1.1 Introduction

Thyroid diseases are the most common endocrine disease in females at reproductive age (Karaca and Akpak, 2015). According to current World Health Organization "W.H.O." statistics more than 3 billion people in the world live in iodine deficient countries. Iodine is the essential ingredient in thyroid hormone synthesis. So if deficient, protein synthesis will be disturbed. The thyroid gland needs iodine to synthesize thyroxine (T4) and triiodothyronine (T3) (Hollowell *et al*., 1998).

In Africa, goiter is endemic in several countries, notably Congo, Uganda, Kenya, and Sudan; the prevalence of goiter is as high as 81% in some parts of these countries (Ekpechi, 1987). In Sudan, endemic goiter and iodine deficiency disorders are serious health problems in many areas. The incidence of goiter among schoolchildren was estimated to be 85% in the Darfur region in western Sudan, 74% in the Kosti area in the center of Sudan, 13.5% in Portsudan in eastern Sudan, and 17% in the capital, Khartoum (Daniels and Dayan, 2006). The prevalence of thyroid nodules is elevated in women in areas of iodine deficiency and increases with advancing age (Bahn and Division, 2011).

Imbalance in production of thyroid hormones arises from dysfunction of the thyroid gland itself, the pituitary gland, which produces thyroid-stimulating hormone (TSH), or the hypothalamus, which regulates the pituitary gland via thyrotropin-releasing hormone (TRH) (Surks *et al.*,2004). The most common presenting clinical features of thyroid disease are the result of hypothyroidism, hyperthyroidism and goiter (Martin and crook, 2006). Hypothyroidism affects between three and ten percent of adults, with incidence higher in women and the elderly (Gharib *et al*., 2004; Fatourechi, 2009; Villar *et al*., 2007).

Thyroid hormones play a vital role in normal human physiology with effects on almost all tissues to influence growth and development, maintain normal cognition, cardiovascular function, bone health, metabolism and energy balance. In recent times we have come to understand the important influence that genetics play in normal and abnormal thyroid function. This has led to a greater knowledge of the intricacies of thyroid hormone action, differences between individuals and resultant disease (Panicker, 2011).

Even unrelated human subjects share about 99.9% of their genome. It has been estimated that 90% of the remaining variation is accounted for by approximately 10 million

common single nucleotide polymorphisms (SNPs), single base changes spread throughout the genome. These are very useful in studying gene-phenotype associations as they occur commonly in the general population, and may either cause changes in gene function themselves, or more frequently are markers of nearby elements that do. (Panicker, 2011)

1.2 Thyroid hormones Physiology

Physiology at multiple levels and in many organs including the hypothalamus, pituitary, thyroid and several peripheral tissues, presumably, they represent redundant, well orchestrated metabolic changes. The same could be said of the alterations in thyroid hormone metabolism in patients with non thyroidal illnesses for which the primary effects of the diseases are sometimes difficult to distinguish from the secondary effects of the altered nutritional state usually present (Danforth and Burger, 1989).

1.2.1 Synthesis of Thyroid hormones

For thyroid hormone synthesis, sufficient supply of the thyroid gland with essential micronutrients such as iodine and selenium is crucial. The most important target tissues of thyroid hormones are the central nervous system, the cardiovascular system and the skeleton (Obregon, 2008).

The daily iodine intake of adult humans varies from less than 10 μg in areas of extreme deficiency to several hundred milligrams for some persons receiving medicinal iodine. Milk, meat, vitamin preparations, medicines, radiocontrast material, and skin antiseptics are important sources (Pennington and Young, 1991; Dunn and Glinoer, 1993).

1.2.1.1 Production of thyroid hormone

The production of thyroid hormone occurs in the follicular cells of the thyroid and involves the following steps cellular uptake of iodide by the Na/I symporter (NIS) located in the basolateral membrane, release of iodide through the apical membrane into the follicular lumen via pendrin and/or other transporters, generation of H2O2 by the enzyme dual oxidase 2 (DUOX2) located in the apical membrane, iodination of tyrosine residues in thyroglobulin (Tg) with generation of mono and diiodotyrosine (MIT, DIT); generation of thyroxine (T4) by coupling of two DITs and of triiodothyronine (T3) by coupling of MIT and DIT. Both iodination and coupling are catalyzed by thyroid peroxidase (TPO) and require H2O2, endocytosis of Tg and hydrolysis by lysosomal enzymes, resulting in

the liberation of MIT, DIT, T4, and T3, deiodination of excess MIT and DIT residues by iodotyrosine dehalogenase (DEHAL), and reutilization of the iodide liberated for thyroid hormone synthesis and secretion of T4 and T3 at the apical membrane by an as yet unknown mechanism (Bjorkman and Elkholm, 1990).

THs can be produced and degraded by iodothyronine deiodinating enzymes, so called deiodinases. These enzymes belong to a selenocysteine containing enzyme family and comprise three types: D1, D2 and D3 (Khorle, 2000).

D1 and D2 are predominantly activating enzymes; both convertT4 to T3 (and rT3 to T2) by outer ring deiodination. D1 is found in liver, kidney, thyroid, and pituitary in humans and D2 in skeletal muscle, central nervous system, pituitary, thyroid, heart, 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 t4 and t3 in a ratio of approximately 17:1.3Conversion 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).

1.2.2 New aspects of thyroid hormone synthesis

A number of molecular causes of thyroid dysgenesis, 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 led to a clearer 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 important functions in the oxidative system of thyrocytes and molecules that play a role in thyroglobulin processing for thyroid hormone liberation and release from the thyroid gland (megalin and cysteine cathepsins) (Havemeyer *et al*. 2006; Jordans *et al.* 2009; Marino *et al.* 2000).

1.2.3 Transport of Thyroid hormones

Thyroid hormone is transported in the circulation tightly bound to different proteins, largely, thyroxine-binding globulin (TBG), transthyretin (TTR), and albumin. However, it is the free fraction of T4 and T3 which is available for metabolism and action in the tissues (Larsen *et al.,* 1998).

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).

1.2.4 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).

Most thyroid hormone actions are initiated by binding of T3 to its nuclear receptors (TRs). Cellular uptake of thyroid hormone does not occur by passive diffusion, but is mediated by specific transporters. These include different members of the monocarboxylate transporter (MCT) and organic anion transporting polypeptide (OATP)

families. TRs are encoded by two genes: THRA which codes for different TRα isoforms and THRB which codes for different TRβ isoforms (Larsen *et al.*, 1998).

1.2.4.1 Novel concepts of transmembrane and intracellular thyroid hormone actions

Previous textbook knowledge of thyroid hormone entry into cells included a diffusion-based passive transmembrane passage for thyroid hormones which are lipophilic but charged molecules. However, an eye-opening revelation was the identification of the MCT8 transporter (SLC16A2) that selectively enables T3 transport into target cells (Friesema *et al*., 2003).

Another unexpected finding of recent research projects was the identification of thyroid hormone derivatives, the so-called thyronamines (Scanlan *et al.,* 2004).

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 trace amine associated receptors (TAARs) (Borowsky *et al*., 2001; 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 dietinduced adiposity and prevents hepatic steatosis, however, without exerting the severe side 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 catecholamines 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 *et al.,* 2010).

1.3 Functions of Thyroid hormones

Thyroid hormones (triiodothyronine and its precursor, thyroxine) are essential for the correct development and maturation of the brain, a process that starts in utero but that extends into postnatal life (Moreno *et al.,* 2008).

The thyroid gland 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 (Procopciuc *et al.,* 2011).

Thyroid hormones are of significant importance for regular functioning of almost all body organs. Thyroxine (T4) is the main hormone and it is transformed into biologically active 3',3,5-triiodothyronine (T3) via 5'-deiodinases of thyroid hormone target cells (Kohrle, 2000).

1.4 Regulation of Thyroid hormones:

The production of thyroid hormone by the thyroid gland is regulated by the hypothalamus-pituitary-thyroid (HPT) axis (Larsen *et al*., 1998).

Thyroid hormone is secreted in response to thyroid-stimulating hormone (TSH), which is synthesized in and released from the pituitary. TSH consists of a (common) α subunit and a TSH-specific β subunit, and exerts its effect via binding to the TSH receptor (TSHR) on the thyroid follicular cells. In turn, TSH production is stimulated by hypothalamic thyrotropin-releasing hormone (TRH). The production of TRH and TSH is down-regulated by thyroid hormones, a process known as negative feedback regulation. Also, other hypothalamic hormones and drugs, such as somatostatin, cortisol, and bromocriptine lower TSH production. Besides the regulation by TSH, thyroid hormone synthesis is also dependent on the availability of iodine (Larsen *et al.,* 1998).

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 (Vassart & Dumont, 1992).

Thyrotropin (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 very important role in the synthesis of thyroid hormone (Guo *et al*., 2005).

1.5 Disorders of thyroid gland

The incidence of thyroid disease is increasing, predominantly among women (Larsen *et al.*, 1998).

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 or hyper functioning of the thyroid gland reflected in the circulating levels of Tri-iodothyronin (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).

1.5.1 Hypothyroidism

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).

1.5.1.1 Causes of hypothyroidism

The common causes which are responsible for the development of primary and secondary or central hypothyroidism. Primary hypothyroidism is due to a disorder of the thyroid gland causing decreased synthesis and secretion of thyroid hormones. Hypothyroidism, which in 50% of the cases is of autoimmune etiology, is observed in chronic autoimmune thyroiditis. In the remaining 50% it is due to other causes or drugs (Amino *et al*., 1976).

Autoimmune thyroiditis is the commonest cause of hypothyroidism; it is known as Hashimoto's disease when there is a non-tender goiter (due to lymphocytic infiltration) or atrophic thyroiditis when the size of the thyroid gland is diminished or normal (Daniels and Dayan, 2006).

Genetic and exogenous factors predispose to the development of chronic autoimmune thyroiditis. The genetic factors recognized so far are few, including genes encoding the major histocompatibility complex (*HLA*), and the gene encoding antigen 4 of cytotoxic T lymphocytes (*CTLA-4*) (Shi *et al*., 1992; Braun *et al.,* 1998).

The mechanisms by which these genes contribute to increased susceptibility to Hashimotos thyroiditis remain obscure. A polygenic factor for autoimmune thyroiditis is suggested by linkage of the disorder to several genetic loci in affected kindred (Allen *et al.,* 2003).

Infectious agents may cause autoimmunity via tissue destruction or molecular mimicry. Higher incidence of antithyroid antibodies has been found in residents of areas with iodine sufficiency than in those with iodine insufficiency, while in cases of iodine insufficiency the presence of autoimmune thyroiditis was correlated with higher iodine excretion in the urine (Tsatsoulis *et al.,* 1999).

Postpartum thyroiditis (which appears during the first year after delivery and affects 5%-10% of women) is due to the presence of antithyroid antibodies which increase after delivery. It presents with mild hyperthyroidism which may be transformed to hypothyroidism and may subside without therapy or may present only with hypothyroidism and should be managed by thyroxine for duration of up to 6 months. However in 25% of cases hypothyroidism may persist for up to 4 or more years. Silent thyroiditis presents with mild, of recent onset hyperthyroidism. It is due to the secretion of thyroid hormones in the circulation, due to cell lysis and subsides in 6-12 weeks or is transformed in 50% of the cases to transient hypothyroidism, which subsides in 2-12 weeks. Rarely, in up to 5% of the cases, hypothyroidism becomes permanent. Iodine insufficiency is a common cause of hypothyroidism (Andersson *et al.,* 2005).

These patients usually have a large goiter. Transient hypothyroidism may occur after the ingestion of large amounts of iodine and is referred to as Wolff- Chaicoff effect, due to the inhibition of hormone synthesis within the thyroid. It appears that there is a mild

enzyme disorder which is corroborated by the ingestion of iodine agents. Increased amounts of iodine are found in contrast agents and in the drug amiodarone. In partial thyroidectomy for hyperthyroidism clinical hypothyroidism has been found in 17% and subclinical in 51.3% whereas in partial thyroidectomy for various disorders clinical hypothyroidism has been found in 27%. In Grave's' disease mild and sometimes transient hypothyroidism is observed during the first 6 months after radioiodine therapy. External radiotherapy of the head and neck, as well as whole body irradiation may cause damage to the thyroid and lead to hypothyroidism. Hypothyroidism appears after a rather large time period (Mercado *et al*., 2001).

Various drugs may cause hypothyroidism, the commonest being the widely used drugs amiodarone and lithium. Interferon-a may also cause hypothyroidism, usually mild. The new tyrosine kinase inhibitor Sunitinib, an anticancer agent, has been shown to cause hypothyroidism (Vetter *et al.*, 2008).

Secondary (central) hypothyroidism is caused by a disorder of the pituitary or the hypothalamus, leading to decreased TSH secretion and consequently to decreased synthesis and secretion of thyroid hormones. Secondary hypothyroidism is also reported as central and is divided in secondary and tertiary when the causes are in the pituitary and the hypothalamus, respectively (Kostoglou-Athanassiou and Ntalles, 2010).

1.5.1.2 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 (Al-Azzam *et al.*, 2014).

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 (Larsen *et al*., 1998).

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).

1.5.1.3 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)

1.5.2 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 thyroxine. Serum TSH has a log-linear relationship with circulating thyroid hormone levels (a 2-fold change in free thyroxine 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. (Hollowell *et al.*, 2002)

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 and Akpak, 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% (Karmisholt *et al.*, 2008; Bennett *et al.*, 2005)

In congenital neonatal hypothyroidism hypothermia, bradycardia, jaundice, feeding unwillingness, apathy, voice hoarseness, constipation and omphalocele 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 (Michalopoulou *et al.*, 1998)

The appearance of symptoms depends on the degree of its severity. In subclinical hypothyroidism most patients do not have symptoms. However, some, which approximate 30%, have. In a study performed in Sweden for estimation of tissue hypothyroidism by a new clinical score, evaluation of patients with various grades of hypothyroidism and controls revealed that 24% of patients with subclinical hypothyroidism had symptoms (Zulewski *et al.,* 1997).

1.5.3 Hyperthyroidism

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 Boelaert, 2012).

Accelerated thyroid function results in secretion of excessive levels of thyroid hormones. The terms ‘‘hyperthyroidism’’ and ‘‘thyrotoxicosis’’ are often used interchangeably; however, hyperthyroidism means that the thyroid gland is functioning more than normal. Therefore, a hyperthyroid patient is thyrotoxic, but a thyrotoxic patient need not have an overactive thyroid and is therefore not actually hyperthyroid (Figge *et al*., 1994).

1.5.3.1 Causes of hyperthyroidism

Common causes of hyperthyroidism include: Grave’s’ disease; toxic multinodular goitre; 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).

1.5.1.2 Signs and symptoms of hypothyroidism

The resulting signs and symptoms can be particularly severe in elderly patients. The most common symptoms are weight loss, irritability, heat intolerance and sweating, fatigue, weakness, gastrointestinal hypermotility, and diarrhea. Common signs are tachycardia and atrial fibrillation, congestive heart failure, tremor, goiter, warm moist skin, and muscle weakness (Iagaru and McDougall, 2007).

Ophthalmopathy occurs concurrently with hyperthyroidism only 40% of the time. In 10% of patients with ophthalmopathy, thyroids hormones are normal, although

hyperthyroidism usually ensues within 18 months. Rarely, ophthalmopathy may be associated with Hashimoto's thyroiditis. Ophthalmopathy may worsen with prolonged hyperthyroidism or with hypothyroidism that occurs following treatment. Restoration of euthyroidism in such patients tends to stabilize or improve the coexisting ophthalmopathy (Ginsberg, 2003).

1.5.3.3 Management of Hyperthyroidism

Treatment for hyperthyroidism includes administration of propylthiouracil (300-600 mg/day total at eight-hour intervals) or methimazole (30-60 mg/day total, administered in two doses), which are thioamides that inhibit hormone biosynthesis by aborting the iodotyrosine residue coupling. Starting dose for the propylthiouracil is 100 mg every six to eight hours. Methimazole is more effective than propylthiouracil but with more side effects. The main purpose of this therapy is to limit the circulating hormone. Surgery and radiotherapy (iodine 131, or I-131) are other options, but they are associated with the risk of creating permanent hypothyroidism. Radioactive iodine therapy is used for patients who have Grave's' disease, as well as severe cardiac compromise, toxic uni- or multinodular goiter or severe reaction to antithyroid drugs (Luong and Nguyen, 2000).

1.5.4 Subclinical hyperthyroidism

Subclinical hyperthyroidism is identified as a low serum TSH concentration (i.e. less than the lower limit of the normal range) and a normal serum TH, in the absence of hypothalamic or pituitary disease, non-thyroidal illness or ingestion of drugs that inhibit TSH (Abalovich *et al*., 2007).

Causes of subclinical hyperthyroidism may be Persistent subclinical hyperthyroidism which can be devided to endogenous (toxic multinodular goitre; solitary toxic (autonomous) nodule; Grave's' disease) or exogenous (either intentional or unintentional over-treatment with levothyroxine) or may be transient subclinical hyperthyroidism such as treatment of clinical hyperthyroidism with antithyroid drugs or radioactive iodine therapy and evolving thyroiditis: (viral, post-partum, silent autoimmune, amiodarone-induced thyroiditis) (Kostoglou-Athanassiou and Ntalles, 2010; Daniels and Dayan, 2006; Cooper and Biondi, 2012).

Other conditions that cause low TSH levels (i.e. below the normal range) but which do not represent SC thyroid disease include early pregnancy, treatment with some drugs like

high dose glucocorticoids or dopamine, or smoking. Normal TSH levels usually return with resolution of the underlying condition. The true prevalence of SC hyperthyroidism is uncertain as the definition used is not consistent across studies (Cooper and Biondi, 2012; Pai and Gong, 2013; Lahner *et al*., 2008).

1.5.5 Goiter

In Africa, goiter is endemic in many countries, notably Congo, Uganda, Kenya, and Sudan; the prevalence of goiter is as high as 81% in some parts of these countries (Elnour *et al*., 2000).

Little is known about the prevalence of goiter in other areas of Sudan. In the areas studied so far, iodine deficiency was known as the principal etiologic factor. Though, consumption of pearl millet, vitamin A deficiency, and protein-energy malnutrition were also suggested as instrumental factors in the etiology of endemic goiter in western Sudan (Osman and Fatah, 1981; Elnour *et al*., 1997).

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 150-300 micrograms (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).

1.5.5.1WHO classification of goiter

Grade 0 – no goiter presence is found (the thyroid impalpable and invisible)

Grade 1 – neck thickening is present in result of enlarged thyroid, palpable, 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 (Lewinski, 2002)

1.5.6 Thyroid nodule

Thyroid nodules are commonly encountered in clinical practice. The prevalence of thyroid nodules in the general population is known to be 4 –7% by palpation alone and 30 –50% by ultrasound but about 5 – 6·5% of them have been found to be malignant (Nam-Goong *et al.*, 2004).

A thyroid nodule is a palpable swelling in a thyroid gland with an otherwise 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 (Welker and Orlov, 2003)**.**

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 cases (Wong and Wheeler, 2000).

1.5.7 Thyroid cancer

Thyroid carcinoma is rare among human malignancies (1%) but is the most frequent endocrine cancer, accounting for about 5% of thyroid nodules. The latter are very frequent in the general population and, according to the method of detection and the age of the patients, their prevalence may approach 20–50% of the general population Currently, thyroid US is the most accurate imaging technique for the detection of thyroid nodules and this procedure is mandatory when a nodule is discovered at palpation (Pacini *et al.*, 2006).

Fine needle aspiration cytology (FNAC) is an important technique that is used for the diagnosis of thyroid nodules; FNAC is a very sensitive tool for the differential diagnosis of benign and malignant nodules. The use of various immunohistochemical markers in

cytologic samples to differentiate papillary thyroid carcinoma from other follicular-derived lesions of thyroid has been explored during the last years but none of the markers appears to be specific enough to be employed as the diagnostic marker for the cytologic diagnosis of papillary thyroid carcinoma (Pacini *et al.*, 2006; Cooper *et al.*, 2009).

1.6 Thyroid Function Testing

Greater sensitivity of assays and more frequent assessment of serum thyroid-stimulating hormone (TSH) levels have resulted in more patients requiring interpretation of abnormal thyroid function test results (Surks *et al.*, 2004).

Assay of free thyroxine (T4) and triiodothyronine (T3) reliably eliminates the difficulties in interpretation of total thyroid hormone levels caused by the common variations in serum thyroid hormone-binding protein, best exemplified by the oestrogen-induced rise in thyronine-binding globulin which increases total thyroid hormone levels. However, free hormone assays are still subject to in-vitro and in-vivo artefacts in severe non-thyroidal illness, severe disturbances of binding proteins, and heparin therapy (Stockigt, 2001).

In patients with overt hypothyroidism, the lack of T_4 feedback leads to TSH levels >20 mIU/L, whereas in milder or subclinical hypothyroidism the TSH levels are between 3 and 20 mIU/L with normal T_4 and T_3 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 (Levey and Klein, 1994; Paschke and Ludgate, 1997).

The most sensitive in-vitro index for thyroid dysfunction autoimmunity is measurement of thyroid peroxidase antibody level, but this can occasionally give false negative results (eg, in juvenile autoimmune thyroiditis). In primary hypothyroidism, a raised level of thyroid peroxidase antibody is evidence for autoimmune chronic lymphocytic thyroiditis. In hyperthyroidism, the role of routine antibody testing is less clear. Diagnosis of Grave's disease is usually possible clinically, and measurement of thyroid peroxidase antibody or TSH-receptor antibody may not contribute to diagnosis or management. However,

measurement of TSH-receptor antibody has a role when the cause of hyperthyroidism is obscure, in assessing the risk of neonatal hyperthyroidism in pregnant women with a history of Grave's disease, and in assessing the risk of relapse after a course of antithyroid drugs in Grave's disease (Hollowell *et al.*, 2002).

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 (Sidawy *et al.*, 1997).

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*., 1998).

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 hypoechogenicity), increased vascularity, microcalcifications, 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).

1.6.1 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 T4 serum concentration is measured at the same time. Free T4 concentrations are raised in nearly all cases of overt 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), 32 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).

1.6.2 Diagnosis of hypothyroidism

TSH and FT_4 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 FT_4 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 FT_4 is decreased and in subclinical when TSH is increased and FT_4 is normal. When TSH is normal or decreased and FT_4 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).

1.7 Genetic Aspects of thyroid function

Genetics play a prominent role in both determination 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).

1.7.1 Type 1iodothyroinine gene and protein

(*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 (chr 1p33-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 type 1 iodothyronine deiodinase enzyme and influences serum thyroid hormone levels, FT3 and FT4 (Procopciuc *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 HapMap 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).

The co translational incorporation of Sec into the deiodinases and other selenoproteins presents significant problems for the cell, which must recognize the UGA as a Sec codon rather than a STOP translation signal. The cloning of D1 led to the identification of the eukaryotic Sec insertion sequence (SECIS) element as a stem-loop structure in the 3_ untranslated regions (UTR) of the D1 and glutathione peroxidase mRNAs. The SECIS element is the signal that recodes the in-frame UGA from a STOP to a Sec codon (Bianco *et al*., 2002).

Peeters *et al* investigated the occurrence and possible effects of SNPs in the deiodinases (*DIO1*; *DIO2*; *DIO3*, the *TSH* receptor and the thyroid hormone receptor-beta) genes.

They identified 8 SNPs of interest, 4 of which had not yet been published. Three are located in the 3-prime untranslated region: a C/T variation at nucleotide position 785 of the *DIO1* cDNA, referred to as *D1a*-C/T *D1b* (allele frequencies, C = 66%, T = 34%); an A/G variation at position 1814, referred to as -A/G (A = 89.7= %, G = 10.3%); and a T/G polymorphism at nucleotide position 1546 of the *DIO3* cDNA, referred to as D3-T/G (T = 85.5%, G = 14.2%). *D1a*-T was associated in a dose-dependent manner with a higher plasma reverse T3 (rT3), a higher plasma rT3/T4, and a lower T3/rT3 ratio. The *D1b*-G allele was associated with lower plasma rT3/T4 and with higher T3/rT3 ratios. The G allele of the *TSHRc*-C/G (asp727 to glu) polymorphism, *TSHRc*-G, was associated with a lower plasma TSH and with lower plasma TSH/free T4, TSH/T3, and TSH/T4 ratios. *TSHRc*-G was associated with a lower plasma TSH (CC, 1.38 _ 0.07, *vs.* GC, 1.06 _ 0.14 mU/liter; *P* _0.04), and with lower plasma TSH/free T4 (*P* _ 0.06), TSH/T3 (*P* _ 0.06), and TSH/T4 (*P* _ 0.08) ratios. The authors concluded that they found significant associations of 3 SNPs in 2 genes (*DIO1*, *TSHR*) with plasma TSH or iodothyronine levels in a normal population (Peeters *et al*., 2003).

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 for rs2235544 (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*-T allele had higher serum free T4 and reverse rT3, lower T3, and lower T3/rT3. The *D1b*-G allele was associated with higher serum T3 and T3/rT3 (De Jong *et al*., 2007).

Procopciuc *et 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 (Procopciuc *et al.*, 2012).

Philibert *et al* genotyped 12 single nucleotide polymorphisms identified in previous genome wide association analyses of thyroid function in DNA contributed by 1555 subjects 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 deiodinase 1(*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 in D2. Ratio of fT3/fT4 in hypothyroid subject was higher than others (Wibowo *et al.*, 2015).

1.7.2 Phosphodiesterase 8B (PDE8B) gene and protein

The human phosphodiesterase type 8B (*PDE8B*) gene is located at human chromosome 5q14.1 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 catalyses 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*, rs 4704397,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 T4 levels, 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 in PDE8B, 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 ($p=1.3\times10^{-11}$) 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 TPOAbs, 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).

1.7.3 *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 messengers 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; Cass and Meinkoth, 1998).

More than 30 point mutations that result in increased constitutive activity in the receptor have been described (Paschke and Ludgate, 1997).

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 harbouring 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).

A number of families have now been identified that are affected by loss-of-function *TSHR* mutations (Sunthornthepvarakul *et al.*, 1995).

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 nonautoimmune thyroid disease (toxic adenoma, 13.2%; multinodular goiter, 9.6%; disseminated autonomy, 21.4%) were not related to significant differences in codon 727 polymorphism 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 is 20.8 (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 0.71 and 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).

Gabriel *et al* Studied 52 normal individuals and 49 patients with Graves' disease; 33.3% of TMNG (P 50.019 vs. normal subjects), 16.3% of Graves' disease patients (P50.10 vs. normal subjects), and 9.6% of normal individuals were heterozygous for the D727E polymorphism. These findings indicate that a polymorphism of codon D727E of *hTSHR* is associated with TMNG, suggesting that its presence is an important predisposing genetic factor in the pathogenesis of TMNG (Gabriel *et al.*, 1999).

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 proband were enrolled and investigated. Six relatives, including his mother and father, were heterozygous for R450H mutation and D727E 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).

1.8 Rationale

Thyroid diseases are an important public health problem, and it is widely distributed in White Nile State in Sudan. However little information are available on assessment for Thyroid Diseases. It is very important to evaluate circulating levels of free T3, free T4 and thyroid stimulating hormones (TSH). Increase sensitivity and specificity of the methods usually used to investigate thyroid diseases achieved through this study using recent techniques, because little is known about advance investigation used for monitoring circulating levels of thyroid hormones. Genetic bases of susceptibility of Sudanese population to thyroid disorders are unknown. Assessment of the genetic bases associated with thyroid diseases in Sudanese may play an important role in the pathogenesis of the disease thus the classification in genetic variation may help in prevention of the disease and in line of treatment.

Accordingly we hypothesis that there is association between genetic variation of the thyroid hormone function and euthyroid goiter among White Nile State population so this study aims to understand the pathogenesis and inheritance of the disease and set genetic baseline for a group of data concerning evaluation and control of patients with Thyroid diseases in Sudan.

1.9 Objectives

General objective

The general aim of this is study was to assess genetic polymorphisms of thyroid related genes and its association with thyroid function and goiter in White Nile State- Sudan.

Specific objectives

1-To measure and compare blood levels of TSH, free T4 and free T3 of test and control group using ELISA technique.

2- To detect gene polymorphism of *TSHR* (rs1991517), *DIO1* (rs11206244 and rs12095080) and *PDE8B* (rs4704397) genes in test and control group using RFLP genotyping.

3- To confirm the results of RFLP genotyping using sequencing technique.

4-To correlate between the SNP in the thyroid related genes and thyroid dysfunction among Sudanese population.

5- To assess the frequency of the genotyping and compare allelic frequency of *TSHR* (rs1991517), *DIO1* (rs11206244 andrs12095080) and *PDE8B* (rs4704397) genes in patients with thyroid diseases and healthy controls.

6-To compare mean concentration of thyroid hormones and thyroid stimulating hormones (FT3, FT4 and TSH) levels of normal allele with mutant allele of each gene in thyroid hormone disorders patients.

2. Materials and Methods

2.1 Study design

Analytical case control hospital based study

2.2 Study area and period

The study was conducted at health insurance hospital in White Nile state in Sudan during the period from June 2013 to September 2016

2.3 Study population

One hundred patients with thyroid disorders were enrolled in this study which classified as thirty hyperthyroidism, thirty hypothyroidism, forty euthyroid goiter and fifty subjects as healthy control**.**

2.3.1 Inclusion criteria

Patients diagnosed with thyroid diseases (Hypothyroidism, hyperthyroidism and euthyroid goiter)

2.3.2 Exclusion criteria

Patients with thyroid diseases 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

2.4 Sampling

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 immuno enzymatic assay. Also 3 ml was collected in container with EDTA anti coagulant for DNA extraction for PCR (polymerase chain reaction) technique.

2.5 Data collection

A questionnaire (see appendix 1) was specifically designed to obtain information which helps in either including or excluding certain individuals in or from study.

2.6 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 2)

2.7 Laboratory Methods

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)

2.7.1 Measurement of free T3 using ELISA technique

Principle

The fT3 test was a solid phase competitive enzyme immunoassay. Patient serum samples, standards, and T3-Enzyme Conjugate were added to wells coated with monoclonal T3 antibody. FT3 in the specimen and the T3 labeled conjugate competed for available binding sites on the antibody. After incubation at room temperature, the wells were washed with distilled water to remove unbound T3 conjugate. On addition of the Substrate (TMB), a color developed only in those wells in which enzyme were present, indicating a lack of serum fT3. The reaction was stopped by the addition of dilute Hydrochloric Acid and the absorbance measured at 450 nm.

This test had been calibrated against in house standards. There is no International standard for this test. (Horworth and Ward, 1972)

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 distilled water 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.

2.7.2 Measurement of free T4 using ELISA technique

Principle

The fT4 test was a solid phase competitive enzyme immunoassay. Patient serum samples, standards, and Thyroxine-Enzyme Conjugate were added to wells coated with monoclonal T4 antibody. After incubation at room temperature, the wells were washed to remove unbound T4 conjugate. On the addition of Substrate (TMB), a color developed only in those wells in which enzyme were present, indicating a lack of fT4. The reaction was stopped by the addition of dilute Hydrochloric Acid and the absorbance was then measured at 450 nm. The intensity of the color formed was proportional to the amount of enzyme present and was inversely related to the amount of unlabelled fT4 in the sample.

This test had been calibrated against in house standards. There was no International standard for this test (Lundberg *et al*., 1982).

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.

2.7.3 Measurement of TSH using ELISA technique

Principle

Specific anti-TSH antibodies were coated onto microtitration wells. Test sera applyed. Then gated anti-TSH labeled with Horseradish Peroxidase enzyme (Conjugate) was added. If human TSH was present in the sample it will combine with the antibody on the well and the enzyme Conjugate, resulting in the TSH molecule being sandwiched between the solid phase and the enzyme linked antibodies. After incubation, the wells were washed to remove unbound labeled antibodies. On addition of the Substrate (TMB), a color developed only in those wells in which the enzyme Conjugate was presented, indicating the presence of TSH. The enzyme reaction stopped by the addition of dilute Hydrochloric acid and the absorbance was then measured at 450nm (Soos and Siddle, 1982).

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 5 seconds.

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.

2.8 Molecular biology techniques

After DNA extracted PCR amplification was performed using Peltier thermal cycler (CONVERGYS® td peltier thermal cycle, Germany) then SNPs were genotyped using RFLP analysis which confirmed by sequencing technique

2.8.1 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 (Chomczynski and Sacchi, 1987).

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: isoamylalcohol 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 (1.5% agarose gel)**

The gel was prepared by mixing 1.5 gm agarose, 100 ml 1X TBE buffer and 4 µl of ethidium bromide (10 mg/ml).

2. **Loading of the samples**

- 2-3µl of the extracted DNA mixed with 2-3 µl of loading buffer.
- 4µl 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 100 to 145 volts for 10-20 min then the gel was viewed under U.V transilluminater (UGENIUS-SYUG –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 µ DNA+ 490 µl H_2O).
- 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 µg/mL

1A260 single stranded DNA = 37 µg/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.

2.8.2 PCR amplification

The purpose of a PCR (Polymerase Chain Reaction) is to make a huge number of copies of a gene. This is necessary to have enough starting template for sequencing. There are three major steps in a PCR, which are repeated for 30 or 40 cycles. This is done on an automated cycler, which can heat and cool the tubes with the reaction mixture in a very short time (Cheng *et al*., 1994).

A. Denaturation at 94°C

During the denaturation, the double strand melts open to single stranded DNA, all enzymatic reactions stop (for example : the extension from a previous cycle).

B. Annealing at 55-60°C

The primers are jiggling around, caused by the Brownian motion. Ionic bonds are constantly formed and broken between the single stranded primer and the single stranded template. The more stable bonds last a little bit longer (primers that fit exactly) and on that little piece of double stranded DNA (template and primer), the polymerase can attach and starts copying the template. Once there are a few bases built in, the ionic bond is so strong between the template and the primer, that it does not break anymore.

C. Extension at 72°C

This is the ideal working temperature for the polymerase. The primers, where there are a few bases built in, already have a stronger ionic attraction to the template than the forces breaking these attractions. Primers that are on positions with no exact match, get loose again (because of the higher temperature) and don't give an extension of the fragment. The bases (complementary to the template) are coupled to the primer on the 3' side (the polymerase adds dNTP's from 5' to 3', reading the template from 3' to 5' side, bases are added complementary to the template).

2.8.3 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. RFLPs can be used to trace inhertitance patterns, identify specific mutations, and for other molecular genetics techniques. Restriction enzymes are proteins isolated from bacteria that recognize specific short sequences of DNA and cut the DNA at those sites. The normal function of these enzymes in bacteria is to protect the organism by attacking foreign DNA, such as viruses (Saiki *et al*., 1985).

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 (CONVERGYS® td peltier thermal cycle, Germany), by using the following primers (Macrogen, Korea) and restriction enzymes(New England Biolab) as seen in table (2.1) 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)

DNA Sequencing 2.8.4

DNA purification and standard sequencing was performed for both strands of *DIO1a* .genes for thirteen samples by Macrogen Company (Seoul, Korea)

Bioinformatics Analysis 2.9

The nucleotides sequences of the genes achieved were searched for sequence similarity using nucleotide BLAST (Atschul *et al*., 1997) (http: //blast.ncbi.nlm.nih.gov/Blast.cgi). Highly similar sequences were retrieved from NCBI and subjected to multiple sequence alignment using the BioEdit (Hall, 1999), Sequences similarities were searched with BLASTP (http://blast.ncbi.nlm.nih.gov/ Blast.cgi CMD =WebandPAGE_TYPEBlastDocs), highly similar sequences were achieved from NCBI and subjected to multiple sequence alignment and evolutionary analysis using the BioEdit software.

2.10 Quality control:

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

2.11 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 significants less than 0.05 levels using *t*- test. The relationship between variables was determined using Chi-square ($\chi 2$) test.

Table (2.1): Primers and restriction enzymes used in this study:

Gene	Primers (´5 - 3')	Restriction enzymes	References
-***TSHR***	F: 5-AACGCCAGGCTCAGGCATAC-3 R: 5-AAGTTCCCCTACCATTGTGA-3	*Nla*III	Peeters *et al*., 2003
-***DIO1a***	F: 5-GAACTTGATGTGAAGGCTGGA-3 R: 5-TAACCTCAGCTGGGAGTTGTTT-3	*Bcl*I	Peeters *et al*., 2003
-***DIO1b***	F: 5-CACATTTAACATGTAACATAG-3 R: 5- CAACAGAGTCATCTAGAAGGGA-3	*Spe*1	Peeters *et al*., 2003
- ***PDE8B***	F: 5-GGCGCTACTCTAGGTTTGGA-3 R: 5-GTCTGCTCCTTGGCTTTTCC-3	*Bsl*1	Grandone *et al.*,2012

3. Results

This study was done during the period of June 2013 to September 2016. This study included hundred (100) subjects as (30) with hypothyroidism, (30) hyperthyroidism and 40 euthyroid goiter and fifty (50) apparently healthy control with no family history of thyroid disease and matched age and sex to measure serum levels of thyroid hormones (free T3, free T4and TSH). In the present study all participants are female.68% (n=68) of patients had family history of thyroid disease with different grade. The mean±SD of the age (years) of the control group (n: 50) and thyroid diseases patients (n: 100) being (36.1±13.3 years) versus (34.8±12.7years).

Table (3.1) Shows the demographic characteristics of the patients and controls which presented insignificant difference *p*-value (0.560) between the mean of serum free T3 levels in euthyroid goiter and control group (2.4±0.7pg/ml) versus (2.3±0.7 pg/ml) also there is insignificant difference *p*-value (0.150) between the mean of F T4 levels in euthyroid goiter and control group (10.9±2.4 pg/ml) versus (11.5±2 pg/ml) and there is significant decrease *p*-value (0.020) of TSH levels in euthyroid goiter when compared with control group (1.03±0.8 pg/ml) versus (1.4±0.6 pg/ml)

Table (3.2) shows the significant association between *DIO1a* gene allelic frequencies and euthyroid goiter (*p*-value= 0.002, OR=4.7) and insignificant association with hypothyroidism and hyperthyroidism (*p*-value= 0.160, 0.080, OR=2.9, 2.4) respectively.

Table (3.3) shows significant association between *DIO1b* gene allelic frequencies and hypothyroidism (*p*-value= 0.001, OR=5.1) and insignificant association with hyperthyroidism and euthyroid goiter (*p*-value 0.320, 0.120, OR=1.8, 2.5) respectively.

Table (3.4) shows significant association between *PDE8B* gene allelic frequencies and hyperthyroidism, hypothyroidism and euthyroid goiter (*p*-value= 0.009, 0.010, 0.008 OR=3.7, 2.6, 2.8) respectively.

Table (3.5) shows significant association between *TSHR* gene allelic frequencies and hyperthyroidism, hypothyroidism and euthyroid goiter (*p*-value= 0.009, 0.004, 0.016 OR=3.1, 3.4, 2.5) respectively.

Table (4.6) shows significant decrease of FT4 level when compare normal allele with mutant allele of *DIO1a* gene in hyperthyroidism *p*-value (0.04) but FT3 and TSH levels are unaffected, Also FT3, FT4 and TSH levels are not change when compare normal allele with mutant allele of *DIO1b* and *TSHR* genes. But *PDE8B* gene shows significant

increase of FT3 and FT4 levels *p*-value (0.001, 0.001) respectively and significant decrease of TSH level *p*-value (0.010) when compare normal allele with mutant allele

Table (3.7): shows the levels FT3, FT4 and TSH in hypothyroidism patients are not affected when compare normal allele with mutant allele of *DIO1a*, *DIO1b*, *PDE8B* and *TSHR* genes.

Table (3.8): shows the levels FT3, FT4 and TSH in euthyroid goiter patients are not changed when compare normal allele with mutant allele of *DIO1a*, *DIO1b*, *PDE8B* and *TSHR* genes.

Figure (3.1): shows. 78% (n=78) of patients were Afro a Aciatic, 12% (n=12) were Nilo Saharian and 10% (n=10) were Niger Kordofain.

Figure (3.2): shows 80% of patients have a goiter where as 20% are not.

Figure (3.3): shows frequencies family history groups which presented most of patients (68%) had family history of thyroid diseases with different grades.

Figure (3.4) shows the frequencies of age group of patients which presented the age group 15-35 years (64%) had high percentage when compared with other age groups as 36-55 years (28%) and 56-75 years (8%)

Figure (3.5): shows the DNA examination shown on 1% agarose electrophoresis.

Figure (3.6): shows PCR results as bands typical in size (565bp) of *DIO1a* gene in addition to marker and negative control

(Figure (3.7) shows the PCR-based restriction analysis of the *DIO1a* by *Bcl*I restriction enzyme which cuts in the T/GATCA region as two fragments of 434 and 131 bp only in the presence of the *DIO1a*-T allele were shown to be TT (polymorphic homozygote) genotype; in the presence of 565 only the fragment were shown to be CC (wild type) genotype; in the presence of 565, 434 and 131 bp, the fragments were shown to be CT (polymorphic heterozygote) genotype.

Figure (3.8): shows multiple sequence alignment of *DIO1a* gene, mutation shown red

Figure (3.9): shows blast alignment of normal (Subject) and mutant (Query) of type1 (*DIO1*) gene the normal residue shown red

Figure (3.10): shows multiple sequence alignment of *DIO1a* gene, new (novel) mutation shown green

Figure (3.11): shows sequencing chromatogram of **A:** *DIO1* novel mutation-positive (containing two peaks with “A” and “C”) and **B:** *DIO1* novel mutation - negative (containing one peak “C”).

Figure (3.12): shows the PCR results showed bands typical in size (486bp) of *DIO1b* gene, addition to marker and negative control

Figure (3.13): shows the PCR-based restriction analysis of the *DIO1b* (rs12095080) gene by *spe*1 restriction enzyme which cuts in the A/CTAGT region as in the presence two fragments of 305 and 181 bp only in the presence of the *DIO1b*-G allele were shown to be GG (polymorphic homozygote) genotype and in the presence of 486 only the fragment were shown to be AA (wild type) genotype also in the presence of 486, 305 and 181 bp, the fragments were shown to be AG (polymorphic heterozygote) genotype.

Figure (3.14): shows the PCR results showed bands typical in size (519bp) of *PDE8B* (rs4704397) gene, in addition to marker and negative control

Figure (3.15): shows the PCR-based restriction analysis of the *PDE8B* (rs4704397) gene by *BSl*I restriction enzyme which cuts in the CCNNNNN/NNGG region as 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 and in the presence of 519 only the fragment were shown to be GG (polymorphic homozygote) genotype and in the presence of 519, 318 and 201bp, the fragments were shown to be AG (polymorphic heterozygote) genotype.

Figure (3.16): shows the PCR results showed bands typical in size (232bp) of *TSHR* gene, in addition to appropriate molecular markers and negative control.

Figure (3.17): shows the PCR-based restriction analysis of the p.D727E polymorphisms of the *TSHR* gene shown on 3.8% agarose electrophoresis by *Nla*III (*Hin*1II) restriction enzyme which cuts in the CATG/G region as in the presence of 129 and 103 bp, the fragments were shown to be GG (polymorphic homozygote) genotype and in the presence of 129, 82and 21bp, the fragments were shown to be be CC (wild type) genotype; in the presence of 129, 103, 82 and 21 bp, the fragments were shown to be CG (polymorphic heterozygote) genotype.

Figure (3.18): shows blast alignment of normal protein (Subject) and mutant (Query) protein of *TSHR* gene, the normal residue shown red

Figure (3.19): shows the biochemistry of the mutation of a aspartic acid into a glutamic acid at position 727.

Figure (3.20): shows the SD structure of the *TSHR* protein showed mutation of aspartic acid into glutamic acid at position 727.

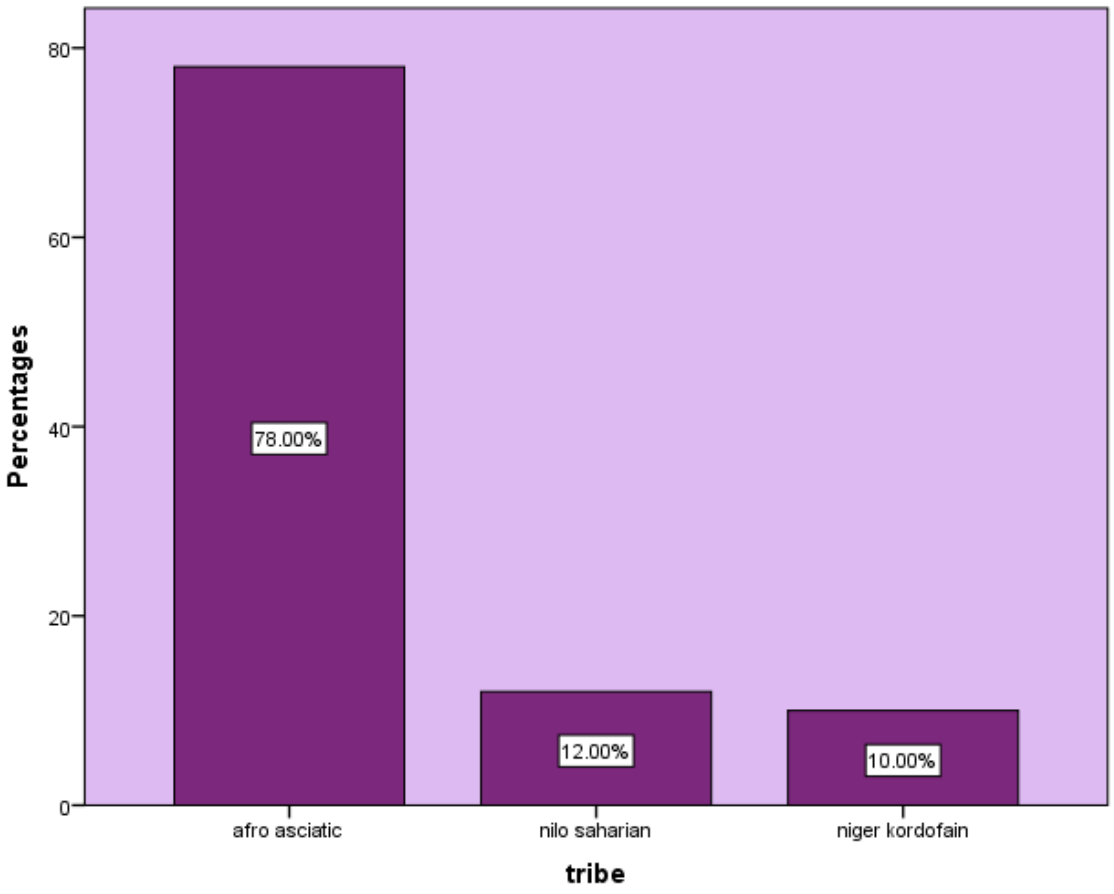


Figure (3.1): Shows frequencies of tribes group in patients

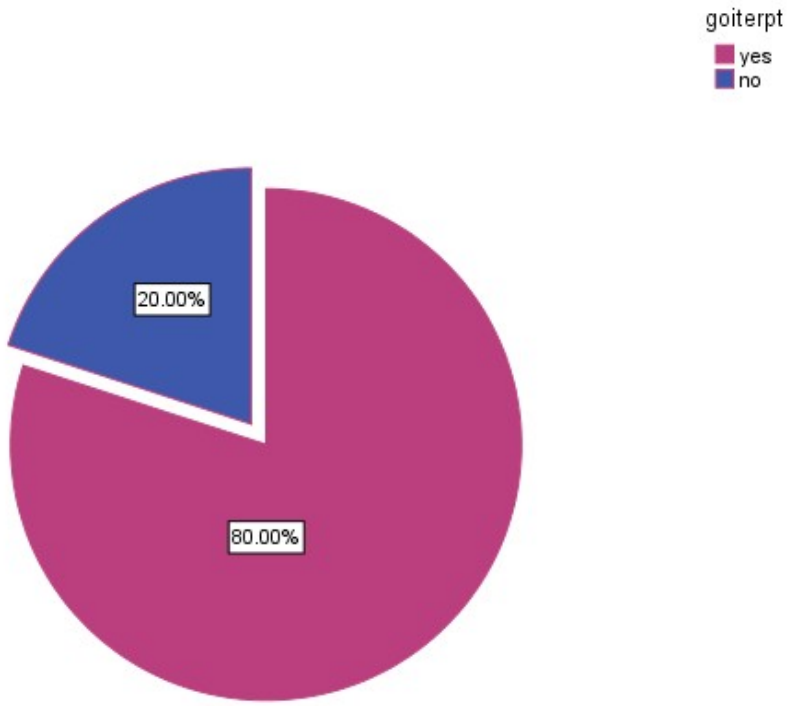


Figure (3.2) shows frequencies of goiter in thyroid disorders patients

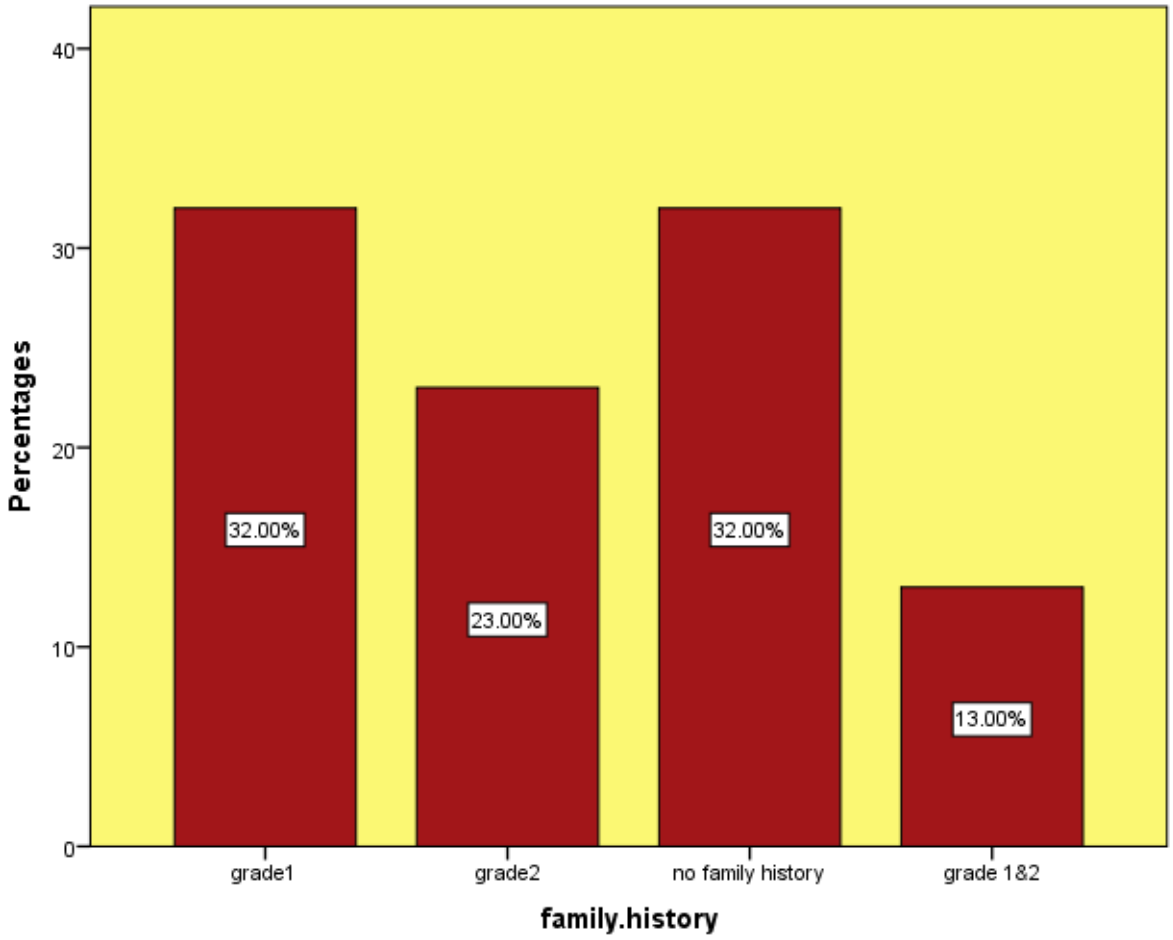


Figure (3.3): Shows frequencies of family history in patients

Key:

Grade 1: First degree relatives

Grade 2: Second degree relatives

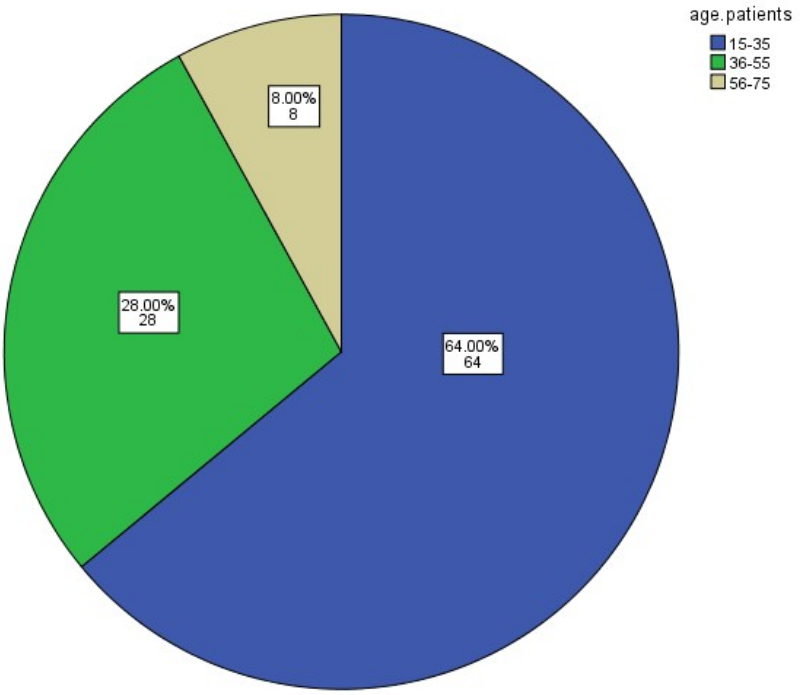


Figure (3.4) shows frequencies of age group within patients

Table (3.1): Comparison of means of FT3, FT4 and TSH levels between euthyroid goiter patients and controls

Variables	euthyroid goiter (n=40) (Mean±SD)	Controls (n=50) (Mean±SD)	*p*-value
FT3	2.40±0.70 pg/ml	2.3±0.7 pg/ml	0.560
FT4	10.1±1.80 pg/ml	11.5±2 pg/ml	0.150
TSH	1.03±0.80 pg/ml	1.4±0.6 pg/ml	0.020

Table (3.2): 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/ OR
	C/C	T/T	C/T	C	T	
Controls (n=50)	86% (n=43)	0 (n=0)	14% (n=7)	93 (93%)	7 (7%)	
Hyperthyroidism (n=30)	76.7% (n=23)	6.7% (n=2)	16.6% (n=5)	51 (85%)	9 (15%)	0.160/ 2.4

Hypothyroidism (n=30)	66.7% (n=20)	3.3% (n=1)	30% (n=9)	49 (81.7%)	11 (18.3%)	0.080/ 2.9
Euthyroid Goiter (n=40)	52.5% (n=21)	5% (n=2)	42.5% (n=17)	59 (73.8%)	21 (26.2%)	0.002 / 4.7

Table (3.3): The association of the *DIO1b* genotypes and allelic frequencies of the *DIO1b* gene in patients and controls

Case study	**Genotype Frequency**			**Allele Frequency**		***p*-value/OR**
	A/A	**G/G**	**A/G**	**A**	**G**	
Controls (n=30)	86.7% (n=26)	0 (n=0)	13.3% (n=4)	56 (93.3%)	4 (6.7%)	
Hyperthyroidism (n=30)	76.7% (n=23)	0 (n=0)	23.3% (n=7)	53 (88.3%)	7 (11.7%)	0.320/ 1.8

Hypothyroidism (n=30)	46.7% (n=14)	0 (n=0)	53.3% (n=16)	44 (73.3%)	16 (26.7%)	0.001 / 5.1
Euthyroid Goiter (n=30)	70% (n=21)	0 (n=0)	9% (n=30)	51 (85%)	9 (15%)	0.120/ 2.5

Table (3.4): The association of the *PDE8B* genotypes and allelic frequencies of the *PDE8B* gene in patients and controls

Case study	**Genotype Frequency**			**Allele Frequency**		***p*-value/OR**
	G/G	**A/A**	**G/A**	**G**	**A**	
Controls (n=30)	33.3% (n=10)	36.7% (n=11)	30% (n=9)	29 (48.3%)	31 (51.7%)	
Hyperthyroidism (n=30)	10% (n=3)	70% (n=21)	20% (n=6)	12 (20%)	48 (80%)	0.009 /3.7

Hypothyroidism (n=30)	10% (n=3)	56.7% (n=17)	33.3% (n=10)	16 (26.7%)	44 (73.3%)	0.010 /2.6
Euthyroid Goiter (n=30)	13.3% (n=4)	63.3% (n=19)	23.3% (n=7)	15 (25%)	45 (75%)	0.008 /2.8

Table (3.5): 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/OR**
	C/C	**G/G**	**C/G**	**C**	**G**	
Controls (n=50)	76% (n=38)	0 (n=0)	30% (n=12)	88 (88%)	12 (12%)	
Hyperthyroidism (n=30)	43.3% (n=13)	3.3% (n=1)	53.3% (n=16)	42 (70%)	18 (30.0%)	0.009 /3.1

Hypothyroidism (n=30)	40.0% (n=12)	3.3% (n=1)	56.7% (n=17)	41 (68.3%)	19 (31.7%)	0.004 /3.4
Euthyroid Goiter (n=40)	52.5% (n=21)	10.0% (n=4)	37.5% (n=15)	57 (71.2%)	23 (28.8%)	0.016 /2.5

Table (3.6): Mean concentration of thyroid hormones (TSH, FT4 and FT3) of mutant genotypes (*DIO1a*, *DIO1b*, *PDE8B* and *TSHR*) compared with normal related genotype in hyperthyroidism

Parameter	**Alleles of *DIO1a* gene (C/T)**		
	Mean±SD	**Main±SD**	***p*-value**
	C	**T**	
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 *DIO1b* gene (A/G)			
	A	**G**	
FT3	12.4±6.5	12.5±8.4	0.900
FT4	48.4±25.4	39.8±16.5	0.400
TSH	0.05±0.06	0.04±0.05	0.800
Alleles of *PDE8B* gene (G/A)			
	G	**A**	
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)			
	C	**G**	
FT3	12.0±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 (3.7): Mean concentration of thyroid hormones (TSH, FT4 and FT3) of mutant genotypes (*DIO1a*, *DIO1b*, *PDE8B* and *TSHR*)compared with normal related genotype in hypothyroidism

Parameter	Alleles of *DIO1a* gene (C/T)		
	Mean±SD	Mean±SD	*p*-value
	C	T	
FT3	0.9±0.3	0.9±0.3	0.600
FT4	6.2±1.7	5.9±2	0.700
TSH	12.4±7.2	15.4±6.3	0.300
Alleles of *DIO1b* gene (A/G)			
	A	**G**	
FT3	0.9±0.3	0.8±0.3	0.200
FT4	6.1±1.8	6.1±1.7	0.900
TSH	13.6±7.6	13.0±5.4	0.900
Alleles of *PDE8B* gene (G/A)			
	G	**A**	
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)			
	C	**G**	
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 (3.8): Mean concentration of thyroid hormones (TSH, FT4 and FT3) of mutant genotypes (*DIO1a*, *DIO1b*, *PDE8B* and *TSHR*) compared with normal related genotype in euthyroid goiter

Parameter	Alleles of *DIO1a* gene (C/T)		
	Mean±SD	Mean±SD	*p*-value
	C	T	
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 *DIO1b* gene (A/G)			
	A	**G**	
FT3	2.4±0.7	2.4±0.8	0.900
FT4	10.1±1.7	10.3±2.0	0.700
TSH	1.1±0.6	0.8±0.8	0.300
Alleles of *PDE8B* gene (G/A)			
	G	**A**	
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)			
	C	**G**	
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

100bp

Figure (3.5): shows the DNA examination shown on 1% agarose electrophoresis. Lane l was loaded with appropriate molecular markers; lane 2 is negative control; lane 3, 4, 5 and 6 are DNA positive

565 bp

Figure (3.6): The PCR results showed bands typical in size (565bp) of *DIO1a* gene. Lane 1 is marker, lane 2 is negative control, lane 3, 4, 5, 6, and 7 are PCR product positive 565pb.

Figure (3.7): The PCR-based restriction analysis of the *DIO1a* (rs11206244) gene shown on 2% agarose electrophoresis. The *Bcl*I restriction enzyme cuts in the T/GATCA region. In the presence two fragments of 434 and 131 bp only in the presence of the *DIO1a*-T allele were shown to be TT (polymorphic homozygote) genotype; in the presence of 565 only the fragment were shown to be be CC (wild type) genotype; in the presence of 565, 434 and 131 bp, the fragments were shown to be C (polymorphic heterozygote) genotype. Lane l was loaded with appropriate molecular markers, lanes 2, 3, 4 and 5 are

homozygous CC subjects; lanes 6 is homozygous TT subjects and lanes 5 and 6 are heterozygous CT subjects

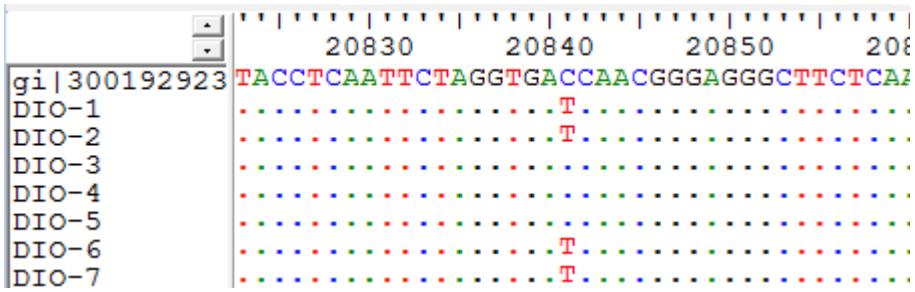


Figure (3.8): Multiple sequence alignment of *DIO1a* gene, mutation shown red

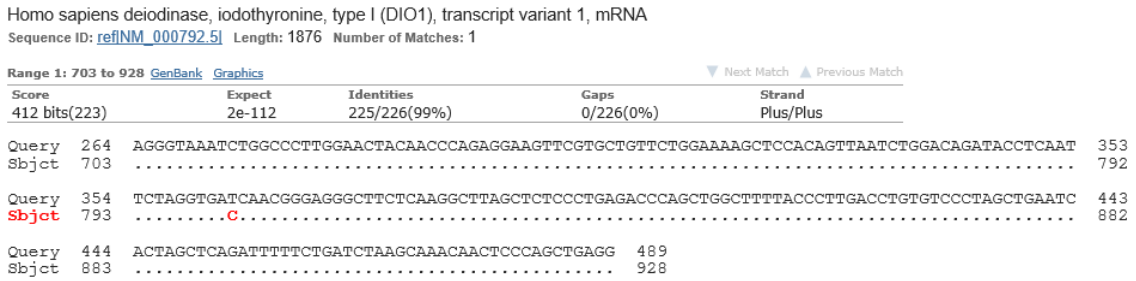


Figure (3.9): Shows blast alignment of normal (Subject) and mutant (Query) of type1 (*DIO1*) gene the normal residue shown red

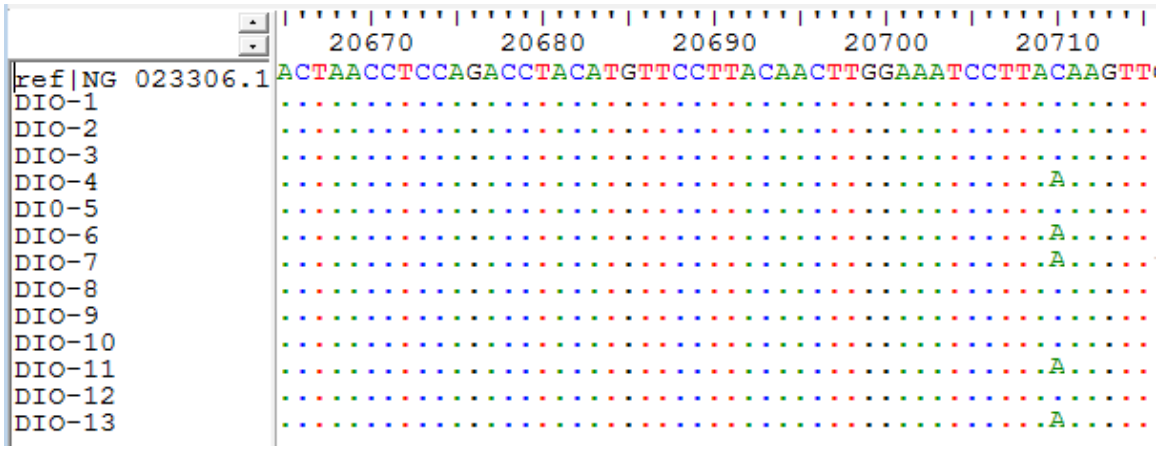
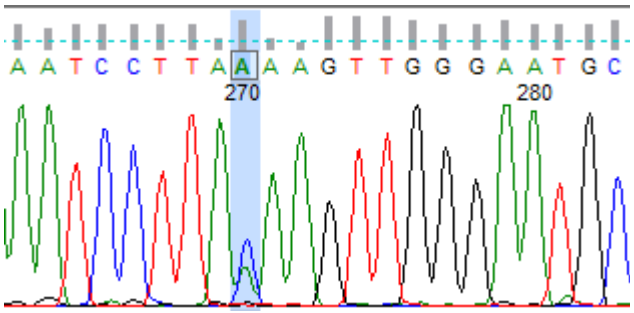


Figure (3.10): Multiple sequence alignment of *DIO1a* gene, new (novel) mutation which shown green

A:



B:

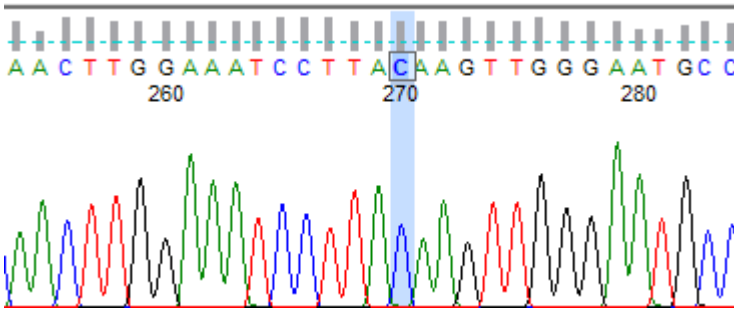


Figure (3.11): Sequencing chromatogram of **A:** *DIO1* novel mutation-positive (containing two peaks with "A" and "C"); **B:** *DIO1* novel mutation - negative.

Figure (3.12): The PCR results showed bands typical in size (486bp) of *DIO1b* gene. Lane 1 is marker, lane

Figure (3.13): The PCR-based restriction analysis of the *DIO1b* (rs12095080) gene shown on 2% agarose electrophoresis. The spe1 restriction enzyme cuts in the A/CTAGT region. In the presence two fragments of 305 and 181 bp only in the presence of the *DIO1b*-G allele were shown to be GG (polymorphic homozygote) genotype; in the presence of 486 only the fragment were shown to be AA (wild type) genotype; in the presence of 486, 305 and 181 bp, the fragments were shown to be AG (polymorphic heterozygote) genotype. Lane l was loaded with appropriate molecular markers, lanes 2, 3 and 6 are homozygous CC subjects; lanes 4, 5 and 7 are heterozygous AG subjects. Homozygous GG subjects not seen

Figure (3.14): The PCR results showed bands typical in size (519bp) of *PDE8B* (rs4704397) gene. Lane 1 is marker, lane 2 is negative control, lane 3, 4, 5, 6,7 and 8 are PCR product positive 519pb.

Figure (3.15): 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 appropriate molecular markers, 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.

Figure (3.16): The PCR-product of the p.D727E polymorphisms of the *TSHR* gene shown on 3% agarose electrophoresis. Lane l was loaded with appropriate molecular markers; lane 2 is negative control; lane 3, 4, 5, 6, 7 and 8 are positive PCR-product 232 bp.

Figure (3.17): The PCR-based restriction analysis of the p.D727E polymorphisms of the *TSHR* gene shown on 3.8% agarose electrophoresis. The *Nla*III (*Hin*1II) restriction enzyme cuts in the CATG/G region. In the presence of 129 and 103 bp, the fragments were shown to be GG (polymorphic homozygote) genotype; in the presence of 129, 82and 21bp, the fragments were shown to be be CC (wild type) genotype; in the presence of 129, 103, 82 and 21 bp, the fragments were shown to be CG (polymorphic heterozygote) genotype. Lane l was loaded with appropriate molecular markers; lane 2 is negative control; lanes 3 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

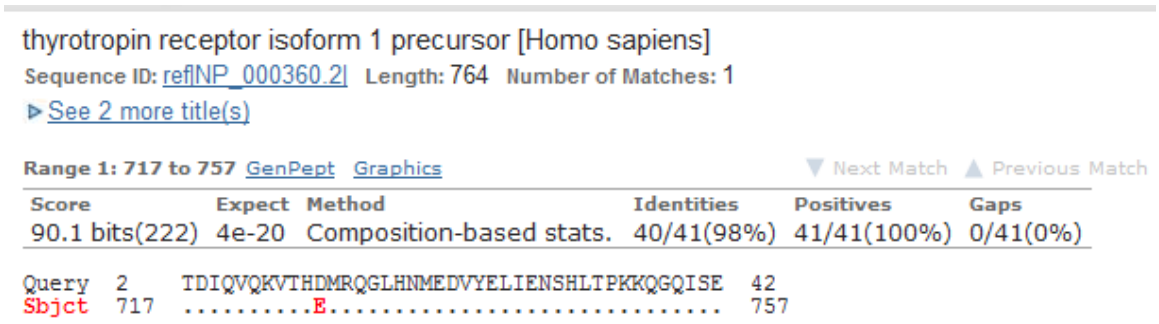


Figure (3.18): Show blast alignment of normal protein (Subject) and mutant (Query) protein of *TSHR* gene, the normal residue shown red

Figure (3.19): Biochemistry of the mutation of aspartic acid into glutamic acid at position 727.

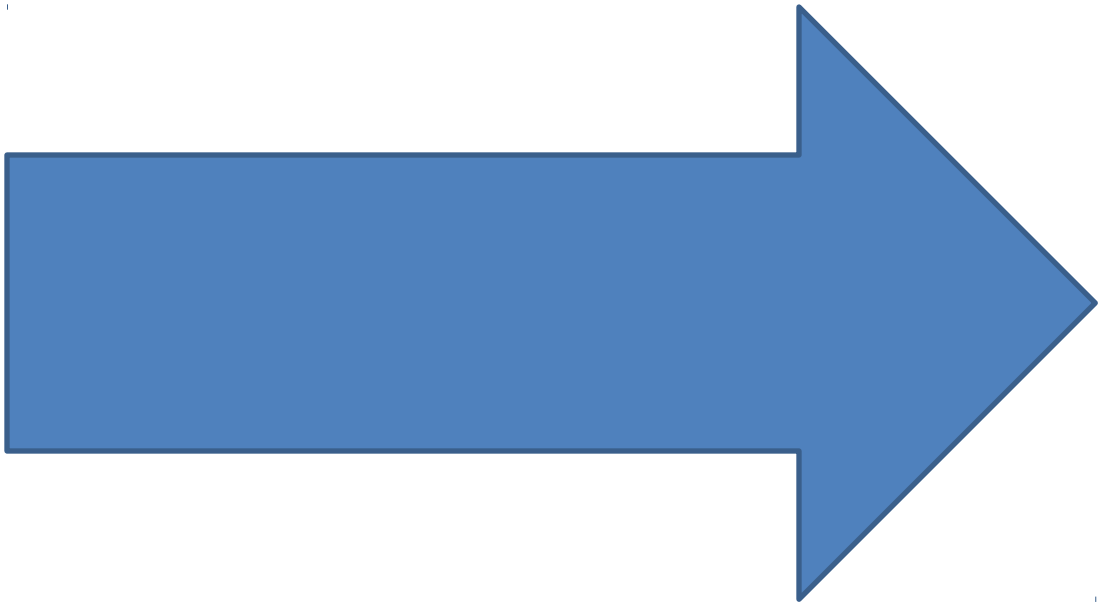


Figure (3.20): SD structure of the *TSHR* protein showed mutation of aspartic acid into glutamic acid at position 727

4.1 Discussion

Thyroid diseases are the most common endocrine disease in females at reproductive age (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 a range of 13% to 87%) (Medani *et al.*, 2011)

Interactions between individual genetic and environmental factors determine the onset of the thyroid disease, as for the genetic factors there are only a few studies. The present study aimed to screen the *DIO1a*, *DIO1b*, *PDE8B* and *TSHR* genes polymorphism and its relation to thyroid diseases (euthyroid goiter, hypothyroidism and hyperthyroidism).

In fact that the afro asciatic tribal group are most population of White Nile State other than Niger Kordofain and Nilo Saharian groups which clearly high prevalence of thyroid diseases among this population, Which classified by Begona in this previos study (Begona *et al.*, 2015).

The present study found that, 80 % of patients had goiter this result in agreement with the results reported by Eltom, *et al* whose showed the prevalence of goiter was estimated to be 85% in Darfur region in western Sudan, 74% in Kosti area in the south of Sudan, 13.5% in Port-Sudan in eastern Sudan, and 17% in the capital, Khartoum (Eltayeb *et al.*, 2008)

The current study found 68 % of patients had family history of thyroid diseases (euthyroid goiter, hypothyroidism and hyperthyroidism) this result was in concordance with the results reported by Tug *et al* whose showed 65% of their patients had family history of goiter (Tug *et al*., 2012).

The present study showed, the prevalence of thyroid disorders (euthyroid goiter, hypothyroidism and hyperthyroidism) was more common in age group15-35 years (61.1%) these findings are partly in agreement with those reported by Biassoni *et al* whom observed in the study among the Bororos a slight but in significant rise in the prevalence of goiter in females from puberty to 45 years, probably related to an increased need for care during pregnancy and lactation. However, no individual over the age of 45 was found to have goiter (Biassoni *et al*., 1998).

The current study provide evidence that, There was significant decrease of the mean of serum TSH level in euthyroid goiter when compared with control group with *p*-value (0.020) while the mean of free T3 and free T4 levels revealed insignificant differences with *p*-value (0.560, 0.150) respectively, this agree with result obtained by Tug *et al* study in goiter turkish population (Tug *et al*., 2012).

The present study revealed that, there was significant association between *DIO1a* gene (rs11206244) allelic frequency and euthyroid goiter *p*-value (0.002) and there was

insignificant association with hyperthyroidism and hypothyroidism *p*-value (0.160 and 0.080) respectively when compared with control group. Interestingly this was first study that explores the association of *DIO1a* gene (rs11206244) polymorphism with hyperthyroidism, hypothyroidism and euthyroid goiter. The previous studies done in healthy and euthyroid individuals as Peeters *et al* referred to as *D1a*-C/T (allele frequencies, C = 66%, T = 34%) in a normal population near to the percentages of this study in healthy control as *D1a*-C/T (allele frequencies, C = 93%, T = 7) (Peeters *et al.*, 2003).

The current study showed that, there was significant association between *DIO1b* gene (rs12095080) allelic frequency and hypothyroidism *p*-value (0.001), in contrast hyperthyroidism and euthyroid goiter showed no asssocition with *DIO1b* gene *p*-value (0.32 and 0.12) respectively when compared with control group. Also previous studies done in healthy and euthyroid individuals as Peeters *et al* referred to as A/G variation at position 1814, referred to as *D1b*-A/G (A = 89.7= %, G = 10.3%) in a normal population near to the percentages of this study in healthy control as A/G variation at position 1814, referred to as *D1b*-A/G (A = 93.3= %, G = 6.7%) (Peeters *et al.,* 2003).

The present study screened the mutations of the *DIO1a* gene by sending thirteen patients samples with thyroid hormone disorders that were selected randomly to standard sequencing as in Figure (3.9). Novel mutation was detected in five patients with thyroid hormone disorders (3 patients with hypothyroidism, one with hyperthyroidism and one with euthyroid goiter) most of them were hypothyroidism. The major determinant of plasma thyroid hormone FT3 levels in peripheral tissues is represented by deiodinase pathways. These extrathyroidal pathways,iodothyronine deiodinase D1 or D2 pathways, are responsible for about 80% of the daily T3 production in healthy subject (Saberi *et al*., 1975; Schimmel *et al*., 1977; Geffner *et al*., 1975).

This novel mutation located in an intronic region. Although little was known about its biological function, several polymorphisms have been described in D1 and D2, of which some are associated with circulating levels of T4, T3, and TSH (Peeters *et al.* 2005; Canani *et al.,* 2005; Mentuccia *et al*., 2002; Panicker *et al*., 2008) it could be the one of causative agent of high incidence of thyroid disorders in White Nile State.

Deiodinase D1 deficiency due to this polymorphism determines lower FT3 serum levels. Higher serum FT4 levels and lower serum FT3 levels in the presence of the T allele of *DIO1a* gene in women with hypothyroidism could be explained on the one hand by the fact that the transformation of FT4 to FT3 in the presence of deiodinase D1 takes place in the liver, which means that in hypothyroidism there is a decreased D1 activity because of the impaired hepatic function. On the other hand, we could explain these results by the fact that the decreased conversion of FT4 to FT3 caused by this novel mutation. Furthermore thyroid hormone disorders cases especially hypothyroidism in this study may have resulted from substitution C to A at position 20710 in the *DIO1* gene as seen in Figure (3.10).

From these results demonstrated that, there was significant association between *PDE8B* gene (rs470439) allelic frequency and hyperthyroidism hypothyroidism and euthyroid goiter when compared with control *p*-value (0.009, 0.010 and 0.008) respectively in fact that this is the first published study of *PDE8B* gene in thyroid diseases.

The present results clearly demonstrated that, there was significant association between *TSHRc*-C/G (Asp727Glu) gene allelic frequency and thyroid disorders (hyperthyroidism, hypothyroidism and euthyroid goiter) when compared with control with *p*-value (0.009, 0.004 and 0.016) respectively. Some previous studies done in healthy individuals as Peeters *et al* referred to as *TSHRc*-C/G (allele frequencies, C = 90.7%, G = 9.3%) and in a normal population near to the percentages of this study in healthy control as *TSHRc*-C/G (allele frequencies, C = 88%, G = 12%) (Peeters *et al.,* 2003) results of this study were disagree with MU HLBERG *et al* They found no significant association in codon 727 polymorphism frequencies between patients with autonomously functioning thyroid disorders (13.3%) and the healthy control group (16.2%) and *p*-value(0.570) (MU HLBERG *et al.,* 2000). Other Assumed that, the CC and CG genotype incidence for the patient group to be 0.71 and 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*-value (0.417 and 0.449, respectively) **(**Tug *et al.*, 2012) this attributed to different population in each study.

This study showed, there was significant decrease when compare normal with mutant allele of *DIO1a* gene rs11206244 (C/T) in serum levels of FT4 *p*-value (0.040, 0.020). In

contrast the serum levels of FT3 *p*-value (0.070, 0.600) and TSH *p*-value (0.190, 0.200) were unchanged in hyperthyroidism and euthyroid goiter respectively, while there was insignificant difference in serum levels of FT3, FT4 and TSH *p*-value (0.600, 0.700 and 0.300) respectively in hypothyroidism patients. These results were agree with results of Roef *et al* whom found significant associations with FT4 concentrations are observed for SNP rs11206244 in DIO1and disagree with De Jong *et al* whome found carriers of the *D1a*-T allele had higher serum free T4 and reverse rT3, lower T3, and lower T3/rT3. Also Procopciuc *et al* found Women with the *D1*-T785 mutated allele had lower FT3 levels, higher FT4 levels than women with the *D1*-C/C genotype (Roef *et al.*, 2013; De Jong *et al.*, 2007; Procopciuc *et al*., *2012*).Therefore this SNP rs11206244 in DIO1 gene had no association to both hyperthyroidism and hypothyroidism but it may contribute to appearance of goiter in euthyroid goiter patients by decreasing FT4 levels.

The current study showed there was insignificant difference when compare normal and mutant allele of *DIO1b* gene (A/G) in serum levels of FT3, FT4 and TSH in hyperthyroidism *p*-value (0.900, 0.400 and 0.800), hypothyroidism *p*-value (0.200, 0.900 and 0.900) respectively and euthyroid goiter patients. these results disagree with results of De Jong *et al* whom found The *D1b*-G allele was associated with higher serum T3 and T3/rT3 (De Jong *et al.*, 2007). So this SNP had no association to serum levels of FT3, FT4 and TSH in this study in hyperthyroidism or euthyroid goiter patients but may contribute to the pathogenesis of hypothyroidism because this SNP is significant associated with hypothyroidism and located in UTR regulatory region and it not associated to thyroid hormones subset FT3, FT4 and TSH this may due to the hypothyroidism already was occurred and negative feedback mechanism of hormones was happened.

The present study revealed that, there was significant increase of serum levels of FT3 and FT4 *p*-value (0.001 and 0.001) respectively also there was significant decrease in serum level of TSH *p*-value (0.010) in hyperthyroidism when compare normal allele with mutant one of *PDE8B* gene (G/A). In contrast there was insignificant difference of serum levels of FT3, FT4 and TSH in hypothyroidism *p*-value (0.200, 0.900 and 0.100) and euthyroid goiter patients *p*-value (0.900, 0.200 and 0.700) respectively. These results agree with results of Groussin *et al.* (2012) whom found a small group of patients with

hypersecreting 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 (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. 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.* Grandone *et al.* and Taylor *et al* (Groussin *et al.,* 2012; Arnaud-Lopez *et al.,* 2008; Shields *et al.,* 2009; Grandone *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 hyperthyroidism *p*-value (0.800, 0.400 and 0.600), hypothyroidism *P*-value (0.700, 0.400 and 0.600) and in euthyroid goiter *p*-value (0.700, 0.700 and 0.100) 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 thyroid disorders hyperthyroidism, hypothyroidism and euthyroid goiter. 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.

4.2 Conclusion

The study concludes that, goiter is more common among all study groups, three types of thyroid disorders are more common in age group15-35 years and afro a sciatic tribal group is dominant. In addition TSH level of euthyroid goiter is decreased while free T3 and free T4 are unchanged. *DIO1a* gene (rs11206244) is associated with euthyroid goiter and no association observed with hyperthyroidism and/or hypothyroidism, novel mutation is detected in *DIO1a* gene and it could be one of the causes of increase thyroid disorders in White Nile State. However *DIO1b* gene (rs12095080) allelic frequency link to hypothyroidism and no link with hyperthyroidism or euthyroid goiter.

Furthermore, *PDE8B* gene (rs470439) allelic frequency is associated with hyperthyroidism, hypothyroidism and euthyroid goiter. Beside that relationship is reported between *TSHRc*-C/G (Asp727Glu) gene and all types of thyroid disorders.

Finally, FT4 level is decreased in hyperthyroidism and euthyroid goiter while FT3 and TSH levels are unchanged when compare normal with mutant allele of *DIO1a* gene rs11206244 (C/T), however mutant allele of *PDE8B* gene revealed increase FT3 and FT4 levels but TSH level is decreased.

So these genes may contribute to pathogenesis of related disorders. In addition genetic polymorphism of *TSHRc* and *PDE8B* genes link with hyperthyroidism, hypothyroidism and euthyroid goiter therefore could be a useful prognostic markers for thyroid disorders.

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4.3 Recommendations

From the results of this study, it was recommended that:

1. Mutation of *DIO1a* gene (rs11206244) should be checked in patients diagnosed goiterous especially whom age ranged 15-35 years old.
2. Mutation of *DIO1b* gene (rs12095080) should be screened in any patients with signs and symptoms of hypothyroidism.
3. Early detection of *PDE8B* gene (rs470439) in symptomatic hyperthyroidism to minimize the incidence of hyperthyroidism.

4. The Novel mutation of *DIO1a* gene need for more studies with large sample size to understand the relation of this SNP with thyroid pathogenesis.

5. I recommended for further studies to evaluate other related genes to thyroid disorders in different study areas in the Sudan and its association with thyroid disorders.

6. Euthyroid goiter patients should be monitored by measure thyroid stimulating hormone level.

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Appendix "1"

Questionnaire

Sudan University of Science and Technology

Genetic Polymorphisms in TSH, T3 and T4 genes in Sudanese with Thyroid Diseases

-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

إعلام موافقة

هذه دعوة منى: الباحثة / سناء عبد الغني يوسف طالبة دكتوراه - مختبرات طبية – كيمياء سريرية – بجامعة السودان للعلوم والتكنولوجيا 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 الغدة الدرقية و لتقييم الجينات المرتبطة بها.

بملء إستمارة بمعلومات تخصكم لها علاقة بموضوع البحث.

أي معلومة تخصكم في الإستمارة سوف تكون سرية.

مشاركتكم في البرنامج تسعدنا وتساعد في إنجاح هدف البحث.

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

يمكنكم الحصول علي إجابة لأي 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

MPSU-125/200-UK

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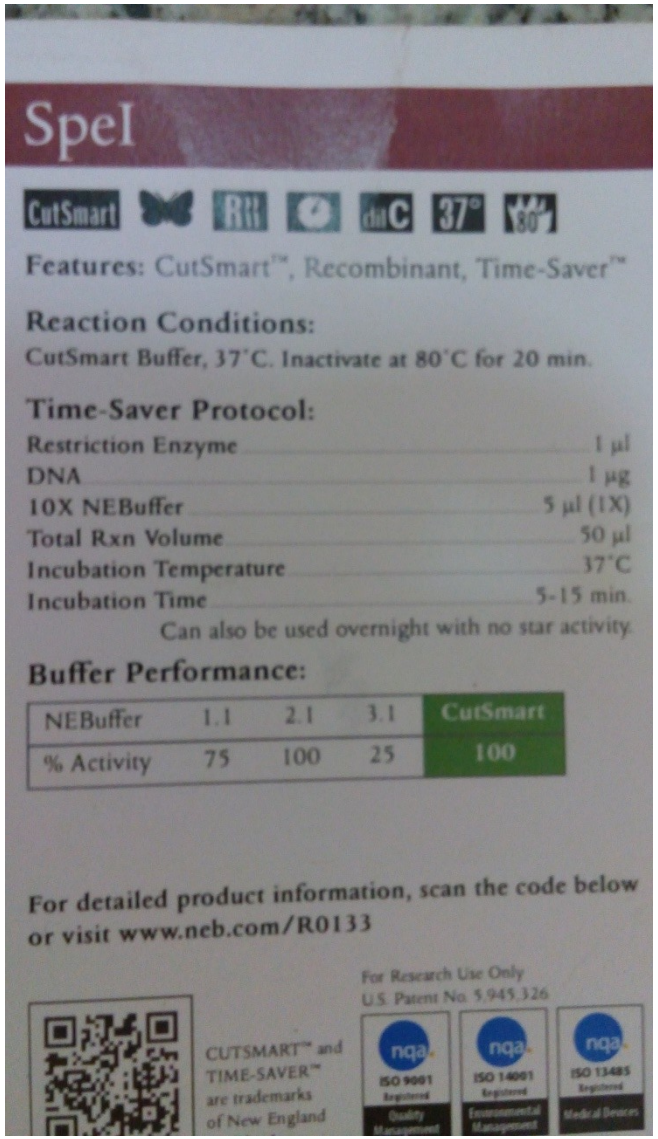

SpeI

CutSmart 37° 80°

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

For Research Use Only
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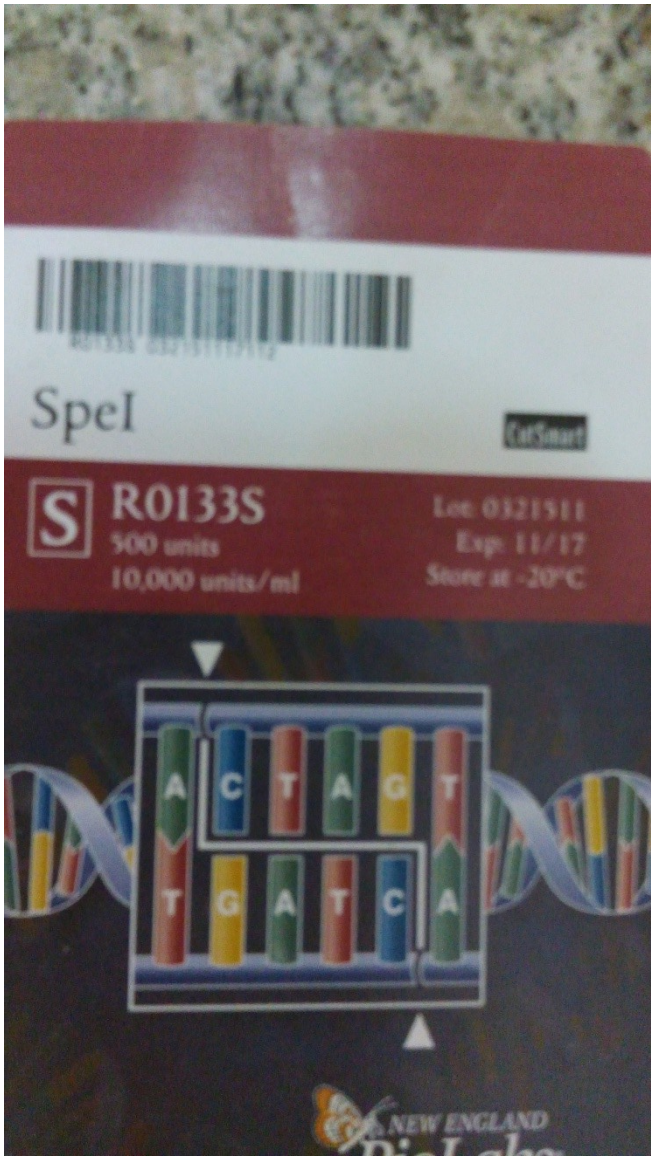
CUTSMART™ and TIME-SAVER™ are trademarks of New England

ISO 9001 Registered Quality Management
ISO 14001 Registered Environmental Management
ISO 13485 Registered Medical Devices



SpeI

CutSmart

S R0133S
500 units
10,000 units/ml

Lot: 0321511
Exp: 11/17
Store at -20°C

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NEW ENGLAND


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CutSmart
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1,000 units
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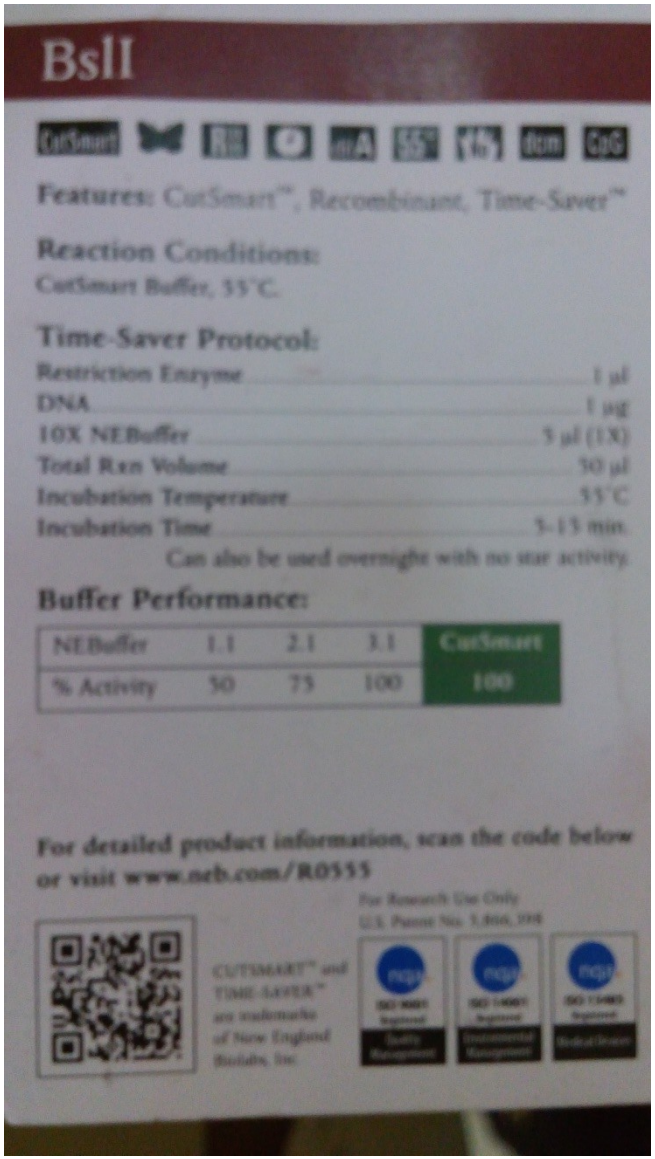
NEW ENGLAND
BioLabs Inc.


BslI
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
For Research Use Only
U.S. Patent No. 5,866,394
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