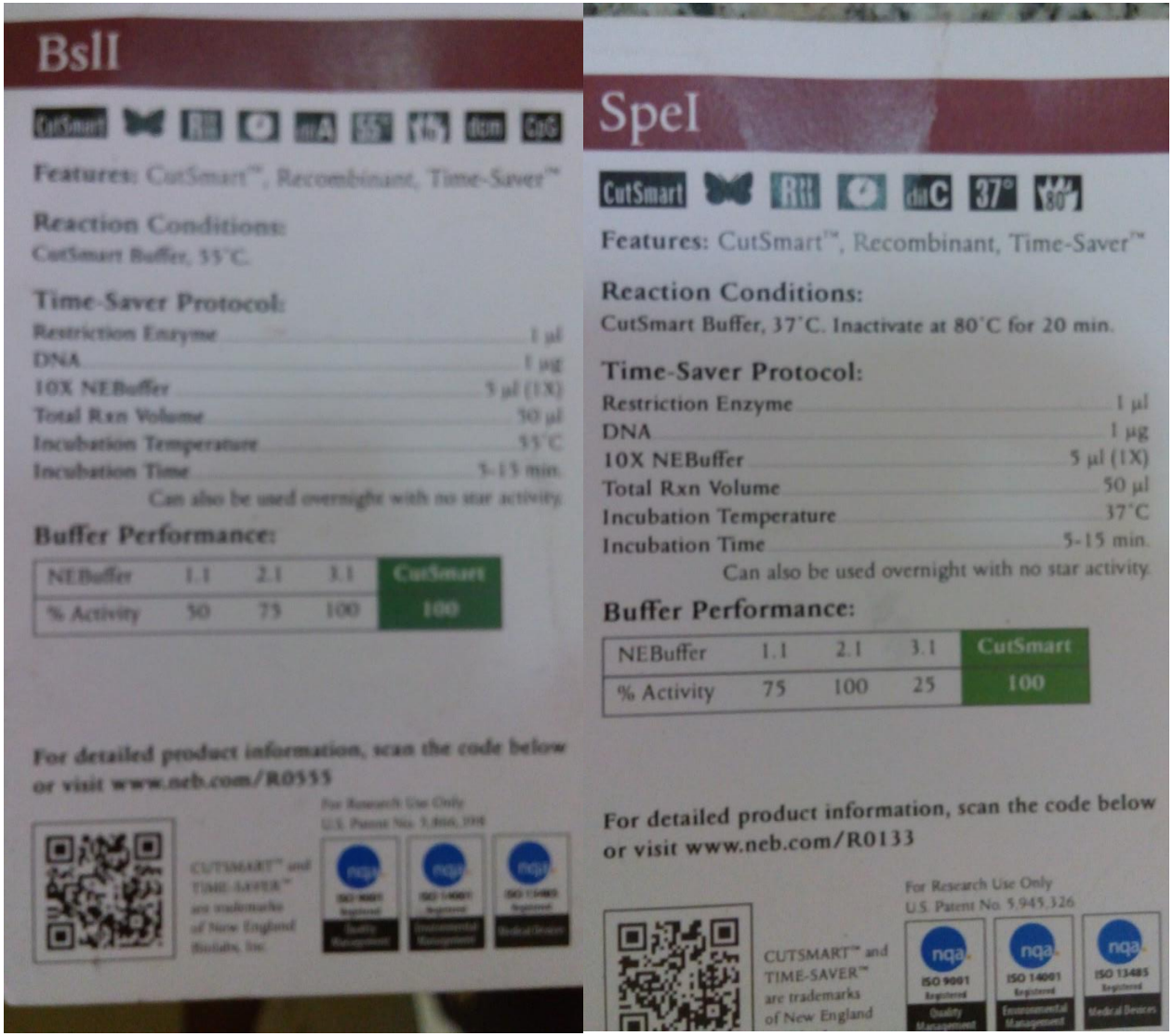

BsII

CutSmart

Features: CutSmart™, Recombinant, Time-Saver™

Reaction Conditions:
CutSmart Buffer, 55°C.

Time-Saver Protocol:

Restriction Enzyme	1 µl
DNA	1 µg
10X NEBuffer	5 µl (1X)
Total Rxn Volume	50 µl
Incubation Temperature	55°C
Incubation Time	5-15 min.

Can also be used overnight with no star activity.

Buffer Performance:

NEBuffer	1.1	2.1	3.1	CutSmart
% Activity	50	75	100	100

For detailed product information, scan the code below or visit www.neb.com/R0555

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U.S. Patent No. 5,866,398

CUTSMART™ and TIME-SAVER™ are trademarks of New England Biolabs, Inc.

SpeI

CutSmart

Features: CutSmart™, Recombinant, Time-Saver™

Reaction Conditions:
CutSmart Buffer, 37°C. Inactivate at 80°C for 20 min.

Time-Saver Protocol:

Restriction Enzyme	1 µl
DNA	1 µg
10X NEBuffer	5 µl (1X)
Total Rxn Volume	50 µl
Incubation Temperature	37°C
Incubation Time	5-15 min.

Can also be used overnight with no star activity.

Buffer Performance:

NEBuffer	1.1	2.1	3.1	CutSmart
% Activity	75	100	25	100

For detailed product information, scan the code below or visit www.neb.com/R0133

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NEW ENGLAND
BioLabs