

# **Chapter One**

## **Introduction**

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## 1.1.Introduction

Graves' disease is an autoimmune disease in which the patient's own immune system produce auto antibodies that attacks the thyroid gland, causing it to produce too much thyroxin. Thyroxin (T4) is a hormone produced by the thyroid gland that has four iodine molecules attached to its molecular structure. T4, as well as other thyroid hormones help regulate growth and control metabolism in the body. The' disease is a form of hyperthyroidism. When thyroxin levels are high the patient's metabolic rate increases; this can have an effect on their physical appearance as well as moods . (Weetman. 2000)

Among patients with hyperthyroidism, 60 to 80 percent have Graves' disease, depending on regional factors, especially iodine intake. The annual incidence in women over a 20-year period is around 0.5 per 1000,37 with the highest risk of onset between the ages of 40 and 60 years; it is thus the most prevalent autoimmune disorder in the United States. Graves' disease is 1/5 to 1/10 as common in men as in women and is children. The prevalence of Graves' disease is similar among whites and Asians, and it is lower among blacks.( Tamai, et al. 1989)

Patient who have graves' disease presented with many signs and symptoms;include:Anxiety,Breast enlargement in men (possible),Difficulty concentrating, Double vision, Eyeballs that stick out (exophthalmoses),Eye irritation and tearing,Fatigue,Frequent bowel movements, Goiter (possible),intolerance, appetite, Increased sweating,Insomnia,Irregular menstrual periods in women, Muscle weakness,Nervousness,Rapid or irregular heartbeat (palpitations or arrhythmia),Restlessness and difficulty sleeping, Shortness of breath with activity,Tremor,andWeight loss . (rarely, weight gain) (Ladenson P. *et al* ., 2007)

Diseases of the thyroid are among the most prevalent of medical conditions, especially in women, but the symptoms can be relatively nonspecific or mild. For this reason, clinicians have been placing increased reliance on the laboratory for assistance in the diagnosis of thyroid disorders. In the 1950s,

only one thyroid test was available, the protein-bound iodine estimate of the serum total thyroxin (T4 concentration which showed a poor sensitivity and specificity for thyroid disease). Technological advances have increased the number of thyroid-related tests available and have progressively improved the specificity, reproducibility and sensitivity of thyroid testing methods, allowing an accurate diagnosis of thyroid status to be made in the majority of cases. However, sensitivity, specificity and standardization issues still result in substantial between-method variability for many of these tests, and analytical interference is still a common problem. Thus many investigations are used now for documentation of thyroid diseases, these include; thyroid function Thyroid-stimulating hormone (TSH) is a glycoprotein hormone secreted by the anterior pituitary. Because of variations in the polysaccharide side chains, TSH occurs in various isoforms, with a range of biological activities. Serum TSH normally exhibits a diurnal variation with a peak shortly after midnight and a nadir in the late afternoon. At the peak of this variation the TSH can be double the value at the nadir. (Weeke and Gundersen ,1978-89)

TSH is now firmly established as the first-line thyroid function test to assess thyroid status for most clinical conditions .The diagnostic superiority of TSH measurement arises principally from the physiological inverse log-linear relationship between circulating TSH and free T4 (FT4) concentrations. An abnormal TSH is the first abnormality to appear in thyroid disease, where other thyroid tests can be normal. Using TSH as a single criterion has been shown to accurately classify the thyroid state of a patient in over 95% of the test(TSH,FT4,FT3) .another advanced tests also were used for documentation of thyroidautoimmunediseases(TPOantibodies and TSH receptor antibodies for graves' disease) (Stockigt J , 2003)

## **1.2 Rationale**

Graves disease in its early stages have not had typical clinical picture , therefore it becomes imperative to implement routine laboratory screening to identify such patients, so that appropriate treatment for thyroid disorders can be instituted or conservative monitoring carried out to anticipate potential future consequences . ( Joshi , 2010)

This study aimed to assess TSH, free thyroid hormone(FT4) and( FT3) in cases have clinical picture of hyperthyroidism ,with or without typical clinical picture of graves disease and so for control group . This to document hyperthyroidism and secondly to assess thyroid auto antibodies to rule out or document graves' disease in Sudanese patients who were clinically diagnosed as graves' disease and not on treatment .

## **1.3 Objectives**

### **1.3.1 General objectives**

To assess TSH ,FT3,FT4, anti thyrotropin receptor antibodies , and anti Thyroperoxidase antibodies as an advanced diagnostic tools for Grave's Disease in Sudanese patients and volunteers .

### **1.3.2 Specific objectives**

1- To measure serum level of thyroid stimulating hormone (TSH), Free thyroxin(FT4),Free triiodothyronine(FT3),thyrotropin receptor antibodies(TRAABS) and Thyroperoxidase antibodies (TPOABS) in study groups.

2- To assess TSH and thyroid hormone ( FT3, FT4) in hyperthyroidism and Graves disease groups. investigate the relationship between thyrotropin receptor antibodies(TRABS) Thyroperoxidase antibodies (TPOABS) in hyperthyroidism and Graves disease groups .

3-To investigate the relationship between thyrotropin receptor antibodies (TRABS), Thyroperoxidase antibodies (TPOABS) in hyperthyroidism and Graves disease group .

4-To correlate between family history and thyrotropin receptor antibodies (TRABS) Thyroperoxidase antibodies (TPOABS) in Graves disease groups .

## **Chapter Two**

### **literature Review**

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### **2.1 thyroid gland**

Thyroid gland is one of most important member of endocrine system that consist of many organs distributing throughout the body and functioning via very specific and potent chemical mediators called thyroid hormones which provide very vital functions. Thyroid gland located in the neck, just below the larynx, the thyroid gland in human is brownish-red organ. (Adams and Purves , 1956)

The thyroid gland consist of two lobed. The lobes connected by narrow band called an isthmus, and is typically asymmetric. The lobes weigh about 20—30 gm each and measured about 4.0cm in length and 2.0—2.5 cm in width and thickness. Thyroid hormones are made primarily of trace element iodine, making iodine metabolism is a key determinant in thyroid function .Iodine is present in sea food, dairy products ,iodine enriched breads, and vitamins. The recommended minimum daily intake of iodine is 150 microgram. If iodine intake drop below 50 microgram daily the thyroid gland is unable to manufacture adequate amount of thyroid hormones and thyroid hormones deficiency-hypothyroidism-result Thyroid cells are organized into follicles. Follicles are spheres of thyroid cells surrounding core of viscous substance termed colloid. The major component of colloid is Thyroglobulin a glycoprotein manufactured exclusively by thyroid follicular cells. Thyroglobulin is rich in amino acid tyrosine. Some of these tyrosil residues can be iodinated, producing the building blocks of thyroid hormones . On the outer side of the follicle, iodine is actively transported into the thyroid cells by the  $N^+/I^-$  symporter located on the basement membranes'. Inside the thyroid cell, iodine diffuse across the cell to the special side of the follicle, which abuts the core of colloid .Here ,catalyzed by membrane –bound enzyme called thyroid peroxidase(TPO), concentrated iodide is oxidized and bound with tyrosil

residues on Thyroglobulin . this results in production of monoiodotyrosine (MIT) and Diiodotyrosine (DIT). This same enzyme also aids in the coupling of two tyrosil residues to form triiodothyronine(T3) (one DIT residue +two MIT residue) or thyroxin (T4)(two DIT residues) .These are the two active form of thyroid hormones. This Thyroglobulin matrix, with branches now holding T4 and T3 ,3 is stored in the core of the thyroid follicle. Thyroid stimulating hormone (TSH) signals the follicular cell to ingest microscopic droplet of colloid by indocytosis inside the follicular cell, these droplets are digested by intracellular lysosomes into T4 and T3 are then secreted by thyroid cell into the circulation ( Juan . *et al.* , 2012)

### **2.1.1 Thyroid-stimulating hormone**

Thyroid-stimulating hormone (TSH) is a glycoprotein hormone secreted by the anterior pituitary. Because of variations in the polysaccharide side chains, TSH occurs in various isoforms, with a range of biological activities. Serum TSH normally exhibits a diurnal variation with a peak shortly after midnight and a nadir in the late afternoon. At the peak of this variation the TSH can be double the value at the nadir.( Weeke and Gundersen , 1978)

TSH is now firmly established as the first-line thyroid function test to assess thyroid status for most clinical conditions. The diagnostic superiority of TSH measurement arises principally from the physiological inverse log-linear relationship between circulating TSH and free T4 (FT4) concentrations. An abnormal TSH is the first abnormality to appear in thyroid disease, where other thyroid tests can be normal. Using TSH as a single criterion has been shown to accurately classify the thyroid state of a patient in over 95% of cases (Kende and Kandapu , 2002)

TSH alone can only be used to assess thyroid status when the pituitary-thyroid axis is stable. Non-thyroidal illness (NTI), pituitary disease and various drugs can all affect the axis and cause discrepancies between TSH levels,



thyroid hormone levels and the clinical state. Glucocorticoids, dopamine and octreotide can all suppress TSH secretion .(Haugen BR., 2009)

The discrepancy between the serum half-life of TSH (1 hour) and that of T4 (1 week) can lead to discordant TSH/FT4 values when thyroid status is in flux. Abnormal TSH levels can persist for weeks or even months after initiation of treatment for thyroid disorders. Measurement of the TSH level is indicated for patients with symptoms suggestive of thyroid dysfunction, reduced bone mineral density, dyslipidaemia, depression, or atrial fibrillation. Over the past few years the TSH reference interval has become controversial. The lower TSH reference limit has been shown to be approximately 0.3 mIU/l irrespective of the population or the method used. In contrast, the setting of the TSH upper reference limit has become contentious with estimates ranging from 2.1 mIU/l to 7.5 mIU/l Multiple factors influence the calculation of the TSH upper reference including population demographics like sex, ethnicity, iodine intake, body mass index, smoking status and age, as well as the failure to exclude the presence of subclinical thyroid disease. Complicating this is the fact that current TSH assays differ in specificity for recognizing different circulating TSH isoforms and that this can give rise to a 1.0 mIU/l difference in TSH values reported by different assays. The TSH reference interval also varies with age and stage of pregnancy ( D. L. Fitzpatrick and M. A. Russell , 2010)

### **2.1.2 Thyroxin(T4)**

The thyroid hormone thyroxin (T4) is physiologically part of the regulating circuit of thyroid gland and has an effect on general metabolism. The major fraction of the total thyroxin is bound to transport proteins (TBG,prealbumin,and albumin).The free thyroxin(FT4) is the physiologically active thyroxin component . (Ekins , 1990 )

This test measures the amount of free thyroxin, or FT4, in the blood. T4 makes up about 90% of thyroid hormones. When the body requires thyroid hormone, the thyroid gland releases stored T4 into circulation. In the blood, . The

concentration of free T<sub>4</sub> is only about 0.1% of that of total T<sub>4</sub>. T<sub>4</sub> is converted into T<sub>3</sub> in the liver or other tissues. T<sub>3</sub>, like T<sub>4</sub>, is also highly protein-bound, but it is the free forms of T<sub>3</sub> and T<sub>4</sub> that are biologically active ( Tietz , 2006) .

### **2.1.3 Triiodothyronine (T<sub>3</sub>)**

Triiodothyronine(T<sub>3</sub>) is one of the thyroid hormones present in serum which regulate metabolism, determination of this hormone concentration is important for diagnosis of euthyroid, hyperthyroid, and hypothyroid status. The major fraction of total triiodothyronine is bound to the transport proteins(TBG,prealbumin,and albumin).Free triiodothyronine (FT<sub>3</sub>) is the physiologically active form of thyroid hormone triiodothyronine (T<sub>3</sub>) Free T<sub>3</sub> is 4 to 5 times more active than FT<sub>4</sub> . (Tietz , 2006)

The serum concentration of T<sub>4</sub> is about 50 times greater than that of T<sub>3</sub>.About 80 % of circulating T<sub>3</sub> is formed following monodiodonation of T<sub>4</sub>.Almost all circulating T<sub>4</sub>(99.97%) and T<sub>3</sub>(99.7 %) hormones are bound to serum proteins. There are three binding thyroid proteins;Thyroxine binding globulin(TBG),transthyritin(TTR) or thyroxin- binding prealbumin(TBPA), and thyroxin binding albumin(TBA). Deiodination of T<sub>4</sub> in peripheral tissues specially the liver and kidneys takes place by the effect of 5deiodonase. Abroximately33 % of T<sub>4</sub>that secreted by thyroid gland each day undergoes monodiodonation to produce T<sub>3</sub>.Another 40% undergoes monodiodonation in the inner ring to produce rT<sub>3</sub>.sever nonthyroidalillness and stress can shift monodiodonation of T<sub>4</sub> to favor rT<sub>3</sub>.Only 0.03 % of T<sub>4</sub> and 0.3 % of T<sub>3</sub> are not bound to proteins. These fractions called free T<sub>4</sub>(FT<sub>4</sub>) and free T<sub>3</sub>(FT<sub>3</sub>),that are the physiologically active portion of thyroid hormones.T<sub>3</sub> is biologically active thyroid hormone and is three to four times more potent than T<sub>4</sub>. T<sub>3</sub> is more active because it is not tightly bound to the serum protein as is T<sub>4</sub> and has grater affinity to target tissue receptor, thus diffusing

more easily into cells than T<sub>4</sub>. Thyroid hormones (THS) play critical roles in differentiation, growth and metabolism. Indeed, THS is required for normal function of nearly all tissues, with a major effect on oxygen consumption and metabolic rate (Stockigt JR and Lim CF, 2009)

#### **2.1.4 Calcitonin**

Ct is a 32 amino- acid- polypeptide, in which the disulphide bridges are essential for biological activity. The physiological role of Ct is unknown. The C cells use the same calcium receptor as do parathyroid cells (Garrett .et al 1995) and high calcaemia is their physiological stimulant. Non physiological stimulants include glucagon,  $\beta$ -adrenergic agonists, alcohol, and gastrin . (Erdogan . et al . , 2006)

Minimal and mild elevations in serum Ct may be seen in C-Cells hyperplasia, renal failure, autoimmune thyroiditis, and hypercalcaemia. Elevated Ct levels may result from non thyroid neuroendocrine tumours. Falsely low Ct levels may occur in the setting of heterophilic antibodies and from a “hook effects”. Optimally, an individual should be followed using the same Ct assay over time and the laboratory should report which Ct assay is being used. Ct values should also be interpreted in the setting of gender specific reference intervals. Moreover, caution has to be taken in interpreting values in children younger than 3 years. (Erdogan . *et al* . , 2006)

The role of stimulated Ct for the diagnosis and follow-up of MTC has recently been pointed out since the classical pentagastrin test is no more available in some European countries. The stimulation test remained very useful to exclude an MTC in an unaffected individual when basal Ct was in the grey zone (15-50 ng/L) as observed in autoimmune thyroiditis with CCH or in neuroendocrine tumours. Another indication for the stimulation test was to detect residual disease or recurrence after surgery for MTC in patients with low basal Ct levels. Patients who have non-detectable and non-stimulable post-operative Ct at two consecutive follow-up visits are considered disease-free, although they

still require yearly follow-up assessments as late recurrence of disease can occur, and there might thus be a need for future complementary surgery. However, it must be remembered that the volume of residual disease is usually very low when only the stimulated Ct level is detectable, and unlikely to be found by imaging until basal Ct is over 150 ng/L. In case of metastatic disease, the response and the peak value could also be indicative of the prognosis. Finally, in genetically predisposed patients with intermediate or low-risk RET proto-oncogene mutations, a prophylactic thyroidectomy is usually advised if basal Ct is lower than 10 ng/L and peak Ct (following pentagastrin stimulation test) is between 50 and 100 ng/L. (Pina . *et al* . , 2013)

### **2.1.5 Disorders of thyroid gland**

The thyroid gland manufactures hormones that regulate the body's metabolism (the process of creating and using energy). When the thyroid produces too much (hyperthyroidism) or not enough (hypothyroidism) hormone, several problems can occur. The most common are, firstly Hashimoto's Disease also known as chronic lymphatic thyroiditis, Hashimoto's disease is the most common cause of hypothyroidism. It can occur at any age but is most common in middle-age women. The disease occurs when the body's immune system attacks and slowly destroys the thyroid gland and its capacity to produce hormone. Mild cases of Hashimoto's disease sometimes present no recognizable symptoms, and the disease can remain stable for years. Symptoms are often subtle, and they are not specific, which means they mimic symptoms of many other conditions, and include ;fatigue ,sleepiness ,depression ,constipation ,mild weight gain ,dry\_skin and/or hair ,heavy and irregular menstruation ,intolerance to cold and enlarged thyroid (goiter). Testing the level of thyroid-stimulating hormone (TSH) is often the initial step when screening for any type of thyroid disorder. If a patient is showing several of the above symptoms, a doctor might order a blood test to check for increased levels of TSH and also low levels of thyroid hormone (T3 or T4). Because Hashimoto's disease is an autoimmune disorder, the blood test would also reveal the

presence of abnormal antibodies that might be attacking the thyroid. There is no known cure for Hashimoto's disease. However, hormone-replacing medication is often used to raise thyroid hormone levels (or lower TSH levels) and, thus, minimize the symptoms of the disease. In rare advanced cases of goiter (an enlarged thyroid), surgery might be necessary to remove part or all of the thyroid gland. Because the disease progresses slowly, it is usually detected at an early stage, remains stable for years, and is easily treated with hormone replacement therapy. Secondly Graves' Disease(GD) named for the physician who first described it more than 150 years ago, Graves' disease is the most common cause of hyperthyroidism (overactive thyroid). It is an autoimmune disorder and occurs when the body's immune system mistakenly attacks the thyroid gland, causing it to overproduce the hormone responsible for regulating metabolism. The disease is hereditary and may develop at any age in men or women, but it is more common in women over the age of 20. Other risk factors include stress, pregnancy, and smoking. With a high level of thyroid hormone in the bloodstream, the body's systems speed up and cause symptoms that are common to hyperthyroidism. Symptoms are not specific, which means they are common to other conditions, and can include: anxiety ,irritability ,fatigue ,increased or irregular heartbeat ,excessive sweating ,difficulty sleeping ,diarrhea or frequent bowel movements ,altered menstrual cycle ,enlarged thyroid (goiter) ophthalmopathy and dermopathy(less common) A simple physical exam can reveal an enlarged thyroid, irritated or bulging eyes, and signs of increased metabolism, including rapid pulse and high blood pressure. The doctor will also call for blood tests to check for high levels of thyroxin (T4) and low levels of thyroid-stimulating hormone (TSH), both of which are signs of Graves' disease. A radioactive iodine uptake might also be administered to measure how quickly the thyroid takes up iodine, which it needs to function properly. A high uptake of iodine is a sign of Graves' disease. There is no treatment to stop the immune system from attacking the thyroid gland and causing it to overproduce hormone. However, the symptoms of Graves' disease can be controlled in several ways, often with a combination of

treatments. Beta blockers are medications used for rapid heart rate, anxiety, and sweating. Anti-thyroid medications are prescribed to prevent the thyroid from producing excessive amounts of hormone. And radioactive iodine is often administered to destroy all or part of the thyroid and render it incapable of overproducing thyroid hormone. Surgery to remove the thyroid gland is an option for patients who cannot tolerate anti-thyroid drugs or radioactive iodine. In most cases, successful hyperthyroidism treatment results in hypothyroidism, and patients must take hormone-replacement medication from that point forward. If left untreated, Graves' disease can lead to heart problems, brittle bones, and, in rare cases, a condition called thyrotoxic crisis, which is an intensification of hyperthyroidism symptoms. But early detection is routine, and because Graves' disease responds well to treatment, the outlook for patients is usually positive. If treated with surgery, there is a very slight risk of damage to the vocal cords because of their proximity to the thyroid. In most cases after the initial treatment, patients can expect to be on a lifetime regimen of hormone-replacement medication, especially if all or part of the thyroid is removed during surgery. Thirdly Goiter which is a noncancerous enlargement of the thyroid gland. The most common cause of goiter worldwide is iodine deficiency in the diet. In the U.S., where iodized salt provides plenty of iodine, goiter is often caused by (and a symptom of) hyperthyroidism (overactive thyroid). Goiter can affect anyone at any age, especially in areas of the world where foods rich in iodine are in short supply. However, goiters are more common after the age of 50 and in women, who are more likely to have thyroid disorders. Other risk factors include family medical history, certain medications, pregnancy, and radiation exposure. If the goiter is not severe, there might not be any symptoms. If the thyroid grows large enough, depending on the size, it may cause one or more of the following symptoms, swelling/tightness in the neck ,breathing and/or swallowing difficulties ,coughing or wheezing ,hoarseness .During a routine physical exam, a doctor will feel the neck area and have the patient swallow. Blood tests will reveal the levels of thyroid hormone, thyroid-stimulating hormone, and antibodies in the

bloodstream, which will diagnose thyroid disorders that are often a cause of goiter. An ultrasound of the thyroid can check for swelling or nodules. Goiter is usually treated only when it becomes severe enough to cause symptoms. If goiter is the result of iodine deficiency, then small doses of iodine can be administered. Radioactive iodine can be used to shrink the thyroid gland. Surgery will remove all or part of the gland. Because goiter is often a symptom of hyperthyroidism, the treatments usually overlap. Goiters are often associated with highly treatable thyroid disorders, such as Graves' disease and are not usually a cause for concern. Goiters themselves are benign. Because there is no pain associated with them, small goiters often go undetected and are not usually treated even if they are diagnosed. Sometimes goiters go away on their own. Sometimes they grow larger and, if left untreated, can present serious complications, such as difficulty breathing and swallowing. Fourthly Thyroid Nodule which are growths that form on or in the thyroid gland. The causes are not always known but can include iodine deficiency and Hashimoto's disease. The nodules can be solid or fluid-filled. Most are benign, but they can also be cancerous in a small percentage of cases. As with other thyroid-related problems, nodules are more common in women than men and the risk in both sexes increases with age. Most thyroid nodules do not cause any symptoms. However, if they grow large enough, they can cause swelling in the neck and lead to breathing and swallowing difficulties, pain, and goiter. Some nodules produce thyroid hormone, causing abnormally high levels in the bloodstream. When this happens, symptoms are similar to those of hyperthyroidism and can include: ,high pulse rate ,nervousness ,increased appetite ,weight loss ,clammy skin. On the other hand, if the nodules are associated with Hashimoto's disease, symptoms will be similar to those associated with hypothyroidism and can include; fatigue ,weigh ain ,hair loss ,dry skin ,and cold intolerance . ( M. P. J. Vanderpump ,1995 )

### **2.1.4.1 Classification of endocrinopathy**

Endocrinopathies are classified as primary, secondary, or tertiary. Primary endocrine disease inhibits the action of downstream glands. Secondary endocrine disease is indicative of a problem with the pituitary gland. Tertiary endocrine disease is associated with dysfunction of the hypothalamus and its releasing hormones. As the thyroid, and hormones have been implicated in signaling distant tissues to proliferate, for example, the estrogen receptor has been shown to be involved in certain breast cancers. Endocrine, paracrine, and autocrine signaling have all been implicated in proliferation thyroid oncogenesis. (Werner and Ingbar's , 2012)

#### **2.1.4.1.1 Hypothyroidism**

Thyroid gland commonly has two major disorders. These are ; hypothyroidism and hyperthyroidism. Hypothyroidism (underactive thyroid) is a condition in which thyroid gland doesn't produce enough of certain important hormones. Women, especially those older than age 60, are more likely to have hypothyroidism. Hypothyroidism upsets the normal balance of chemical reactions in the body. It seldom causes symptoms in the early stages, but, over time, untreated hypothyroidism can cause a number of health problems, such as obesity, joint pain, infertility and heart disease. While hyperthyroidism is the medical term to describe the signs and symptoms associated with an over production of thyroid hormone . ( Werner and Ingbar's , 2012)

#### **2.1.4.1.2 Hyperthyroidism(thyrotoxicosis)**

Is a condition caused by the effects of too much thyroid hormone on tissues of the body. The term thyrotoxicosis is applied to a group of syndrome caused by high level of free thyroid hormones in the circulation Although there are several causes of hyperthyroidism, most



of the symptoms patients experience are the same regardless of the cause .Because the body's metabolism is increased, patients often feel hotter than those around them and can slowly lose weight even though they may be eating more. The weight issue is confusing sometimes since some patients actually gain weight because of an increase in their appetite. Patients with hyperthyroidism usually experience fatigue at the end of the day, but have trouble sleeping. Trembling of the hands and a hard or irregular heartbeat (called palpitations) may develop. These individuals may become irritable and easily upset. When hyperthyroidism is severe, patients can suffer shortness of breath, chest pain, muscle weakness, increased bowel movement, and light or absents menstrual period. Usually the symptoms of hyperthyroidism are so gradual in their onset that patients don't realize the symptoms until they become more severe. This means the symptoms may continue for weeks or months before patients fully realize that they are sick. In older people, some or all of the typical symptoms of hyperthyroidism may be absent, and the patient may just lose weight or become depressed. The level of thyroid hormones are not significantly changed during thyroid storm . Thyroid storm can occur in any patient with uncontrolled thyrotoxicosis who is subjected to stress. Infection is common precipitating event ,general anathesia,surgery,therapeutics use of Iodine131 ,or with drawl of anti thyroid drugs also can precipitate the condition . ( Werner and Ingbar's , 2012)

#### **2.1.4.1.2.1 Graves disease**

Graves disease is long standing autoimmune hyperthyroidism in which the immune system generate auto antibodies that directed to thyroid gland antigens which causing long standing disease that represent complex pathological condition which is difficult for complete recovering and affect different members of society . ( Juan . *et al* . ,2012)

#### **2.1.4.1.2.1.1Historical background**

Graves' disease is named for the doctor who first described it in Ireland-Robert J. Graves. He noticed it in a patient in 1835. The disease is also referred to as Basedow's disease—named after a German, Karl Adolph van Basedow, who described the disease in 1840. He didn't know that Graves had described the same disease just a few years earlier. The term Basedow's disease is more commonly used in continental Europe; in America, it's called Graves' disease . (Young . *et al* . , 1976)

#### **2.1.4.1.2.1.2Clinical Manifestations of graves' disease**

The severity and duration of Graves' disease and the age of the patient determine the manifestations of hyperthyroidism. The most common symptoms are nervousness, fatigue, a rapid heartbeat or palpitations, heat intolerance, and weight loss. These symptoms are present in more than half of all patients who have the disease. With increasing age, weight loss and decreased appetite become more common, whereas irritability and heat intolerance are less common. Atrial fibrillation is rare in patients who are younger than 50 years old but occurs in up to 20 percent of older patients. Approximately 90 percent of patients who are younger than 50 years old have a firm, diffuse goiter of variable size , as compared with about 75 percent of older patients. Nonspecific laboratory findings include high serum concentrations of bilirubin, aminotransferases, ferritin, and sex hormone-binding globulin. The rate of bone resorption is increased. Hypercalciuria is frequent, but hypocalcaemia is rare. Glucose intolerance and, rarely, diabetes mellitus may accompany hyperthyroidism. Among patients who are treated with insulin for diabetes, hyperthyroidism Increases the insulin requirement . (Nordyke . *et al* . , 1988) .

#### **2.1.4.1.2.1.3 Autoimmunity and graves' disease**

Graves' disease shares many immunologic features with autoimmune hypothyroidism, including high serum concentrations of antibodies against Thyroglobulin, thyroid peroxidase, and possibly the sodium–iodide co transporter in thyroid tissue . (Weetman and DeGroot , 1999 )

The serum concentrations of these antibodies vary among patients, and the antibodies themselves may modify the stimulatory effects of thyroid-stimulating antibodies. In some patients, the simultaneous production of antibodies that block the thyrotropin receptor reduces the stimulatory action of thyroid-stimulating antibodies. For these reasons there is no direct correlation between serum concentrations of thyroid-stimulating antibodies and serum thyroid hormone concentrations in patients with Graves' hyperthyroidism . (Bottazzo. et al. , 2006)

The thyroid-stimulating antibodies cause not only thyroid hyper secretion but also hypertrophy and hyperplasia of the thyroid follicles, which have a columnar and folded epithelium and little colloid The result is the characteristic diffuse goiter. Lymphocytic infiltration is often present, occasionally resulting in the formation of germinal centers. These intrathyroidal lymphocytes are a major source of auto antibodies, with contributions from the cervical lymph nodes and bone marrow . (Weetman . et al . ,1984)

Anti thyroid drugs ameliorate the histologic changes . ( Young . *et al.* ,1976)

#### **2.1.4.1.2.1.4 Graves disease and pregnancy**

Ideally, women with Graves' hyperthyroidism should avoid pregnancy until their hyperthyroidism is adequately treated, because the rate of fetal loss in untreated women is high. When Graves' hyperthyroidism occurs or recurs during pregnancy, an antithyroid drug should be given

in the lowest dose necessary to maintain the woman's serum free thyroxin concentration in the upper part of the normal reference range or just above this range.( Mandel . *et al.* , 1994)

Combination therapy with an antithyroid drug and thyroxin must be avoided because the dose of antithyroid drug needs to be higher in patients who are also receiving thyroxin therapy, and little of the thyroxin reaches the fetus, resulting in fetal hypothyroidism. There is little difference between propylthiouracil and methimazole in terms of the potential of causing fetal hypothyroidism, despite the theoretically lower risk of transplacental transfer of propylthiouracil as a result of higher levels of drug binding to serum proteins.( Momotani . *et al.* , 1997)

Properly monitored treatment with an antithyroid drug is safe in pregnant women. There is a weak association between aplasia cutis congenital and maternal use of methimazole or carbimazole during pregnancy. The risk is uncertain, but studies to assess the frequency of this complication have had insufficient power to establish that no risk exists . ( Wing ,1994)

Since propylthiouracil is equally effective and has not been suspected of having teratogenic effects, it is usually used in pregnant women with hyperthyroidism(Mandel . *et al.* , 1994)

For unknown reasons, serum concentrations of thyroid-stimulating antibody decline and thyrotropin-receptor–blocking antibodies sometimes appear during pregnancy . (Kung and Jones. ,1998)

As a result, there is often spontaneous remission of hyperthyroidism in the last trimester of pregnancy, in which case therapy with antithyroid drugs can be stopped. Hyperthyroidism is present in the fetuses and neonates of 1 to 5 percent of women who have Graves' disease during pregnancy. It is caused by the transplacental passage of thyroid-

stimulating antibodies; nearly all mothers of affected fetuses and neonates have very high serum concentrations of thyrotropin-receptor antibody. The risk of neonatal hyperthyroidism can be assessed by measuring maternal serum thyrotropin-receptor antibodies at the beginning of the third trimester; this test is particularly useful in women who are still taking an antithyroid drug at this time . (Davies . *et al* . , 1998) , (Laurberg , 1998)

In fetuses, hyperthyroidism causes poor intrauterine growth and a heart rate of more than 160 beats per minute. In neonates, the symptoms and signs include tachycardia, hyperactivity, irritability, and weakness. Mothers who are taking low doses of any antithyroid drug may breast-feed safely, but the baby's thyroid status should be evaluated periodically. Mild-to-moderate ophthalmopathy often improves spontaneously, and only simple measures are needed . Severe ophthalmopathy, in particular impaired vision, improves in about two thirds of patients who are treated with high doses of glucocorticoids, orbital irradiation, or both Orbital decompression is effective in patients with optic neuropathy and exophthalmoses, either as the initial treatment or after the failure f glucocorticoids treatment .The place of other medical treatments is unclear. By stimulating the thyrotropin receptor, antibodies have a crucial pathogenic role in Graves' disease. Genetic and environmental factors interact through unknown mechanisms to increase the risk of Graves' disease. The frequent association of ophthalmopathy with hyperthyroidism suggests a common autoimmune response, which may be the result of the expression of thyrotropin in the orbits. Current treatments for Graves' hyperthyroidism are effective, but often at the expense of iatrogenic hypothyroidism, whereas the treatment of ophthalmopathy remains unsatisfactory. Further understanding of the immunologic processes involved should allow the development of better diagnostic methods and treatments . ( Bartalena .*et al* . , 1997)

#### **2.1.4.1.2.1.5 Genetic Factors for Graves disease**

The rate of concordance for Graves' disease is about 20% among monozygotic twins, and the rate is much lower among dizygotic twins, indicating that genes make only a moderate contribution to susceptibility. No single gene is known to cause the disease or to be necessary for its development. There is a well-established association with certain HLA alleles that varies among racial groups. In whites, HLA-DR3 and HLA-DQA1\*0501 are positively associated with Graves' disease, whereas HLA-DRB1\*0701 protects against it . (Hye Won Jang, 2011)

The risk of Graves' disease in the HLA-identical siblings of an affected patient is much lower than the risk in a monozygotic twin,) indicating the involvement of non-HLA genes. Graves' disease is associated with polymorphisms of the cytotoxic T-lymphocyte antigen 4 (CTLA-4 ) gene in several racial groups.(65,68)24,27 This association may reflect an effect of certain CTLA-4 alleles on the function of autoreactive T cells, because other organ-specific autoimmune disorders are also associated with CTLA-4 polymorphisms. When a CTLA-4 molecule, rather than a CD28 molecule, on a T cell engages CD80 or CD86 co stimulatory molecules on antigen-presenting cells, the T cell is inactivated. Linkage analysis has identified loci on chromosomes 14q31, 20q11.2, and Xq21 that are associated with susceptibility to Graves' disease,(69,70)28-30 but confirmation of the importance of these loci will require screening large numbers of families with multiple affected members. There is no clear genetic susceptibility to the development of ophthalmopathy . (Hashizume . *et al* . , 1991)

#### **2.1.4.1.2.1.5 Complications of Graves' disease**

Clinically evident ophthalmopathy occurs in about 50 percent of patients, in 75 percent of whom the eye signs appear within a year

before or after the diagnosis of hyperthyroidism. However, studies reveal evidence of ophthalmopathy, in the form of enlarged extra ocular muscles, in most patients without clinical signs . (Perros . *et al* . , 1993)

Older men are at highest risk of severe ophthalmopathy. The prevalence of clinically evident ophthalmopathy is lower in Asians than in whites About 90 percent of patients with ophthalmopathy have hyperthyroidism; the remainder have autoimmune hypothyroidism or are euthyroid at presentation. The most frequent signs of ophthalmopathy are eyelid retraction or lag and per orbital edema. Although a minor degree of eyelid retraction (1 to 2 mm) may be due to sympathetic over activity and can occur in patients with any type of hyperthyroidism, more marked retraction is likely to be due to Graves' ophthalmopathy. Exophthalmoses (proptosis) occurs in up to a third of patients, and diplopia occurs in 5 to 10 percent. Compression of the optic nerve at the apex of the orbit may cause visual loss but is rare. Clinicians can estimate the activity of the eye disease by awarding a point for each of the following signs: retro bulbar pain, pain on eye movement, eyelid erythema, conjunctival injection, chemosis, swelling of the caruncle, and eyelid edema . This score can be used in addition to objective findings of worsening, including increasing proptosis, decreasing visual acuity, and decreasing eye movement, to assess the level of activity of ophthalmopathy. (Mourits . *et al* . , 1997)

Localized dermatopathy is most frequent over the anterolateral aspects of the shin , but it can occur at other sites, especially after trauma. – Dermopathy occurs in 1 to 2 percent of patients with Graves' disease, almost always in the presence of severe ophthalmopathy . (Tellez . *et al* . , 1992)

patients with hyperthyroidism, 60 to 80 percent have Graves' disease, depending on regional factors, especially iodine intake. The annual

incidence in women over a 20-year period is around 0.5 per 1000,<sup>37</sup> with the highest risk of onset between the ages of 40 and 60 years; it is thus the most prevalent autoimmune disorder in the United States.<sup>38</sup> Graves' disease is 1/5 to 1/10 as common in men as in women and is children. The prevalence of ves' disease is similar among whites and Asians, and it is lower among blacks . (Tamai . *et al* . ,1989)

unusual in The chief risk factor for Graves' disease — female sex — is *in* Grapart the result of the modulation of the autoimmune response by estrogen. In some patients, adverse events (such as bereavement, divorce, and job loss) precede the onset of Graves' disease, supporting the possibility of a role for stress as an initiating factor in the disease by means of neuroendocrine pathways . ( Vander and Tunb,1999)

Smoking is weakly associated with Graves' hyperthyroidism and strongly associated with the development of ophthalmopathy . (Burch and Wartofsky , 1993)

In regions of iodine deficiency the hyperthyroidism ; including Graves' hyperthyroidism may be induced by this deficiency, iodine supplementation precipitates Graves' hyperthyroidism and other types of hyperthyroidism, by means of the Jod–Basedow phenomenon. Lithium therapy is usually associated with hypothyroidism and goiter, but paradoxically, immunologic effects of the drug treatment, possibly through In patients with the acquired immunodeficiency syndrome, highly active antiretroviral therapy has been associated with Graves' hyperthyroidism and may be related to the resulting increase in the numbers or change in the function of CD4+ T cells . (Vitti .*et al* . , 1997)

Graves' hyperthyroidism also occurs in patients with multiple sclerosis who are treated with the Campath-1H monoclonal antibody directed against T cells . (Maugendre . *et al* . , 1999)



There is no evidence that infection affects the susceptibility to Graves' hyperthyroidism or directly induces it

**Predisposing Factors**

:Susceptibility to Graves' disease is determined by a mixture of genetic, environmental, and endogenous factors, which are responsible for the emergence of auto reactivity of T and B cells to the thyrotropin receptor. The mechanisms involved are unknown . ( Weetman . *et al.* , 1994

## **Chapter three**

### **Materials and Methods**

## **Materials and Methods**

### **3.1 Study design**

This was across sectional, case control hospital based study ,done in Sudanese hyperthyroidism to asses thyroid hormones,TPO,and, and TRA antibodies as tools for diagnosis of graves' disease .

### **3.2 Study area and period**

The study was done in Khartoum state during the period June 2011 to June 2013 .

### **3.3 Study population**

The study was conducted on 100 Sudanese patients with hyperthyroidism ;50 hyperthyroidism without Graves disease and 50 patients had Graves disease . All the patients were newly discovered, and 50 apparently healthy volunteers (as control group). Age and sex were matches .

### **3.4 Inclusion criteria**

Sudanese patients with hyperthyroidism, Graves disease, who were newly diagnosed, during 3 months and attend outpatients clinic at Omdurman Teaching Hospital .

### **3.5 Exclusion criteria**

Hospitalized patients, patients with severe psychiatric illness, patients on treatment for hyperthyroidism or for graves' disease, individuals with hypothyroidism, sub clinical hyperthyroidism also patients with other endocrine disease .

### **3.6 Ethical consideration**

All the participants were full informed about the aims and benefit of this study . Inform consent was obtained from all the participants in this study .

### **3.7 Data collection and samples process**

Data was collected by carefully designed questionnaire. Venous blood samples(5mls)were taken from each participant by using disposable syringe the blood sample was allowed to clot for 20 min at room temperature and then serum was obtained after centrifugation at 300rpm.the obtained clear serum was stored at -60°C till used .

#### **3.7.1 Measurement of TSH,FT4,andFT3**

##### **3.7.1.1 TSH**

##### **3.7.1.1 .1TSH principle (Appendix I)**

Biotinylated monoclonal TSH specific antibody and monoclonal TSH specific antibody labeled with ruthenium complex react to form sandwich complex that become bound via interaction to streptavidin coated micro particles .the reaction mixture is aspirated into measuring cell where the micro particles are magnetically captured into the surface of the electrode and measured . (Surks, 1990)

##### **3.7.1.1.2 Procedures for TSH (appendix I)**

fully automated chemical analyzer was used for quantitative study of thyrotropin(TSH), In the course of operation the reagents , specimens, and control sera were brought to room temperature.(approx 20 °C),and was placed on sample/reagents disk of the analyzer (ambient temperature 20-25 °C), Reagent bottles caps were opened manually before use and was closed manually after use .Since every Elecsys TSH reagent set has bar-coded label containing the specific information

required for calibration of particular reagent lot. The predefined master curve was adapted to the analyzer by the use of Elecsys TSH Cal Set .(Calibration must be performed once per reagent lot .Precicontrol TSH Universal 1 and 2 was used for quality control every 24 hours .the analyzer automatically calculate TSH concentration of each sample in  $\mu\text{IU/ml}$ . (Surks , 1990)

### **3.7.1.2 Free thyroxin(FT4)**

#### **3.7.1.2.1 Test principle for free thyroxin (appendix-II)**

T4 specific antibody labeled with ruthenium complex bound to T4. The complex become bound to the solid phase via interaction of biotin and streptavidin. Then the reaction mixture is aspirated into measuring cell where the micro particles are magnetically captured to the surface of electrode and measured .(Surks , 1990)

#### **3.7.1.2.2 Procedures for FT4 (appendix-II)**

fully automated chemical analyzer was used for quantitative study of tetraiodothyronine(FT4),In the course of operation the reagents ,specimens, and control sera were brought to room temperature.(approx  $20\text{ }^{\circ}\text{C}$ ),and was placed on sample/reagents disk .Reagent bottles caps were opened manually before use .Since every Elecsys FT4 reagent set has bar-coded label containing the specific information required for calibration of particular reagent lot. The predefined master curve was adapted to the analyzer by the use of Elecsys FT4 Cal Set.PrecicontrolFT4 Universal 1 and 2 was used for quality control .the analyzer automatically calculateFT4 concentration of each sample in  $\text{pmol/l}$ ,  $\text{ng/dl}$  or  $\text{ng/l}$  . (Surks , 1990)

### **3.7.1.3 Free tetraiodothyronine(FT3)**

#### **3.7.1.3.1 Test principle for free tetraiodothyronine (FT3)**

T3 specific antibody labeled with ruthenium complex bound to T3, then the complex become bound to solid phase via interaction of biotin and streptavidin. Then the reaction mixture is aspirated into measuring cell where the micro particles are magnetically captured to the surface of electrode and measured. (Surks, 1990)

#### **3.7.1.3.2 procedures for FT3 (appendix III)**

fully automated chemical analyzer was used for quantitative study of triiodothyronine(FT3). In the course of operation the reagents (appendix-I), specimens, and control sera were brought to room temperature. (approx 20 C°), and was placed on sample/reagents disk of the analyzer. Reagent bottles caps were opened manually before use. Since every Elecsys FT3 reagent set has bar-coded label containing the specific information required for calibration of particular reagent lot. The predefined master curve was adapted to the analyzer by the use of Elecsys FT3 Cal Set. Precicontrol FT3 Universal 1 and 2 was used for quality control. the analyzer automatically calculate FT3 concentration of each sample pmol/ml, pg/ml or ng/dl. (Surks, 1990).

### **measurement of thyrotropin receptor antibodies (TSHRABS) and Thyroperoxidase antibodies (TPODABS) levels**

#### **3.7.1.4 SHRABS ( appendix-1V )**

##### **3.7.1.4.1 Principles for TSHRABS ( appendix-1V )**

The anti TSH Receptor (TRABS) fast Elisa (IgG) test kit provides a quantitative in vitro assay for human auto antibodies against thyroid-stimulating hormone (TSH) receptor (TRABS). the test kits contain micro plate strips, each with 8 break-off reagent well coated with TSH-

Receptor, patient sera are incubated in the wells, if samples are positive, specific antibodies bind to the TSH-Receptors. thyroid stimulating human monoclonal autoantibody(M22,in the form of M22-peroxidase), is added. Bound antibodies are able to inhibit its binding of. Peroxidase substrate 3,3',5,5 tetramethyl-benzidine(TMB) is added to catalyzing color reaction The intensity of the color formed is proportional to the concentration of antibodies against TSH-receptor.

#### **3.7.1.4.2 Procedure for(TRABS)**

The second procedure used in this research is enzyme linked immunosorbent assay (ELISA) for measurement of thyroid autoimmune antibodies(THYROTROPIN RECEPTOR ANTIBODIES AND THYROPEROXIDASE ANTIBODIES) in which reagents of EUROPIAN ORIGIN-(EUROIMMUN )was used . The ELISA test kit provides quantitative in vitro assay for human auto antibodies of the IgG class against thyrotropin receptor and Thyroperoxidase in human serum or plasma.

Reagents, control sera, Calibrator, and patients samples were brought to room temperature. 75 µL of sample buffer was transferred to each of the micro plate wells used. Then 75 µL calibrators, negative and positive control and patients sera into individual micro plate wells, according to the pipetting protocol. The reagent wells were covered and were incubated for 1 hour at room temperature (20°C -25°C) on micro plate shaker (500rpm). Wells were manually emptied. Automatically wells were washed once with 450 µL working strength wash buffer. 100 µL of reconstituted M22-peroxidase was pipetted into each well, and was incubated for 25 minutes at room temperature (20°C - 25°C). Automatically wells were washed 3 times with 450 µL working strength wash buffer. 100 µL of peroxidase substrate TMB was pipetted into each of the micro plate wells, was incubated for 25 minutes at room temperature (20°C -25°C) and was protected from sunlight. 50 µL of stop

solution were pipetted into each of the micro plate wells in the same order and in the same speed .the intensity of the color formed was measure photo metrically at 450 nm and reference wavelength between 620nm and 650 nm ,within 30 minutes. Master curve was plotted by using calibrator I,II,III absorbance's ,from which patients results were obtained.

### **3.7.1.5 TPOABS (appendix-1V)**

#### **3.7.1.5.1 Principles**

assay for human auto antibodies of IgG class against Thyroglobulin in serum or plasma. The test kits contains micro titer strips each with 8 break-off of reagent wells coated with Thyroperoxidase.

Diluted patient samples are incubated in the micro titer plates( wells). In the case of positive samples ,specific IgG antibodies(also IgA and IgM)will bind to the antigens. To detect the bound antibodies, second incubation is carried out an Then enzyme-labeled antihuman IgG(enzyme conjugate ) ,which is capable of promoting a colour reaction is added to the reaction micro titer plates..the intensity of the formed colour is proportional to the concentration of antibodies to Thyroperoxidase..

#### **3.7.1.5.2 procedure**

The second procedure used in this research is enzyme linked immunosorpent assay (ELISA) for measurement of thyroid autoimmune antibodies(THYROTROPINRECEPTORATIBODIES AND THYROPEROXIDASE ANTIBODIES) in which reagents of EUROPIAN ORGIN-(EUROIMMUN )was used. The ELISA test kit provides quantitative in vitro assay for human auto antibodies of the IgG class against thyrotropin receptor and Thyroperoxidase in human serum or plasma.



Reagents, control sera, Calibrator, and patients samples are brought to room temperature. 100  $\mu$ L calibrator (I, II, III), 100  $\mu$ L positive and negative control, and 100  $\mu$ L of diluted patient serum were transferred into individual micro plate wells, according to pipetting protocol then they were incubated for 30 minutes at room temperature (+18 °C to +25 °C). Micro plates wells were washed automatically three times with 400  $\mu$ L working strength wash buffer. 100  $\mu$ L of enzyme conjugate (peroxidase –labeled anti human IgG) were pipeted into each of micro plate wells, and was incubated for 30 at room temperature (+18 °C to +25 °C). Then wells were emptied, and washed as described previously. 100  $\mu$ L of chromogen/substrate solution was pipeted into each of the micro plates wells. and was incubated for 15 at room temperature (+18 °C to +25 °C). 100  $\mu$ L of stop solution was pipeted into each of the micro plates wells in the same order and at the same speed as the chromogen/substrate was introduced. Photometric measurement of the colour intensity was made at wavelength 450nm and reference wavelength between 620nm and 650 nm, within 30 minutes. Master curve was plotted by using calibrator I, II, III absorbance's, from which patients results were obtained.

### **3.4 Quality control**

The accuracy and precision of all methods used in this study were checked each time by using commercial control sera (normal and pathological human control sera).

### **3.5 Data analysis**

Data collected in this study were analyzed by using SPSS. The mean and standard deviation. T-test were used to compare between the means, multiple comparisons (two way ANOVAs test) were used for comparisons (P-value < 0.05 was considered significant)

## **Chapter Four**

### **4.Results**

## Chapter Four

### 4.Results

One hundred and fifty Sudanese were enrolled in this study. 100 are cases, their means of age was (  $33.2 \pm 10.04$  years) , and 50 volunteers their means of age was (  $34.82 \pm 11.13$  ) years (4.1).

Study showed there was insignificant differences in means of age between the case and control groups , P value ( 0.39 ) , also The study shows significant decrease in mean  $\pm$  SD of TSH in cases with hyperthyroidism (  $0.024 \pm 0.028$  U/L ) compared to control group (  $3.28 \pm 0.9$  U/L ) , Significant increase of mean  $\pm$  SD of FT4 in cases with hyperthyroidism (  $29.96 \pm 32$  ng/dl ) compared to control group , (  $2.86 \pm 0.45$  ng/dl ) P value is ( 0.00 ) , Significant increase of mean  $\pm$  SD of FT3 in cases with hyperthyroidism (  $17.86 \pm 16.35$  pg/ml ) compared to control group (  $1.43 \pm 0.3$  pg/ml ) P value is ( 0.00 ) , Significant increase of mean  $\pm$  SD of TRABS in cases with hyperthyroidism (  $5.35 \pm 6.7$  U/ml ) compared to control group, (  $0.7 \pm 0.3$  U/ml ) P value is (0.00) , Significant increase of mean  $\pm$  SD of TPOABS in cases with hyperthyroidism (  $13.51 \pm 12.9$  Pg/ml ) compared to control group, (  $5.04 \pm 1.90$  Pg/ml ) P value is ( 0.00 ) , Significant increase of mean  $\pm$  SD of TSH in cases with graves (  $0.02 \pm 0.016$  U/L ) compared to control group, (  $3.28 \pm 0.92$  U/L ) P values Significant increase of mean  $\pm$  SD of FT4 in cases with graves (  $32.6156 \pm 36.46$  ng/dl ) compared to control group, (  $2.8660 \pm 0.45$  ng/dl ) P value is (0.00) Significant increase cases with of mean  $\pm$  SD of FT3 in cases with graves (  $21.41 \pm 20.6$  Pg/ml ) compared to control group, (  $1.4300 \pm 0.37$  Pg/ml ) P value is ( 0.03 ) Significant increase of mean  $\pm$  SD of TRAbs in graves (  $9.32 \pm 7.7$  U/ml ) compared to control group , (  $1.377 \pm 0.099$  U/ml ) P value is ( 0.00 ) , Significant increase of mean  $\pm$  SD of TPOAbs in cases with graves (  $32.6156 \pm 36.46$  Pg/ml ) compared to control group, (  $5.04 \pm$

1.90 Pg/ml ) P value is (0.00)

Table(4.1): Comparison between means of TSH, FT3,FT4, TPO, TRA in Sudanese patients with hyperthyroidism , Sudanese patients with hyperthyroidism and clinically diagnosed without graves' disease , and Sudanese patients with graves' compared with control group,

Study group	Means_+SD	P value
TSH U/L Hyperthyroidism (100) Control (50)	----- 0.024±0.02 3.28±0.9	0.00*
FT4 ng/dL Hyperthyroidism (100) Control(50)	----- -29.96±32 2.86±0.45	0.00*
F T3 pg/mL  (Hyperthyroidism 100) Control (50)	  7.86±16.35 1.43±0.3	0.00*
TPO Pg/mL Hyperthyroidism (100) Control (50)	 13.51±12.9 5.04±1.90	0.00*
TRA Ab U/mL Hyperthyroidism (100) Control (50)	 5.35±6.7 0.7±0.3	0.00*
TSH U/L		0.00*

Hyperthyroidism without graves (50)	----- 0.028±0.035	
Control (50)	3.28±0.9	
FT4 ng/dL		
Hyperthyroidism without graves' disease (100)		0.00*
Control (50)	27.30±28.8 2.86±0.45	
F T3 pg/mL		0.00*
Hyperthyroidism without graves' disease (100)	----- 14.3±9.50	
Control (50)	1.43±0.37	
TPO Pg/mL		0.00*
Hyperthyroidism without graves' disease (100)		
Control (50)	27.30±28.8 2.86±0.45	
TRA Ab U/mL		0.00*
Hyperthyroidism without graves' disease (100)		
Control (50)	1.37±0.09 0.71±0.30	
TSH U/L		0.00*
Hyperthyroidism with graves' disease (50)		
Control (50)	0.02±0.016 3.28±0.92	

FT4 ng/dL		0.00*
Hyperthyroidism with graves' disease (50)	32.6156±36.46	
Control (50)	2.86±0.45	
F T3 pg/mL		0.00*
Hyperthyroidism with graves' disease (50)	21.4±20.6	
Control (50)	1.43±0.37	
TRABS U/mL		0.00*
Hyperthyroidism with graves' disease (50)	9.3242±7.71	
Control (50)	0.71±0.30	
TPO Pg/mL		0.00*
Hyperthyroidism with graves' disease (50)	32.6156±36.46	
Control (50)	5.04±1.90	

\*\*Independent sample T.test was used for comparison , value considered significant at level <0.05

Table(4.2): Comparison between means of TSH, FT3,FT4, TPO, TRA in Sudanese patients with hyperthyroidism and clinically without Graves disease compared with Graves disease group.

Study group	Means_+SD	P value
TSH U/L		0.117
Graves	0.024±0.028	
hyperthyroidism	3.28±0.9	
FT4 ng/dL		0.421
Grave	32.6156±36.46	
hyperthyroidism	27.30±28.8	
F T3 pg/mL		0.03*
Grave	21.41±20.6	
hyperthyroidism	14.30±9.50	
TPOABS Pg/mL		0.00*
Grave	21.7800±13.88	
hyperthyroidism	5.0480±1.92	
TRABS U/ml		0.00*
Graves	9.32±7.7	
Hyperthyroidism	1.377±0.099	

\*Independent sample T.test was used for comparison. P value considered significant at level <0.05

Table (4.3) : cross tabulation between hyperthyroidism, Graves disease and family history.

			Cases		Total
			Graves	hyperthyroidism	
family history	Yes	Count (n)	22	14	36
		Patient %	44.0%	28.0%	36.0%
	No	Count (n)	28	36	64
		Patient %	56.0%	72.0%	64.0%



table (4.4): cross tabulation between TPO antibodies and cases of graves ..

		Causes		Total	Relative risk (RR)
		Graves disease	control		
TPO	positive Count	8	0	8	2.19
	TPO grave	100.0%	.0%	100.0%	
	negative Count	42	50	92	
	TPO grave	45.7%	54.3%	100.0%	
Total	Count	50	50	100	
		50.0%	50.0%	100.0%	

Table (4.5): cross tabulation between TRA antibodies and cases of graves' disease .

		Cause		Total	Relative risk (RR)
		patient	control		
TRA grave	positive Count	45	1	46	10.565
	% TRA Graves	97.8%	2.2%	100.0%	
	negative Count	5	49	54	
	% TRA Graves	9.3%	90.7%	100.0%	
Total	Count	50	50	100	
		50.0%	50.0%	100.0%	

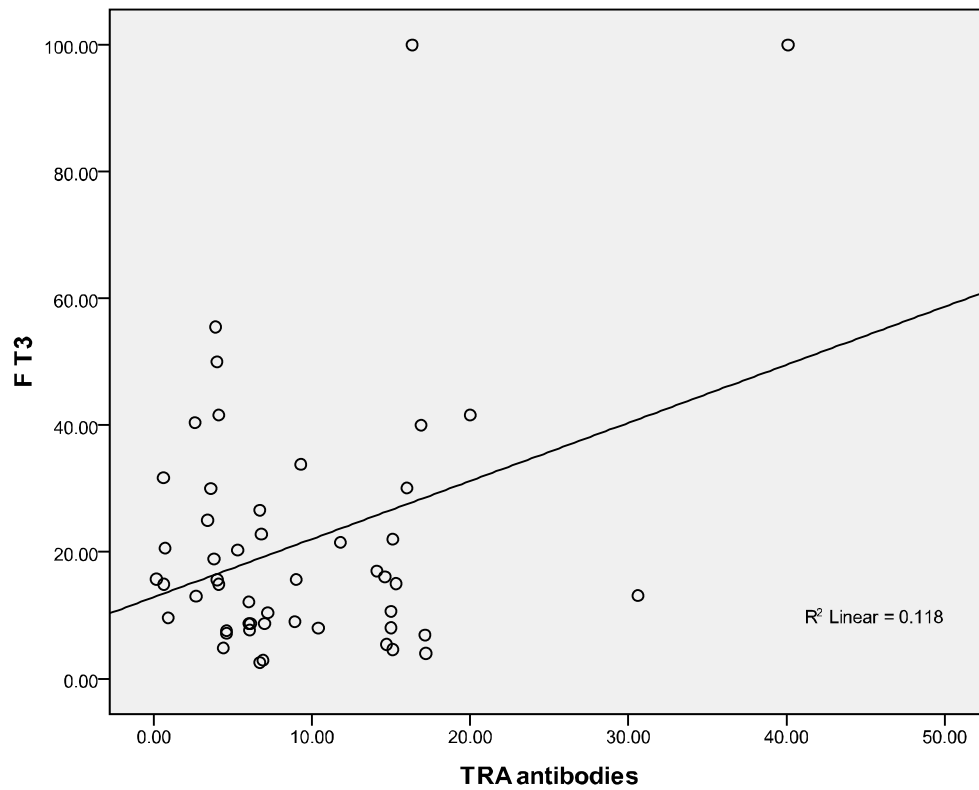
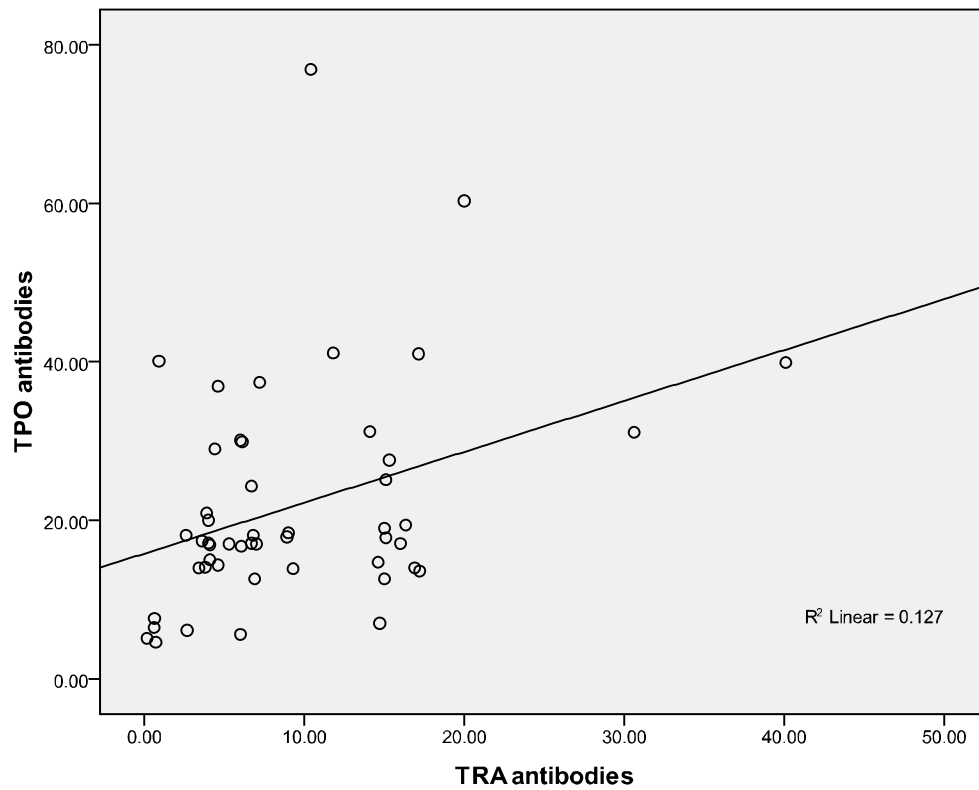


Fig (4.1) : shows correlation between TRA antibodies and FT3 among Sudanese patients with Graves disease. Significant positive correlation ( $p=0.0115$ ,  $r=0.118$ )



Fig(4.2): shows correlation between TRA antibodies and TPO among Sudanese patients with Graves disease, Significant positive correlation ( $p= 0.011$ ,  $r=0.127$ )

## **Chapter Five**

### **5.Discussion ,Conclusion, and Recommendations**

## Chapter Five

### 5. Discussion , Conclusion, and Recommendations

#### 5.1 Discussion

Thyroidreceptorantibodies(TRABS),andThyroperoxidaseantibodies(TPOABS), Free thyroxin and free triiodothyronine are recently used as advanced laboratory test for diagnosis of thyroid autoimmune disease instead of T4andT3.Studeis show significant increase of FT4,FT3 and thyroid autoantiboies(TRABS and TPOABS) in 100 patient clinically diagnosed as hyperthyroidism compaired to 50 volunteers as control group(disease free population) of graves' disease as autoimmune hyperthyroidism where TPOABS show significant increase in both autoimmune hyperthyroidism and hypothyroidism .FT4 and FT3 are recently used instead of T4 and T3 as they are not affected by the level of thyroxin binding proteins.

This study showed significant increase in mean of TRABS in patient group(patients with graves' disease and hyperthyroidism without diagnosis of graves) compared to control group , P value is 0.00.This agree with that reported by( B. Rees Smith . *et al* . , 2004) , ( K. Kamij , 2003) , ( Schott M . *et al* . , 2008) . ( Rees Smith B, 2001) , ( Gupta MK , 2000) , and that reported by ( Bolton J . *et al* , 1999 )

This study showed significant increase in the mean of TRABS in patients with graves (untreated)compared to hyperthyroidism without diagnosis of graves , P value is 0.00 .This agree with that reported by ( B. Rees Smith . *et al* 2004) , ( K. Kamij , 2003 ) , ( Schott M . *et al* . ,2000 ) , ( B.Rees Smith , 2001) ,( Orgiazzi , 2000) , ( Gupta MK , 2000 ) , and that reported by( Bolton J . *et al* . , 1999 )

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2004 ) , ( K. Kamij , 2003 ) , ( B . Rees Smith . , 2001) , ( Orgiazzi J , 2000 ) , ( Gupta MK , 2000) , and that reported by ( Bolton J . *et al* . , 1999 )

This study showed significant increase in the mean of TRABS in patients with hyperthyroidism compared to control group , P value is 0.00. This agree with that reported by ( Schott M . *et al.* ,2008 ) , ( B. Rees , 2004) , ( K. Kamij , 2003 ) , ( B . Rees Smith , 2001) , ( Orgiazzi , 2000 ) , ( Gupta MK ,2000 ) , and that reported by ( Bolton J. *et al* . , 1999 ) .

This study showed significant increase in mean of TPOABS in patient group(patients with graves' disease and hyperthyroidism without diagnosis of graves) compared to control group , P value is 0.00.This agree with that reported by ( Rapoport B and Mclachlan ,1994 ) , ( Kende M and Kandapu , 2002 ) ,( Utiger .*et al* . , 2005 ) , ( Chardes T . *et al* . ,2002 ) ,and ( McLachlan SM and Rapoport B , 2000 ) .

This study showed significant increase in the mean of TPOABS in patients with graves (untreated)compared to hyperthyroidism without diagnosis of graves , P value is 0.00. This agree with that reported by ( Rapoport B and Mclachlan , 1994 ) , ( Kende M, and Kandapu . S 2002 ) , ( Utiger . , *et al* , 2005 ) , (Chardes T . *et al* . , 2002 ) ,and ( McLachlan and SM Rapoport B , 2000 ) .

This study showed significant increase in the mean of TPOABS in patients with graves (untreated)compared to control group , P value is 0.00. This agree with that reported by ( Rapoport B and Mclachlan , 1994 ) , ( Kende M and Kandapu , 2002 ) , (Utiger . *et al* . , 2005) , (Chardes T. *et al* . , 2002 ) , and ( McLachlan and SM Rapoport B , 2000 ) .

This study showed significant increase in the mean of TPOABS in patients with hyperthyroidism compared to control group , P value is 0.00. This agree With reported by ( Rapoport B and Mclachlan , 1994 ) , ( Kende M and Kandapu , 2002 ) , (Utiger . *et al* . , 2005) , (Chardes T. *et al* . , 2002 ) , and ( McLachlan and SM Rapoport B , 2000 ) .

This study showed significant increase in mean of FT4 in patient group(patients with graves' disease and hyperthyroidism without diagnosis of graves) compared to control group , P value is 0.00.This agree with that reported by ( Schott M . *et al* . , 2008 ).

This study showed significant increase in the mean of FT4 in patients with graves (untreated)compared to hyperthyroidism without diagnosis of graves , P value is 0.00.This agree with that reported by ( Wheeler MH and Lazarus ,1994 ) ,( Ekins RP , 1990) and with that reported by ( Passing H . *et al* 1988 )

This study showed significant increase in the mean of FT4 in patients with graves (untreated)compared to control group , P value is 0.00.This agree with that reported by ( Wheeler MH and Lazarus JH.,1994), ( Ekins RP , 1990 )and with that reported by (Passing H . *et al* . , 1988 )

This study showed significant increase in the mean of FT4 in patients with hyperthyroidism compared to control group , P value is 0.00.This agree with that reported by ( Wheeler MH and Lazarus JH.,1994), ( Ekins RP , 1990 )and with that reported by (Passing H . *et al* . , 1988 )

This study showed significant increase in mean of FT3 in patient group(patients with graves' disease and hyperthyroidism without diagnosis of graves) compared to control group , P value is 0.00.This agree with that reported by (Tietz NW , **1995** ),( Becker DV. *et al* , 1990 ),( Klee GG ,1996 ), ( Demers LM and that reported by Spencer, (2002 ),

This study showed significant increase in the mean of FT3 in patients with graves (untreated)compared to hyperthyroidism without diagnosis of graves , P value is 0.00.This agree with that reported by (Tietz NW , 1995 ),( Becker DV. *et al* , 1990 ),( Klee GG ,1996 ), ( Demers LM and that reported by Spencer ,2002

This study showed significant increase in the mean of FT3 in patients with graves (untreated)compared to control group , P value is 0.00.This agree with that reported by (Tietz NW , 1995 ),( Becker DV. *et al* , 1990 ),( Klee GG ,1996 ), ( Demers LM and that reported by d Spencer , (2002 )



This study showed significant increase in the mean of FT3 in patients with hyperthyroidism without diagnosis of graves' disease compared to control group , P value is 0.00.This agree with that reported by (Tietz NW , **1995** ),( Becker DV. *et al* , 1990 ),( Klee GG ,1996 ), ( Demers LM and that reported by Spencer , (2002 )

This study showed significant decrease in mean of TSH in patient group(patients with graves' disease and hyperthyroidism without diagnosis of graves) compared to control group , P value is 0.00.This agree with that reported by ( Kende M and Kandapu S ,2002 ) , ( Passing H .*et al* . , 1988 ) (Tietz NW , 1995) , and agree with that reported by ( Surks MI . *et al* 1990 )

This study showed significant decrease in the mean of TSH in patients with graves (untreated)compared to control group , P value is 0.00.This agree with that reported by ( Kende M and Kandapu S ,2002 ) , ( Passing H .*et al* . , 1988 ) (Tietz NW , 1995) , and agree with that reported by ( Surks MI . *et al* 1990 )

This study showed significant decrease in the mean of TSH in patients with hyperthyroidismwithoutdiagnosisofgravesdiseasecomparedtocontrolgroup( $0.028 \pm 0.035$ ),( $3.28 \pm 0.92$ ),P value is 0.00.This agree with that reported by ( Kende M and Kandapu S ,2002 ) , ( Passing H .*et al* . , 1988 ) (Tietz NW , 1995) , and agree with that reported by ( Surks MI . *et al* 1990 )

Thyroidreceptorantibodies(TRABS),andThyroperoxidaseantibodies(TPOABS), Free thyroxin and free triiodothyronine are recently used as advanced laboratory test for diagnosis of thyroid autoimmune disease instead of T4andT3.Studeis show significant increase of FT4,FT3 and thyroid

## 5.2 Conclusion

This study concluded that -:

- (1) Serum TRAbs , TPOABS , FT4 , FT3 concentrations was significantly increase in patients with untreated graves' disease, moderately increase in cases of hyperthyroidism, compared to control groups.
- (2) Serum TSH concentration was significantly decrease in patients with untreated graves' disease, moderately decrease in cases of hyperthyroidism, compared to control groups.
- (3) 44% of patients with graves' disease have had family history for graves' disease
- (4) FT3,TRAbs,andTPOABS,show significant correlation for patients with graves' disease.
- (5) Age in patients with graves' disease ,show significant correlation to FT4
- (6) TRAbs in patients with graves' disease ,show significant correlation to TPOabs and FT3
- (7) TPOAbs in patients with graves' disease ,show significant correlation to TRAbs
- (8) FT3 in patients with graves' disease ,show significant correlation to TRAbs
- (9)Gender percentage in Sudanese with hyperthyroidism (male: female) is 45:55%

### **5.3 Recommendations**

(2) Thyrotropin (TSH) should be done for patient suspected with hyperthyroidism.

(3) Free thyroxin (FT4) and Free triiodothyronine (FT3) should be used instead of total T4 and total T3 for laboratory diagnosis of hyperthyroidism.

(4) Thyrotropin (TSH) ,FT4,and FT3 should be done for patient suspected with hyperthyroidism

(5) Thyroid receptor auto antibodies should be requested for hyperthyroidism patients, where it will show positive results for long standing hyperthyroidism (Graves disease).

(6) For further documentation of graves' disease thyroidperoxidase antibodies should be quantitated

(7) Combinations between FT3,TRAbs,andTPOABS can be used for assessment of cases of graves' disease.

(8) A combination between FT3,andTRAbs, can be used for assessment of cases of graves' disease.

(9) This research should be done by using large sample size .

(10) ELISA whenever possible should used For such serious laboratories findings .

## References'

**Bahn RS, Heufelder AE** , (1993) , Pathogenesis of Graves' ophthalmopathy , N Engl J Med, 329,1468-1475

**Bartalena L, Marcocci C, Pinchera A** , ( 1997) , Treating severe Graves' ophthalmopathy , BaillieresClinEndocrinolMetab , 11 , 521-536

**Becker DV ,Bigos ST,Gaitan E,Morris JC,Rallison ML,Spencer CA et al** , (1990) , Optimal use of blood tests for assessment of thyroid function (letter). , JAMA , 269 , 273

**blackburn GF , et al** , (1991) , Electrochiluminescence, Detection for Development of immunoassay and DNA Prop assay for clic chem. , Nature biotechnology ,37:1534-1539.

**Boelaert K, Torlinska B** , (2010) , Older subjects with hyperthyroidism present with a paucity of symptoms and signs , J Clin Endocrinol Metab , 95,2715-2726.

**Bolton J, Sanders J, Oda Y, Chapman C, Konno R, Furmaniak J, Rees Smith B** , (1999) , Measurement of thyroid-stimulating hormone receptor autoantibodies by ELISA , Clinical Chemistry , 45 , 2285-2287

**Bottazzo GF, Pujol-Borrell R, Hanafusa T, Feldmann M** , ( 2006) , Role of aberrant HLA-DR expression and antigen presentation in induction of endocrine autoimmunity , Lancet , 2, 1115–1119.

**Burch HB, Wartofsky L** , ( 1993) , Graves' ophthalmopathy, . Endocr Rev , 14 , 747-793

**Chardès T, Chapal N, Bresson D, Bès C, GiudicelliV,LefrancMP, Péraldi-Roux S ( 2002).** "The human anti-thyroid peroxidase, Clinical and experimental immunology, 2 , 195–204.

**D. L. Fitzpatrick and M. A. Russell** , 2010 , Diagnosis and management of thyroid disease in pregnancy , Obstetrics and Gynecology Clinics of North America , 37. No. 2 , 173–193 .

**Davies TF, Larsen PR. Thyrotoxicosis. In: Kronenberg HM, Melmed S, Polonsky KS, Larsen PR, eds. Williams , (2008) , Textbook of Endocrinology , 11th ed ,chap 11.**

**Davies TF, Roti E, Braverman LE, degroot LJ , (1998) , Thyroid controversy stimulating antibodies, J Clin Endocrinal Metab , 83, 3777-3785**

**Demers LM and Spencer , ( 2002) , CA Laboratory support for the diagnosis and monitoring of thyroid disease ,Clinica Chimica Acta , 293 , 1-29 .**

**EkinsRP(1990), Measurement of free hormones in blood, Pub Med ,**

**Erdogan MF, Gursoy A, Kulalaksizoglu M(2006): Long-term effects of elevated gastrin levels on calcitonin secretion. J Endocrinol Invest , 29 (9) , 771–775**

**Fukata S, Kumar` K, Sugawara M.( 2007) Granulocyte colony-stimulating factor (G-CSF) does not improve recovery from ant thyroid drug-induced agranulocytosis , Chang Gun Med J 2007,30,242-8**

**Garrett JE, Tamir H, Kifor O, Simin RT, Rogers KV, Mithal A, Gagel RF, Brown EM , (1995) , Calcitonin-secreting cells of the thyroid express an extracellular calcium receptor gene , Endocrinology ,136 , 5202-211**

**Gerding MN, Terwee CB, Dekker FW, Koornneef L, Prummel MF, Wiersinga WM , ( 1997) , Quality of life in patients with Graves' ophthalmopathy is markedly decreased , Thyroid , 7 , 885-889 .**

**Gupta MK. (2000) Determinazioni di laboratorio , clinical chemical Acta ,293-1-29**

**Hashizume K, Ichikawa K, Sakurai A, et al , (1991) , Administration of thyroxin in treated Graves' disease , N Engl J Med , 324 , 947-953**

**Haugen BR , (2009) , Drugs that suppress TSH or cause central hypothyroidism , Best Pract Res Clin Endocrinol Metab , 23(6) , 793-800**

**Hye Won Jang, Hyun Won Shin, Hyun-Jung Cho, Hee Kyung Kim, Ji In Lee, Sun Wook Kim, Jong-Won Kim, Jae Hoon Chung** ,Immunological Investigations. ,BMJ,Jan 2011, Vol. 40, No. 2: 172-182

**International Agranulocytosis and Aplastic Anemia Study** , (1988) , Risk of agranulocytosis and aplastic anemia in relation to use of ant thyroid drugs , BMJ , 297 , 262-265

**Kamij.K** , (2003) , TSH receptor antibody measurement in patients with various thyrotoxicosis andHashimoto's thyroiditis: a comparison of two-step assays, coated plate ELISA using porcine TSH receptor and coated tube radio assay using human recombinant , Endocrine Journal , 50 , 113-1163

**Kende M, Kandapu S** , (2002) , Evaluation of thyroid stimulating hormone (TSH) alone as a first-line thyroid function test (TFT) in Papua New Guinea. P N G Med J , 45(3-4) , 197-199

**.Kende M** , ( 2003) ,The role of thyrotropin receptor antibody assay , Thyroid.Jun,Vol 20 NO 7:715-725)

**Kenten JH ,et al**( 1991) Rapid Electrochemiluminescence .Assay of polymerase chain reaction Products. Clin chem. (9):1626-1632 .

**Klee GG** , (1996) , Clinical usage recommendations and analytic performance goals for total and free triiodothyronine measurements , Clin Chem , 42 ,155-159

**Kung AWC, Jones BM** , ( 1998) , A change from stimulatory to blocking antibody activity in Graves' disease during pregnancy , J ClinEndocrinolMetab , 83 , 514-518

**Ladenson P, Kim M. Thyroid. In: Goldman L, Ausiello D, eds**, (2007) , Laboratory support for the diagnosis and monitoring of thyroid disease , Cecil Medicine , 23rd ed. chap 244.

**Laurberg P, Nygaard B, Glinoe D, Grussendorf M, Orgiazzi J** , (1998) , Guidelines for TSH-receptor antibody measurements in pregnancy , *EurJEndocrinol* , 139 , 584-586

**Leland JK** , (1990) , Powell MJ *Electrogenerated Chemiluminescence: An Oxidative-Reduction Type ECL Reaction/Sequence Using Tripropyl Amine* , *J Electrochem Soc* , 137(10) , 3127-3131

**M. P. J. Vanderpump , W. M. G. Tunbridge , J. M. French , D. Appleton , D. Bates , F. Clark , J. Grimley Evans , D. M. Hasan , H. Rodgers , F. Tunbridge , and E. T. Young** .1995, Disorders of thyroid gland, *Clinical Endocrinology*, 43, 55–68

**Mandel SJ, Brent GA, Larsen PR** , (1994) , Review of ant thyroid drug use during pregnancy and report of a case of aplasia cutis , *Thyroid* , 4(1), 129-133

**Maugendre D, Gatel A, Campion L, et al** , (1999) Antithyroid drugs and Graves' disease , *Clin Endocrinol* , 50 , 127-132

**Mciver B, Rae P, Beckett G, Wilkinson E, Gold A, Toft A** , ( 1996) , Lack of effect of thyroxine in patients with Graves' hyperthyroidism who are treated with an ant thyroid drug , *N Engl J Med* , 334 , 220-224

**McLachlan SM, Rapoport , B (2000)** , Autoimmune response to the thyroid , *Int. Rev. Immunol* , 19 (6) , 587–618.

**Momotani N, Noh JY, Ishikawa N, Ito K** , (1997), Effects of propylthiouracil and methimazole on fetal thyroid status in mothers with Graves' hyperthyroidism , *J Clin Endocrinol Metab* , 82:3633-3636

**Mourits MP, Pummel MF, Wiersinga WM, Koornneef L** . (1997) Clinical activity score as a guide in the management of patients with Graves' ophthalmopathy. *Clin Endocrinol*, 47, 632, 914

**Orgiazzi J** , (2000) , Anti-TSH receptor antibodies in clinical practice , Endocrinology and Metabolism Clinics of North America , 29 , 339-355

**Parle JV, Holder RL, Roberts LM, Hobbs FD, Wilson S, et al** , Association between serum free thyroxin concentration and atria fibrillation , Arch Intern Med , 167 , 928

**Passing H, Bablok W, et al** , (1988) , A general regression procedure for method Transformation , Clin Chem Clin Biochem , 26 , 783-790

**Perros P, Crombie AL, Kendall-Taylor P** , (1995) , Natural history of thyroid associated ophthalmopathy , Clin Endocrinol , 42 , 45-50

**Perros P, Crombie AL, Matthews JNS, Kendall-Taylor p** , (1993) Age and gender influence the severity of thyroid-associated ophthalmopathy: a study of 101 patients attending a combined thyroid-eye clinic, Clin Endocrinol , 38 , 367-372

**Pina G, Dubois S, Murat A, Berger N, Niccoli P, Peix JL, Cohen R, Guillausseau C, Charrie A, Chabre O, Cornu C, Borson-Chazot F** , (2013), Is basal ultrasensitive of calcitonin capable of substituting for the pentagastrin-stimulation test , Clin Endocrinol , 78 , 358-364.

**Prummel MF, Wiersinga WM, Mourits MP, Koornneef L, Berghout A, van der Gaag R** , (1990), Effect of abnormal thyroid function on the severity of Graves' ophthalmopathy , Arch Intern Med , 150, 1098-1101

**Rapoport B, McLachlan SM** , (1994) , Thyroid peroxidase as an auto antigen in autoimmune thyroid disease , Endocrine Rev , 3 , 96-102

**Rees Smith B. Thyroid auto antibodies** , (2001) , The Scandinavian Journal of Clinical and Laboratory Investigation Supplement , 61 , 45-52

Rev;11:5



**Ryan SP, Nicholas NMJ**, (1994) , The Thyroid and Parathyroid Glands. In: Anatomy for diagnostic imaging , Nicholas NMJ W.B Saunders. Philadelphia , 35-37

**Ryan Wallace.(2014)** Reference Library

**Saller.S Schilddruesenkrankheiten.B** , (1995) , Diagnose and Therapie , Berliner Medizinische Verlagsanstalt GmbH , 2 , 30-32,60-62

**Stockigt J** , (2003) Assessment of thyroid function: towards an integrated laboratory , clinical Clin Biochem,.;24(4) , 109-22.

**Stockigt JR and Lim CF**. 2009. Medications that distort in vitro tests of thyroid function, with particular reference to estimates of serum free thyroxine . Best Pract Res Clin Endocrinol Metab . 23(6) . 753-767

**Surks MI ,Chopra IJ,Maiash CN ,Nicoloff JT ,Solomon DH** , (1990) , American thyroid association Guidelines for the use of the laboratory tests in thyroid disorders , JAMA , 263 , 1529-1532

**Tamai H, Kasagi K, Takaichi Y, et al** , (1989) , Development of spontaneous hypothyroidism in patients with Graves' disease treated with antithyroidal drugs: clinical, immunological, and histological findings in 26 patients , J Clin Endocrinol Metab , 69 , 49-53.

**Tellez M, Cooper J, Edmonds C** , (1992) , Graves' ophthalmopathy in relation to cigarette smoking and ethnic origin , Clin Endocrinol , 36 , 291-294.

**Tietz NW** , (1995) , Clinical guide laboratory to laboratory tests , Third edition, 612

**Tietz NW** , (2006) , Clinical Guide to Laboratory Tests, Fourth Edition , 1053

**Toft AD and Weetman AP** , ( 1998), Screening for agranulocytosis in patients treated with antithyroid drugs , Clin Endocrinol , 49 , 271-271

**Tonacchera.M, Ferrarini. E, Dimida.A, Agretti.P, Marco. G. De, Pinchera.A ,Sanders.J M. Evans, T. Richards, Furmaniak.J, Smith. B. Rees (2006) . A new assay for thyrotropin receptor auto antibodies , 14 , 830**

**Vander pump MPJ, Tunb ridge WMG ,(1999) , The epidemiology of autoimmune thyroid disease , Totowa N.J , 15 ,141-62**

**Vitti P, Rago T, Chiovato L, et al., (1997) Clinical features of patients with Graves' disease undergoing remission after ant thyroid drug treatment ,Thyroid , 7 , 369-375**

**Weeke J and Gundersen HJ, (1978), Circadian and 30 minutes variations in serum TSH and thyroid in normal hormones subjects , Acta Endocrinol ,89(4) , 659-672.**

**Weetman AP and DeGroot L ,(1999),Autoimmunity to the thyroid gland,. , J Clin Endocrinol Metab ,84 , 702-10**

**WEETMAN AP, MCGREGOR AM, WHEELER MH, HALL R , (1984) ,EXTRA THYROIDAL SITES OF AUTOANTIBODY SYNTHESIS IN GRAVES' DISEASE , CLIN EXP IMMUNOL ,56 ,330-336**

**Weetman AP, Pickerill AP, Watson P, Chatterjee VK, Edwards OM (1994) , Treatment of Graves' disease with the block-replace regimen of ant thyroid drugs: the effect of treatment duration and immunogenetic susceptibility on relapse, QJM , 87 , 337-341**

**Werner & Ingbar's, Hypothyroidism ,(2012),The Thyroid, A Fundamental and Clinical Text. 10, 523-525**

**Wheeler MH ,Lazarus JH, (1994),Diseases of the thyroid ,journey of American medical association ,18 , 107-115**

**Wing DA, Millar LK, Koonings PP, Montoro MN, Mestman JH. A , (1994)comparison of propylthiouracil versus methimazole in the**

treatment of hyperthyroidism in pregnancy, Am J Obstetric Gynecology ,170 , 90-95.

**Young RJ, Sherwood MB, Simpson JG, Nicol AG, Michie W, Beck JS** ,(1976) ,Histometry of lymphoid infiltrate in the thyroid of primary thyrotoxicosis patients relation of extent of thyroiditis to preoperative drug treatment and preoperative hypothyroidism , J ClinPathol ,29 ,398-402

## **Appendices**