بسم الله الرحمن الرحيم

Sudan University of Science and Technology

College of Graduate Studies

Assessment of Stimulating Hormone and Free T4 among Positive and Negative Tpo Abs Hypothyroidism among Saudi Arabia Patients

قياس الهرمون المحفز للغدة الدرقية و الثايروكسين لدى مرضى خمول الغدة الدرقية سالبي وموجبي اجسام البيروكسيداز المضاده في المملكه الهربيه السعوديه

A Dissertation Submitted in Partial Fulfillment of the Requirements for M.Sc. Degree in Medical Laboratory Science (Clinical Chemistry)

By:

Samah Basheer Mohammed Nasir

B.Sc. (honor) Of Medical Laboratory Science (Clinical Chemistry) - Faculty of medical laboratory sciences -University of Gazira - 2011

Supervisor:

Dr. Abdelgadir Ali Elmugadam

Associate Professor of Clinical Chemistry-Medical Laboratory Science

February 2021

قال تعالي:

{رَبِّ أَوْزِعْنِي أَنْ أَشْكُر نِعْمَتَك الَّتِي أَنْعَمْت عَلَي وَعَلَى وَالِدَي وَأَن أَعْمَل صَالِحًا تَرْضَاه وَأَدْخِلْنِي بِرَحْمَتِك فِي عِبَادِكَ الصَّالِحِينَ}

صدق الله العظيم

(سورة النمل:الآية19)

Dedication

I dedicate this work to those who were the causes of my success after god Allah

To whom I saw the way of my life and from whom I drew my strength and my self-esteem, to the endless struggle, to the great who taught me the meaning of persistence and that no thing impossible with faith power and proper planning

My great father

To my love the closest one to my heart **my mother**, my **sisters** ,**brother** and to whom always keep supporting me very important consultant in my life my beautiful aunt U. **SaniaMukhtar**

To my small family

My lovely husband the one who stand beside me supportive at every time in all my stages **Dr. Amr** , my beautiful daughter **Hayoma .**

Acknowledgements

The first and last thanks and grateful always for **Allah** who create me and everything in a best form for his greatest blessing that he is **Allah** the only lord of the worlds.

With full respect I would like to thank **Dr. Abdelgadir Elmugadam** for his patience, time, and continuous encouragement, and professional supervising and being areal mentor for me.Thanks to **Dr. Rami** for his help in statistical analysis.

Thanks for my father, my sister who supported me and help me financially and morally and all other aspects to enroll this study.

Thanks for all colleagues for help and support. Thanks for all the participants whom voluntarily participate in this study. Thanks for my hospital Mohammed DossaryHospital which allow me to use laboratory material and patient's data to fulfill my research.

Abstract

Thyroid gland disorders (hypothyroidism) one of the commonest problems now days in all ages and nationalities, the aim of this study To Asses serum levels of anti-TPO antibody and thyroid function test parameters (FT4 and TSH) in positive and negative hypothyroidism in Saudi Arabia.

This was hospital-based cross sectional comparative study, performed in period from January 2018 to December 2019 in Saudi Arabia. One hundred and fifty (150) participants, divided into three groups as positive tpo ab hypothyroidism, negative tpo ab hypothyroidism and healthy individuals, with different ages and nationalities, blood specimens collected and serum separated and stored in -20 C till used: TSH and fT4 were measured by Vitros 5600 and TPO abs by Cobas e 411.

The study results revealed a significant increase in TSH level in positive tpo hypothyroidism and negative tpo hypothyroidism with p value: 0.00, and significant decrease in FT4 level in positive tpo hypothyroidism and negative tpo hypothyroidism with p value 0.00, while TPO abs showed significant increase in positive tpo hypothyroidism with p value 0.00.There was no significant correlation between age and positive tpo hypothyroidism p value 0.274 r=0.158, and no significant correlation between age and negative tpo hypothyroidism p value 0.808 r=0.035.

In conclusion: this study showed significant increase of TPO in positive tpo hypothyroidism, with increase levels of TSH in positive tpo hypothyroidism negative tpo hypothyroidism.

مستخلص الدراسة

تعد اضطرابات الغدة الدرقية (قصور الغدة الدرقية) من أكثر المشاكل شيوعًا في الوقت الحاضر في جميع الأعمار والجنسيات ، والهدف من هذه الدراسة هو تحديد مستويات مصل الأجسام المضادة لـ TPO ومعايير اختبار وظيفة الغدة الدرقية (FT4 و TSH) لدى مرضى قصور الغدة الدرقيه موجبي وسالبي اجسام البيروكسيداز المضاده .كانت هذه دراسة مقطعية مستعرضة على أساس المستشفى ، أجريت في الفترة من يناير 2018 إلى ديسمبر2019. تم تسجيل 150 مشاركة مقسمة إلى 50 في كل مجموعة الى موجبي وسالبي قصور الغدة الدرقية وفرد سليم من مختلف الأعمار والجنسيات في المملكة العربية السعودية ، فصل المصل: TSH و تم قياس FT4 في فيتروس 5600 و TPO ab في cobas e 411

أظهرت نتائج الدراسة زيادة معنوية في مستوى TSH في قصور الغدة الدرقية الإيجابي و قصور الغدة الدرقية السلبي بقيمة p: 0.00 ، وانخفاض معنوي في مستوى FT4 في قصور الغدة الدرقية الإيجابي و قصور الغدة الدرقية السلبي بقيمة p 0.00 ، بينما أظهر TPO abs زيادة معنوية في قصور الغدة الدرقية. قصور الغدة الدرقية موجب tpo بقيمة p 0.00. لم يكن هناك ارتباط معنوي بين العمر وقيمة قصور الغدة الدرقية الإيجابية r = 0.158 tpo p 0.274 ، ولا يوجد ارتباط معنوي بين العمر وقيمة قصور الغدة الدرقية السالبة r = 0.035 tpo 0.808.

في الختام: أظهرت هذه الدراسة زيادة معنوية في TPO في قصور الغدة الدرقية الإيجابي ، مع زيادة مستويات TSH في قصور الغدة الدرقية الإيجابي في حالة قصور الغدة الدرقية السلبي.

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Abbreviations

Abbreviation	Meaning
CTLA-4	Cytotoxic T lymphocyte associated Ag 4
HRP	Horseradish peroxidase
IgG	Immune globulin
Mcg	Micro gram
MHC	Major histocompatibility complex
NR	Normal range
QC	Quality control
RIA	Radio Immune Assay
RT3	Reverse 3’3’5tri iodithyronin
SLE	Systematic lupus erythromatous
SPSS	Statistical package for the social science
T3	3’3’5 tri iodothyronine
T4	Thyroxin
TH	T helper T cell
TG	Thyroglobulin
TPO	Thyroid peroxidase enzyme
TSH	Thyroid stimulation hormone

Chapter one

Introduction, Rationale and Objectives

1. Introduction, Rationale and Objectives

1.1. Introduction

The thyroid gland is positioned in the lower anterior neck and is shaped like a butterfly, thyroid lies below Adam's apple, along the front of the trachea ,Brownish-red in color, the thyroid is rich with blood vessels, Nerves important for voice quality also pass through the thyroid (Beynon and Pinneri ., 2016) .

The thyroid secretes several hormones, collectively called thyroid hormones; the main hormone is thyroxin, also called T4. Thyroid hormones act throughout the body, influencing metabolism, growth and development, and body temperature, during infancy and childhood, adequate thyroid hormone is crucial for brain development (Mendoza and Anthony.,2017).

In parts of the world where severe iodine deficiency exists, neither the mother nor the fetus can produce thyroid hormone and both develop hypothyroidism, the impact is most severe on the fetus because hypothyroidism leads to mental retardation and cretinism (Jonklaas *etal.,* 2014).

Some Thyroid Conditions like: Goiter a general term for thyroid swelling.

Goiters can be harmless, or can represent iodine deficiency or a condition associated with thyroid inflammation called Hashimoto's thyroiditis .Hyperthyroidism: Excessive thyroid hormone production. And Hypothyroidism: Low production of thyroid hormone. Thyroid damage caused by autoimmune disease is the most common cause of hypothyroidism (Faggiano *et al*.,2011).

There are two biologically active thyroid hormones: thyroxin (T4) and3, 5, 3'-triiodothyronine (T3). They differ in that T4 has two iodine atoms on its phenyl (outer) ring, whereas T3 has only one , The compound formed if an iodine atom is removed from the inner ring of T4 is 3,3',5'-triiodothyronine (reverse T3 [rT3]), which has no biological activity.T4 is solely a product of the thyroid gland, whereas T3 is a product of the thyroid and of many other tissues, in which it is produced by de iodination of T4. The thyroid gland contains large quantities of T4 and T3 incorporated in thyroglobulin, the protein within which the hormones are both synthesized and stored (Köhrle, 2018).

1.2 Rationale:

Thyroid gland one of the most glands in the human body responsible about many main functions like metabolism, growing, controlling many process. to the best of our knowledge no published data concerning increase prevalence of hypothyroidism and Hashimoto's thyroiditis were done in Saudi Arabia in eastern province while many cases were coming to endocrinology physician in Mohammed Dossary Hospital in Al khobar complains present with thyroid dysfunction such as weight gain , lack of energy , sleepiness , cold tolerance ,difficulty swallowing ,throat feel swollen , period disturbance , voice is hoarse and hair loss, so we need to good out come in differentiate between hypothyroidism and Hashimoto'sthyroiditis**.**

1.3. Objectives:

General objective:

To assess the serum levels of anti-TPO antibody and thyroid function test parameters (FT4 and TSH) among positive and negative hypothyroidism in Saudi Arabia patients.

Specific objectives:

1- To measure and compare serum TSH, freeT4,and Tpo abs among positive and negative hypothyroidism patients in Saudi Arabia.

2-To compare biochemical parameters TSH, FT4 and Tpo abs between subgroups of the patients.

3- To correlate serum TSH, FT4 and Tpo Abs with patient's variables (sex, age).

Chapter two

Literature Review

2.Literature Review

2.1. Hypothyroidism (under active thyroid):

Hypothyroidism is a condition in which the thyroid gland does not produce enough of certain crucial hormones. Hypothyroidism may not cause noticeable symptoms in the early stage, over time, untreated hypothyroidism can cause number of health problems, such as obesity, joint pain, infertility and heart disease (Chaker *et al* ., 2017).

2.1.2 Signs and symptoms:

Its vary depending on the severity of the hormone deficiency; problems tend to develop slowly, often over a number of years, started with fatigue and weight gain, generally include:

Fatigue ,Increase sensitivity to cold ,Constipation ,Dry skin ,Weight gain ,Puffy face ,Hoarseness ,Muscle weakness ,Elevated blood cholesterol level ,Muscle aches, tenderness and stiffness ,Heavier than normal or irregular menstrual periods ,Slower heart rate ,Depression ,Impaired memory ,Enlarged thyroid gland [goiter] (Koehler *et al* .,2018).

Table2.1. major symptoms and signs of hypothyroidism

Mechanism	Symptoms	Signs
Slowing of metabolic processes	Fatigue and weakness Cold intolerance Dyspnea on exertion Weight gain Cognitive dysfunction Mental retardation (infantile onset) Constipation Growth failure	Slow movement and slow speech Delayed relaxation of tendon reflexes Bradycardia Carotenemia
Accumulation of matrix substances	Dry skin Hoarseness Edema	Coarse skin Puffy faces and loss of eyebrows Periorbital edema Enlargement of the tongue
Other	Decreased hearing Myalgia and paresthesia Depression Menorrhagia Arthralgia Pubertal delay	Diastolic hypertension Pleural and pericardial effusions Ascites Galactorrhea

Major symptoms and signs of hypothyroidism: (Konca C e*t al.,* 2016)

2.1.3 Causes of hypothyroidism :

There can be a number of causes including:-Autoimmune disease, hyperthyroidism treatments, radiation therapy, thyroid surgery and certain medications (Almandoz and Gharib, 2012) .

Autoimmune disease:

The most common cause of hypothyroidism is an autoimmune disorder known as hashimoto's thyroiditis (Caturegli *et al*.,2014**).**

2.1.4 Effect on Health:

Anti-thyroid antibodies target specific parts of the thyroid gland, including:

Thyroid peroxidase (TPO): TPO is an enzyme that plays an important role in making thyroid hormones.

Thyroglobulin (TG): This substance also helps body make thyroid

hormones.

Thyroid stimulating hormone (TSH) receptor: TSH sticks to the receptor on thyroid cells, which causes the gland to make and release thyroid hormone into the blood (Fröhlich and Wahl ,2017).

The antibodies can damage the gland, make it swell, and affect how it works ,this can lead to medical conditions like:

Hashimoto's disease. This is the most common form of underactive thyroid, called hypothyroidism. When the thyroid gland is inflamed, it can't make hormones as well as it normally does. , Over many years, the thyroid becomes damaged; this leads to a drop in thyroid hormone levels in the blood. When the levels get too low, body's cells can't get enough thyroid hormone and they can't work as they should (Liontiris and Mazokopakis ,2017).

Signs of Hashimoto's disease include:

Feeling very tired or sluggish, being very sensitive to cold, Weight gain, Body or joint pain, Feeling depressed (Lorini *et al*., 2003**).**

Graves' disease . This happens when antibodies cause the cells in the gland to work overtime. An overactive thyroid, or hyperthyroidism, makes and releases too much thyroid hormone into blood, when that happens, and all the body functions tend to speed up (Subekti and Pramono,2018**).**

Symptoms of Graves' disease include:

Anxiety or irritability ,Bulging eyes ,Sensitivity to heat ,Unexplained weight loss, Shaking hands or fingers Tiredness ,Fast or irregular heartbeat (Subekti and Pramono, 2018).

2.2 Hashimoto thyroiditis differential diagnoses :

The following auto immune phenomena may occur or be found in association with hashimoto's thyroiditis : Addison disease ,Autoimmune gastritis (pernicious anemia) Chronic active hepatitis ,Idiopathic hypo parathyroidism ,Primary biliary cirrhosis, Primary ovarian or testicular failure , Rheumatoid arthritis ,Systemic Lupus Erythromatous (SLE) ,Systemic sclerosis (sclerodema) ,Type 1 diabetes mellitus and Vitiligo (Adetti G, 2014) .

Differential diagnosis: Diffuse toxic goiter (Graves 'disease) ,Euthyroid sick syndrome ,Goiter ,Hypo pituitarism (pan hypopituitarism) ,Lithium induced goiter ,Nontoxic goiter ,Thyroid lymphoma ,Toxic nodular goiter ,Type 1 poly glandular autoimmune disease ,Type 2 poly glandular autoimmune disease (Subekti and Pramono,2018) .

The name Hashimoto's thyroiditis is derived from the 1912 pathology report by Hashimoto describing patients with goiter and intense lymphocytic infiltration of the thyroid as "Struma lymphomatosa (Akamizu and Amino, 2017).

Some clinicians reserve this term only for patients with hypothyroidism, however, many people with thyroid antibodies do not have hypothyroidism, and others have no goiter or even have an atrophic thyroid gland. These are considered manifestations of the same disorder with differing clinical phenotypes. The presence of serum thyroid autoantibodies may be sufficient evidence for Hashimoto's disease, this logic is based upon the observation that thyroid antibodies correlate well with the presence of a lymphocytic infiltrate in the thyroid gland at

autopsy examination of individuals with no history of thyroid failure (Ewa*et al*., 2019).

Hashimoto's thyroiditis is primarily a disease of women, with a sex ratio of approximately 7:1; it can also occur in children .Variant mild forms of Hashimoto's thyroiditis have been given names such as silent (or painless) thyroiditis and postpartum thyroiditis, both of which are transient but may be followed years later by thyroid failure (Ewa *et al*., 2019).

the usual course of Hashimoto's thyroiditis is gradual loss of thyroid function , Among patients with this disorder who have mild (subclinical) hypothyroidism, exhibited as slight increases in TSH and the presence of thyroid antibodies, overt hypothyroidism occurs at a rate of approximately 5 percent per year (Akamizu and Amino, 2017).

Overt hypothyroidism, once present, is permanent in nearly all cases, except in some children and postpartum women in whom it is often transient.(Stagnaro *et al*.,2011)

2.3 Thyroid antigens:

Several antigen-specific T cells directed against thyroid antigens in chronic autoimmune thyroiditis.

The major antigens are :Thyroglobulin (TG) ,Thyroid peroxidase (TPO, historically known as the "microsomal" antigen) and thyroid-stimulating hormone (TSH) receptor (Catureglie*t al* .,2014) .

2.3.1 Thyroglobulin and thyroid peroxidase:

TG is synthesized by follicular cells and secreted into the lumen of the thyroid follicle, where it is stored as colloid. TPO catalyzes the iodination

of tyrosine residues of TG to form mono iodotyrosine and di iodotyrosine (Lewis and David , 2013) .

The induction of experimental autoimmune thyroiditis in mice using either TG or TPO as antigen provides evidence for their potential role in the pathogenesis of Hashimoto's thyroiditis in humans. The *TG* gene may be a susceptibility gene for autoimmune thyroid disease coding for TG variants of different immunogenicity (Hooshang et*al*.,2017).

When stimulated by TSH, the thyroid follicular cells take up TG from the colloid. The TG is then cleaved by peptidases yielding thyroxin (T4) and tri iodothyronine (T3), which are released into the extracellular fluid. Some TG is detectable in the serum of normal subjects, and the concentration may be increased in patients with any thyroid disease.

TPO is a key enzyme in thyroid hormonogenesis. It is located on the luminal surface of the microvilli of thyroid epithelial cells (Citterio *etal*.,2019) .

2.4 Role of B cell:

B cells from thyroid tissue of patients with Hashimoto's thyroiditis are activated, as indicated by their ability to secrete thyroid antibodies spontaneously in vitro. Thus, the thyroid gland is a major site of thyroid antibody secretion, Additional evidence for this is the decline in serum thyroid antibody concentrations that occurs after surgery and during administration of anti-thyroid drugs to patients with this disorder .However, there is also evidence that extra thyroidal lymphoid tissues may contribute to antibody production (Rydzewska,2018) .

2.4.1 Antibodies to TG and TPO:

Nearly all patients with Hashimoto's thyroiditis have high serum concentrations of antibodies to thyroglobulin (Tg) and thyroid peroxidase (TPO) , These antibodies are also found, although usually in lower concentration, in patients with other thyroid diseases including Graves' disease and in many subjects with no clinical or biochemical evidence of thyroid dysfunction but who presumably have a mild thyroiditis . On average, up to 20 percent of all women may have such antibodies depending on the different assays reported (Rotondi M, 2014).

2.4.2 Function of thyroid antibodies:

TG and TPO antibodies are polyclonal and are usually immunoglobulin G1 (IgG1) or IgG3 antibodies, but may be of any subclass. The polyclonality of these autoantibodies is strong evidence that they are a secondary phenomenon to the thyroid damage inflicted initially by T cells, their polyclonality also indicates their variable ability to fix complement (primarily IgG1 and IgG3) and pass through the placenta varies. However, complement-dependent antibody-mediated cytotoxicity may indeed contribute to thyroid damage in patients with Hashimoto's thyroiditis. In addition, TPO antibodies may inhibit TPO enzyme activity, although the significance of such in vitro observations are in dispute. This is because the importance of these actions, in comparison with T cell- and cytokine-mediated apoptosis, is most likely only minor, more important is the potential role of thyroid antibody-secreting B cells in presenting attached thyroid antigen to the T cells (Eleonore and Richard, 2017).

2.5 **Genetic susceptibility:**

It is clear that there is genetic susceptibility to Hashimoto's thyroiditis, and much has been learned in recent years concerning the susceptibility genes for this disorder in particular and for autoimmune thyroid disease in general (Tomer , 2010).

Evidence for genetic susceptibility to Hashimoto's thyroiditis includes the following observations:

The disease in families, sometimes alone and sometimes in combination with Graves' disease, the sibling recurrence risk is >20, It occurs with increased frequency in patients with Down syndrome and Turner syndrome. There is linkage to certain alleles of a small number of immune-related genes including the genes for cytotoxic T-lymphocyte-associated antigen 4 (CTLA-4) and CD40, T cell surface molecules involved in T cell activation. The thyroglobulin (TG) gene (*TG*) has been linked to autoimmune thyroid disease and has been suggested to code for TG forms with different immune reactivity (Tomer, 2010).

2.6 **Precipitating Factors :**

Infection, stress, sex steroids, pregnancy, iodine intake, and radiation exposure are the known possible precipitating factors for Hashimoto's thyroiditis. Fetal microchimerism within the maternal thyroid is also a possibility **(**Iddah and Macharia ,2013**) .**

Infection: No infection is known to cause or even to be closely associated with Hashimoto's thyroiditis in humans, although thyroiditis can be induced in experimental animals by certain viral infections.

Patients with sub-acute granulomatous thyroiditis (presumed to be a viral infection) and congenital rubella may have thyroid antibodies for a few months after their illnesses, and the infections could initiate expression of major histocompatibility complex (MHC) class II molecules in the thyroid gland. However, neither disorder is known to be commonly followed by chronic thyroiditis although evidence of thyroid autoimmunity may persist (Strieder and Prummel ,2003).

Stress: The second case of hyperthyroidism described by Parry in 1825 was a 21-year-old woman whose symptoms began four months after she had been thrown accidentally down the stairs in a wheelchair. Subsequently, stress of various types has been linked to Graves' hyperthyroidism. The proposed mechanisms include induction of immune suppression by non-antigen-specific mechanisms, perhaps due to the effects of cortisol or corticotrophin-releasing hormone on immune cells, followed by immune hyperactivity leading to autoimmune thyroid disease. Such a mechanism might be operative in postpartum thyroiditis, which occurs three to nine months after delivery. However, there is currently no evidence linking emotional or psychological stress to Hashimoto's thyroiditis most probably because of the long natural history of the disease requiring a large part of the gland to be damaged before thyroid function is compromised. Any major stress may have occurred many years earlier (Tomer and Huber ,2009).

Gender — more women than men have Hashimoto's thyroiditis, suggesting a role for sex steroids. However, older women may be more likely to have Hashimoto's thyroiditis than younger women, suggesting that the presence or absence of estrogen may not be the important factor

.Yet, in chickens, androgens protect against thyroiditis induced by immunization with thyroglobulin (TG) (Yin*et al*., 2007).

Pregnancy — pregnant women must generate tolerance for their fetus. During pregnancy, there is a marked increase in CD4+CD25+ regulatory T cells which lead to diminished function of both T cells and B cells , and the rebound from this immune suppression is thought to contribute to the development of postpartum Hashimoto's thyroiditis .Pregnancy-associated immune changes are associated with a shift to Th2 T cells and a shift in cytokine profiles .Approximately 20 percent of patients with postpartum thyroiditis go on to develop classical Hashimoto's disease in later years (Yin *et al*., 2007) .

Iodine intake — Mild iodine deficiency is associated with a lower prevalence of Hashimoto's disease and hypothyroidism, while excessive intake is associated with a higher prevalence. As an example, in China, autoimmune thyroiditis was found in 0.3 percent of those with mildly deficient iodine intake and 1.3 percent of those with excessive iodine intake. Similarly, high iodine-containing drugs, such as amiodarone, often precipitate autoimmune thyroiditis, although a variety of mechanisms have been suggested (Walsh *et al* .,2006) .

Radiation exposure: Environmental radiation exposure may increase the possibility of developing markers of autoimmune thyroid disease, although the evidence for this and developing autoimmune hypothyroidism are conflicting (Huber*et al* .,2002)**.**

Fetal microchimerism — fetal cells have been identified within maternal thyroid glands in patients with autoimmune thyroid. Such cells may initiate graft versus host reactions within the thyroid gland and play

a significant role in the development of Hashimoto's thyroiditis)to date, however, this remains hypothetical (Ando and Davie ,2013) .

2.7**Previous Studies:**

Syed M *et al*, 2006.Studied Relationship between Anti-Thyroid Peroxidase Antibody and Thyroid Function Test In Mohammad Afkhami-Ardekani in Yazd University of medical science in Iran in 2006

The aim of their study was evaluate the relationship between serum levels of anti-TPO antibody And thyroid function test parameters (T3, T4, and TSH) in patients with thyroid disease.

the data was collected 2425 subjects suspected of having thyroid disease referred to Yazd central medical laboratory by physicians during a 2 year period, the concentrations of serum anti-TPO antibody (ELISA) and T3, T4, and TSH (RIA) were measured.

Result was 53.53% of the patients were 20 to 39 years old. 2135 patients (88.04%) were Female and 290 (11.96%) were male. The levels of T3, T4, and TSH in individuals with normal and raised anti-TPO antibody titers was significantly different ($P<0.0001$). A correlation between TSH and T4 levels and abnormal anti-TPO antibody was detected ($P=0.002$). The **Conclusion** results confirm the correlation between thyroid function test and anti-TPO antibody values, indicating the clinical significance of this antibody and suggesting a through clinical examination and follow up of individuals with high anti-TPO antibody titer.

Matthias *et* al ., (2008) study Long-Term Follow-Up of Anti thyroid Peroxidase Antibodies in Patients with Chronic Autoimmune Thyroiditis (Hashimoto's Thyroiditis) Treated with Levothyroxine. The aim of their study was to compare the serum levels of anti-thyroid peroxidase antibodies (TPO-Ab) in patients with Hashimoto's thyroiditis decline during levothyroxine treatment .The data was collected by using of TPO-Abs concentrations in 36 women and 2 men (mean age 51 ± 16 years; range 19–81 years) with Hashimoto's thyroiditis as defined by the following criteria: elevated plasma TPO-Ab and typical hypoechogenicity of the thyroid in high-resolution sonography at first presentation or during follow-up and low pertechnetate uptake in thyroid scintigraphy. When first studied 17 women and 1 man were not yet taking levothyroxine. The remaining 20 patients were receiving levothyroxine. At initial examination 18 patients had serum thyroid-stimulating hormone (TSH) concentrations above normal. Results of up to eight (mean = 5.8) measurements obtained over a mean period of 50 months while patients were receiving levothyroxine were analyzed. In addition, serum TSH, free triiodothyronine (fT3), and free thyroxin (fT4) were measured, and ultrasound of the neck was performed at each follow-up examination. The result showed that the in terms of TPO-Abs levels, 35 of 38 patients (92%) had a decrease, 2 patients had undulating levels, and 1 patient had an inverse hyperbolic increase in her TPO-Abs levels. In the 35 patients in who there were decreasing TPO-Abs values, the mean of the first value was 4779 IU/mL with an SD of 4099 IU/mL . The mean decrease after 3 months was 8%, and after 1 year it was 45%. Five years after the first value, TPO-Ab levels were 1456 ± 1219 IU/mL, a decrease of 70%. TPO-Ab levels became negative, < 100 IU/mL, in only six patients, a normalization percentage of 16%. There were no correlations between changes in thyroid volume and changes in TPO-Ab. In the

conclusion Serum TPO-Abs levels decline in most patients with Hashimoto's thyroiditis who are taking levothyroxine, but after a mean of 50 months, TPO-Abs became negative in only a minority of patients.

Mariotti *et* al ., (1990). Studied ant thyroid Peroxidase Autoantibodies in Thyroid Diseases. The aim of their study was to detect anti-TPO antibodies (anti-TPO Abs) employing purified antigen. The data was collected by using investigation anti-TPO Abs were assayed by a newly developed monoclonal antibody-assisted RIA in a large number (n = 715) of subjects, including 119 normal controls and 596 patients with different autoimmune or non-autoimmune thyroid diseases; Anti-TPO Ab were detected in 10 of 119 (8.4%; range, 11–210 U/mL) normal controls, 134 of 181 (74%; range, 11–74.000 U/mL) patients with Graves' disease, all but 1 of 144 (99.3%; range, 11–90.000 U/mL) with Hashimoto's thyroiditis (n – 98) or idiopathic myxedema (n = 46), 20 of 180 (11.1%; range, 11–6.700 U/mL) with miscellaneous non autoimmune thyroid diseases, 16 of 83 (19.2%; range, 11–6.600 U/mL) patients with differentiated thyroid carcinoma, and in none of 8 patients with sub-acute thyroiditis. The result showed that the highest anti-TPO Ab concentrations were found in untreated hypothyroid Hashimoto's thyroiditis, but no simple relationship between anti-TPO Ab levels and thyroid function was observed. Anti-TPO Ab significantly decreased in patients with Graves' disease after treatment with methimazole and in those with hypothyroid Hashimoto's thyroiditis or idiopathic myxedema during L-T4 administration. A highly significant positive correlation ($r = 0.979$; $P < 0.001$) was found between anti-M Ab titers by passive hem agglutination (PH; available in 650 sera) and the corresponding average anti-TPO Ab by RIA; discrepant results were almost exclusively limited to sera with negative or low (1:100–1:400) anti-M Ab titers. Analysis of

these discrepant data indicated higher autoimmune disease specificity and sensitivity of anti-TPO Ab RIA tests compared to anti-M Ab by PH. Absorption studies showed that interference of anti-Tg Ab was responsible for anti-M Ab-positive tests in occasional anti-TPO Ab negative anti-M Ab-positive sera from autoimmune thyroid disease patients. Anti-TPO Ab determination by RIA was unaffected by circulating thyroglobulin concentrations up to more than 10,000 ng/mL. In the conclusion anti-TPO Ab assay by monoclonal antibody assisted RIA appears to be more sensitive and specific for thyroid autoimmune diseases than anti-M Ab determination by PH. Since the assay is easy to perform and employs only tracer amounts of purified antigen, these characteristics should allow its rapid diffusion to the clinical routine.

Fatima *et* al., (2013). Studied of Serum Trace Elements and Vitamin Levels in Hashimoto's Thyroiditis: Single Centre Experience from Turkey. The aim of their study was to determine levels of serum trace elements and vitamins, and to find out possible correlations between these elements and vitamins with thyroid function tests and thyroid autoantibody levels in patients having Hashimoto's thyroiditis (HT). The data was collected by using 51 pre menauposal women with untreated HT, aged 18 to 56 years without any known chronic diseases or chronic medicine usage, and 27 healthy premenauposal women aged 19 to 42 years old. Trace elements (selenium, zinc, copper, iron levels) and vitamins [A, E, B12, 25-OH-D, 1, 25(OH) 2D and folic acid levels] were evaluated in patient and control groups. The result showed that consequently, serum trace elements and vitamin B12 levels did not significantly differ in patients with HT and control group. Thyroid functioning tests and autoantibody levels did not show any correlation with the levels of trace elements, vitamin A, vitamin E and 25-OH

vitamin D. A correlation was detected between vitamin B12 and Anti thyroid peroxidase levels. In the conclusion the negative correlation between vitamin B12 and Anti thyroid peroxidase levels may demonstrate the necessity to screen the patients with HT for atrophic gastritis. We believe that more comprehensive studies with larger sample sizes are needed in which patients are randomized according to their nutritional status.

Susan *et* al., 2011. Studied Thyroid Antibodies in Women with Autoimmune Thyroid Disease. The aim of their study was to assess whether females with Graves' disease or Hashimoto thyroiditis are more likely than age-matched controls to have thyroid antibodies before clinical diagnosis and to measure the timing of antibody seroconversion. The data was collected by using assessed thyroid antibodies in the serum of 522 females, active-duty, and military personnel including: 87 Graves' disease cases, 87 Hashimoto thyroiditis cases, and 348 age matched controls. One serum sample was available at the time of the clinical diagnosis (±6 months); three additional samples were retrieved from the repository up to 7 years before the clinical diagnosis, for a total of 2088 samples. The result showed that in Hashimoto thyroiditis, TPO antibodies were found in about 66% of the cases at all-time points. TG antibodies showed a similar stationary trend, at a lower prevalence of about 53%at all-time points. No TSH-R antibodies were found. In Graves' disease, TPO antibodies gradually increased from 31% at 5–7 years prior to diagnosis to 57% at diagnosis and TG antibodies from 18 to 47%. TSH-R antibodies were present before diagnosis and showed an increasing prevalence from 2, 7, 20, to 55%. In the conclusion Antibodies to TG, TPO, and TSH-R precede by years the development of the diagnostic

autoimmune thyroid diseases phenotype. Overall, the presence of thyroid antibodies in apparently healthy individuals should not be neglected.

Chapter three

Materials and Methods

3.Materials and Methods

3.1 Study design

This was a hospital-based cross sectional comparative study.

3.2 Study area and duration

This study was conducted in Mohammed Dossary Hospital in (Saudi Arabia) during the period from January 2018 to December 2019.

3.3 Study population:

The study included 150 subjects classified based on tpo ab to positive and negative un treated hypothyroidism each one 50 participants and 50 randomly as healthy individual.

Inclusion criteria

-Random Patients of both sexes known to have un treated hypothyroid and were enrolled to participate in this study.

-Random patients of both sexes known to have untreated auto immune thyroid disease(positive tpo abs hypothyroidism).

-random patients with different ages in all group (positive and negative tpo ab hypothyroidism)

-random nationalities (Asian and African)

Exclusion criteria

- Patients with interfere diagnosis with increasing Tpo ab like Graves ' disease, toxic thyroiditis .Idiopathic myxedema.

-treated patient from hypothyroidism .

3.4 Ethical consideration

All participants were signed informed consent was obtained from each participant (Appendix I) . informed consent and satisfied with the study objectives recruited in this study. Privacy and confidentiality for each participant were guaranteed.

3.5 Sample Size

The sample of the study consists of 150 participants divided between patient suspected hypothyroidism, hashimoto thyroiditis and healthy individual.

3.6 Data collection

Checklist were used for data collection from patient medical file.

3.7 Specimen collection

About 5ml of venous blood were collected by using sterile disposal plastic syringe and applying aseptic standard non traumatic vein puncture technique. And informed consent was obtained verbally before blood sample collection every sample was emptied in Lithium heparin anticoagulant container and then centrifuged (3000 rpm) for 10 minutes, the plasma was stored at - 20◦c until analyzed.

3.8 Estimation of TSH level:

3.8.1 Principle of TSH level:(appendix II)

TSH test is performed using Vitros TSH reagent pack and the Vitros TSH calibrators on the Vitros 5600 integrated system using Intellicheck technology. An immunometric immunoassay technique is used, which involves the simultaneous reaction of TSH present in sample with biotinylated antibody (mouse monoclonal anti –whole TSH) and horseradish peroxidase (HRP)- labeled antibody conjugate (mouse monoclonal anti –TSH B-sub unit). The antigen –antibody complex is captured by streptavidin on the well,UN bound materials are removed by washing.

The bound HRP conjugate is measured by a luminescent reaction. A reagent containing luminogenic substrate (aluminol derivative and peracid salt) and electron transfer agent. Is added to the wells, the HRP in the bound conjugate catalyzes the oxidation of the luminol derivative,

producing light. The electron transfer agent (a substitute acetanilide) increases the level of light produced and prolong its emission. The light signals are read by the system .the amount of HRP conjugate bound directly proportional to the concentration of TSH present.

3.8.2 Procedure of TSH level

Before running sample in vitros 5600 machine which is automated one, run the quality control after pass go to sample, in machine add around 80 ul from patient sample to the reagent well which is coated by AB, then incubate at 37 C for 29 min then add signal reagent to the medium (emitted one with HRP) then incubate again for 8 min then it read by illuminator as figures

Normal range from 0.46_4.68 m1IU/L

3.9 Estimation of FT4 level(appendix III)

3.9.1 Principle of FT4 level:

FT4 test is performed using Vitros TSH reagent pack and the Vitros FT4 calibrators on the Vitros 5600 integrated system using Intellicheck technology. A direct labeled antibody, competitive immunoassay technique is used. FT4 present in sample competes with ligand on the modified well surface for a limited number of binding sites on a horseradish peroxidase (HRP)_labeled antibody conjugate (sheep anti – FT4). The well surface has been modified to act as a ligand for UN combined conjugate. Unbound materials are moved by washing .the test design, with optimal reagent concentration, ensures that disturbance of the T4/ binding protein equilibrium is so small as to be negligible. The bound HRP conjugate is measured by a luminescent reaction. A reagent containing luminogenic substrate (a luminol derivatives and peracid salt) and an electron transfer agent, is added to the wells. The HRP in the bound conjugate catalyzes the oxidation of the luminol derivative,

producing light. The electron transfer agent (a substitute acetanilide) increases the level of light produced and prolong its emission. The light signals are read by the system .the amount of HRP conjugate bound directly proportional to the concentration of FT4 present.

3.9.2 Procedure of FT4 level:

Before running sample in vitros 5600 machine which is automated one, run the quality control after pass go to sample, in machine add around 25 uL from patient sample to the reagent well which is coated by AB, then incubate at 37 C for 16 min then add signal reagent to the medium (emitted one with HRP) then incubate again for 8 min then it read by illuminator as figures

N R: from 0.78 _2.19 ng/dL

3.10Estimation of TPO Abs level: (appendix IV)

3.10.1 Principle of TPO Abs level:

By using Cobas e 411 machine Competition principle. Total duration of assay: 18 minutes.

First incubation: 20 µL of sample are incubated with anti-TPO-antibodies labeled with a ruthenium complex.

Second incubation: After addition of biotinylated TPO and streptavidin-coated micro particles, the anti-TPO antibodies in the sample compete with the ruthenium-labeled anti-TPO antibodies for the biotinylated TPO antigen. The entire complex becomes bound to the solid phase via interaction of biotin and streptavidin.

The reaction mixture is aspirated into the measuring cell where the micro particles are magnetically captured onto the surface of the electrode. Unbound substances are then removed with ProCell. Application of a

voltage to the electrode then induces chemiluminescent emission which is measured by a photo multiplier.

• Results are determined via a calibration curve which is instrument-specifically generated by 2-point calibration and a master curve provided via the reagent barcode.

3.10.2 Procedure of TPO Abs level:

Before running sample in Cobas e411 machine which is automated one , run the quality control (QC) after pass go to sample , in machine add around 20 uL from patient sample to the TPO reagent , then incubate at 37 C for 18 min then then it induces chemiluminescent emission which is measured by a photomultiplier read

N R: 34 IU/Ml (>34 = positive)

3.11 Quality control

The precision and accuracy of all methods used in this study were checked by commercially prepared control sample before it is application for the measurement of test and control samples.

3.12 Statistical analysis

For analysis of data, Statistical Package for Social Sciences software, version 23.0 (IBM SPSS Inc., Chicago, IL) was used. Initially, all information gathered via data master sheet then coded into variables. Both descriptive and inferential statistics involving Independent T-test, one way ANOVA-Test (Analysis of variances) and Pearson's Correlation Test were used to present results. A p-value of less than 0.05 was considered statistically significant .P value<0.05 that' s considered as statistically significant. P value>0.05 that' s considered as statistically insignificant.

Chapter four

Results

4-RESULTS

This chapter present tables and figures show ages, sex and means of serum TSH, FT4 and TPO abs

Table (4.1) show demographic data of study groups showing positive and negative hypothyroidism against healthy individual

This study showed that mean of serum TSH level were 16.17 mIU/L +-24.91 with range (0.5_100) mIU/L, while FT4 (0.10__2.20) ng /dl with mean 0.67+-0.35 ng/dl

And TPO abs from (0.00_1000) IU/ml (**Table 4.2**) .

The study showed that 88 (58.7%) were female and 62 (41.3 %) were male (**Table 4.3).**

The age range were less than 20 years 9 participant (6%), 20_40 years 99 (66%) and more than 40 years around 42 (28%) (**Table** 4.4).

Figure (2) showed nationality distribution among case groups divided to 20 % African and 80 % Asian subject

Nationality distribution with different countries e.g. .(Bangladesh, Egypt , Philippine, Indian , Jordan ,Lebanese ,Pakistani , Saudi Sudanese ,Syrian ,and Yemeni an) less group were Philippine , Lebanese , Syrian and Yemeni an with 4 participant in each and highest group were Indian participants by 45 (**Table 4.5**).

There were statistically significant increase in the mean of TSH level in negative tpo hypo thyroids (5.2_100) mIU/L with mean 22.97 mIU/L and Positive tpo hypothyroidism (4.8_100) mIU/L with mean 23.09 mIU/L , while normal level in case control (0.5_4.5) mIU/L with mean 2.46 mIU/L.(**Table 4.6**)

Statistically showed significant increase in TSH level in Positive tpo hypothyroidism and negative tpo hypothyroidism with p value: 0.00, and significantly decrease in FT4 level in Positive tpo hypothyroidism and negative tpo hypothyroidism with p value 0.00, for TPO abs also show significantly increase in Positive tpo hypothyroidism with p value 0.00 **(Table 4.7)**

Table (4.8) show in significant cooperation between sex and TPO Abs level in Positive tpo hypothyroidism disease with p value 0.857.and insignificant between sex and TPO Abs level in negative tpo hypothyroidism p value 0.797

Table (4.9) show no significant correlation between age and Positive tpo hypothyroidism disease p value 0.274 r=0.158, and no significant correlation between age and negative tpo hypothyroidism p value 0.808 r=0.035.

Table (4.1) Demographic data of study groups showing positive and negative hypothyroidism against healthy individual

Variable		Pos Tpo	Neg Tpo	Healthy individual	Total number
Age /years	Less than 20 years	1	5	3	9
	20-40 years	35	33	31	99
	More than 40 years	14	12	16	42
Sex	Male	17	22	23	62
	Female	33	28	27	88
Tsh mIU/L		4.8_100	5.2_100	0.5_4.5	150
FT4 ng/dl		0.2_0.7	0.1_0.7	0.8_2.2	150
Tpo abs IU/ml		39.2_1000	3_21.6	0_24	150

Table (4.2): Represent study parameters mean and SD against study case groups

Descriptive Statistics		Number	Minimum	Maximum	Median	Mean	Std. Deviation
Positive tpo hypothyroidism	Age (Years)	50	14	61	33	36	11
	TSH mIU/L	50	4.8	100	10.65	23.09	26.24
	FT4 ng/dL	50	0.2	0.7	0.60	0.53	0.16
	TPO IU/Ml	50	39.2	1000	315.05	397.64	298.22
Negative tpo Hypothyroidism	Age (Years)	50	9	57	31	32	10
	TSH mIU/L	50	5.2	100	9.34	22.97	30.14
	FT4 ng/dL	50	0.1	0.7	0.43	0.41	0.17
	TPO IU/Ml	50	3	21.6	13.00	13.10	4.74
Healthy individuals	Age (Years)	50	11	67	35	36	12
	TSH mIU/L	50	0.5	4.5	2.34	2.46	1.28
	FT4 ng/dL	50	0.8	2.2	1.02	1.07	0.26
	TPO IU/Ml	50	0	24	4.50	5.76	4.98

Table (4.3): Shows sex distribution among study group

Variables		Groups			Total
		Positive tpo hypothyroidism	Negative tpo Hypothyroidism	Healthy individuals	
Sex	Male	17	22	23	62
		27.40%	35.50%	37.10%	100.00%
	Female	33	28	27	88
		37.50%	31.80%	30.70%	100.00%
Total		50	50	50	150
		33.30%	33.30%	33.30%	100.00%

Table (4.4): Shows age distribution among study group

Variables		Groups			Total
		Positive tpo hypothyroidism	Negative tpo Hypothyroidism	Healthy individuals	
Age groups	Less than 20 years	1	5	3	9
		11.10%	55.60%	33.30%	100.00%
	20-40 years	35	33	31	99
		35.40%	33.30%	31.30%	100.00%
	More than 40 years	14	12	16	42
		33.30%	28.60%	38.10%	100.00%
Total		50	50	50	150
		33.30%	33.30%	33.30%	100.00%

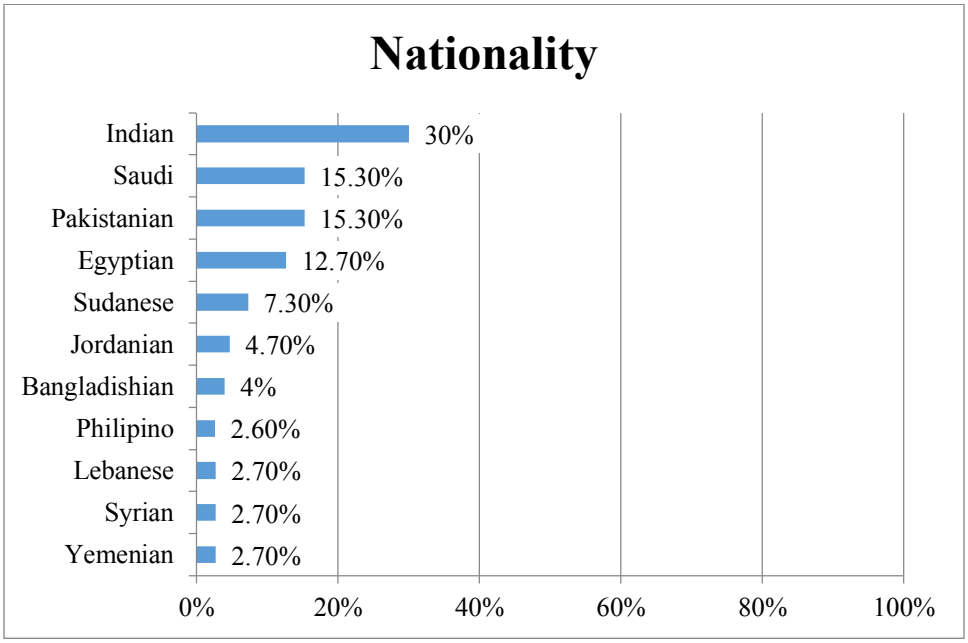

Figure (4.1): Shows nationality distribution among study groups

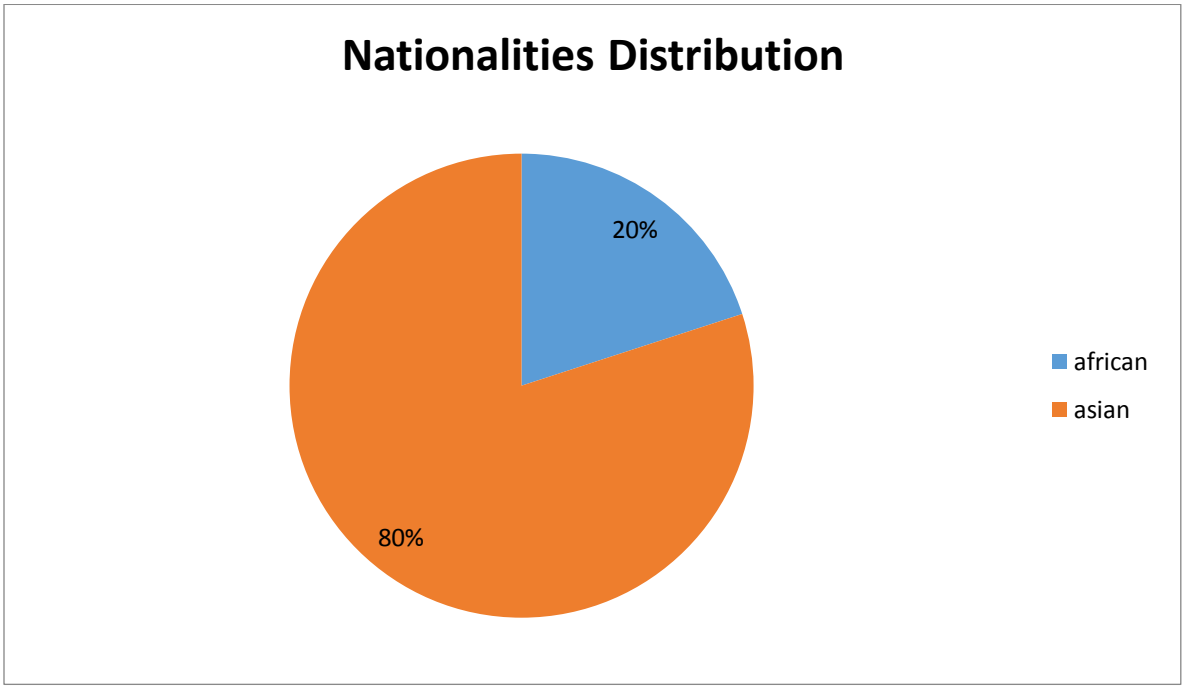

Figure (2) Show national distribution among case groups divided to African and Asian subject

Table (4.5): Shows the nationality distribution among study groups

Variables		Groups			Total
		Positive tpo hypothyroidism	Negative tpo Hypothyroidism	Healthy individuals	
Nationality	Bangladishian	2	0	4	6
		33.30%	0.00%	66.70%	100.00%
	Egyptian	4	11	4	19
		21.10%	57.90%	21.10%	100.00%
	Philipino	0	2	2	4
		0.00%	50.00%	50.00%	100.00%
	Indian	17	14	14	45
		37.80%	31.10%	31.10%	100.00%
	Jordanian	5	1	1	7
		71.42%	14.29%	14.29%	100.00%
	Lebanese	2	0	2	4
		50.00%	0.00%	50.00%	100.00%
	Pakistanian	8	11	4	23
		34.80%	47.80%	17.40%	100.00%
	Saudi	9	9	5	23
		39.13%	39.13%	21.74%	100.00%
	Sudanese	2	2	7	11
		18.20%	18.20%	63.60%	100.00%
	SYRIAN	1	0	3	4
		25.00%	0.00%	75.00%	100.00%
	Yemenian	0	0	4	4
		0.00%	0.00%	100.00%	100.00%
Total		50	50	50	150
		33.30%	33.30%	33.30%	100.00%

Table (4.6): Represent comparison mean and SD for age and study parameters

Total						
Descriptive Statistics	Number	Minimum	Maximum	Median	Mean	Std. Deviation
Age (Years)	150	9	67	33	35	11
TSH mIU/L	150	0.50	100.00	6.82	16.17	24.91
FT4 ng/dL	150	0.10	2.20	0.62	0.67	0.35
TPO IU/Ml	150	0.00	1000.00	13.77	138.83	250.97

Table (4.7): Comparison between biochemical parameters in case groups

One Way ANOVA-Test						
Variables		Number	Mean	Std. Deviation	Std. Error	P value
TSH mIU/L	Positive tpo hypothyroidism	50	23.09	26.24	3.71	0.000001
	Negative tpo Hypothyroidism	50	22.97	30.14	4.26	
	Healthy individuals	50	2.46	1.28	0.18	
FT4 ng/dL	Positive tpo hypothyroidism	50	0.53	0.16	0.02	0.000001
	Negative tpo Hypothyroidism	50	0.41	0.17	0.02	
	Healthy individuals	50	1.07	0.26	0.04	
TPO IU/Ml	Positive tpo hypothyroidism	50	397.64	298.22	42.17	0.000001
	Negative tpo Hypothyroidism	50	13.10	4.74	0.67	
	Healthy individuals	50	5.76	4.98	0.70	

One way a nova test was used to compare between means, P value>0.05 that's considered as statistically insignificant

Table (4.8) Comparison between biochemical parameters and sex among study groups

Independent T-Test							
Variables		Sex	Number	Mean	Std. Deviation	Std. Error Mean	P value
Positive tpo hypothyroidism	TSH mIU/L	Male	17	25.13	24.93	6.05	0.698
		Female	33	22.04	27.21	4.74	
	FT4 ng/dL	Male	17	0.51	0.16	0.04	0.537
		Female	33	0.54	0.16	0.03	
	TPO IU/Ml	Male	17	408.42	286.03	69.37	0.857
		Female	33	392.08	308.51	53.70	
Negative tpo Hypothyroidism	TSH mIU/L	Male	22	16.23	20.64	4.40	0.163
		Female	28	28.27	35.36	6.68	
	FT4 ng/dL	Male	22	0.41	0.19	0.04	0.903
		Female	28	0.41	0.16	0.03	
	TPO IU/Ml	Male	22	13.29	4.44	0.95	0.797
		Female	28	12.94	5.03	0.95	
Healthy individuals	TSH mIU/L	Male	23	2.22	1.27	0.27	0.232
		Female	27	2.66	1.27	0.24	
	FT4 ng/dL	Male	23	1.12	0.30	0.06	0.181
		Female	27	1.02	0.20	0.04	
	TPO IU/Ml	Male	23	5.04	4.98	1.04	0.353
		Female	27	6.37	4.99	0.96	

Independent T-Test used to compare between means, P value>0.05 that's considered as statistically insignificant

Table (4.9): show the Pearson correlation and p value for age among case groups

Correlations					
Variables			TSH	FT4	TPO
Positive tpo hypothyroidism	Age (Years)	Pearson Correlation	0.158	-0.178	0.073
		Sig. (2-tailed)	0.274	0.217	0.612
		Number	50	50	50
		Strength	Weak	Weak	Weak
		Direction	Positive	Negative	Positive
Negative tpo Hypothyroidism	Age (Years)	Pearson Correlation	0.035	0.236	0.242
		Sig. (2-tailed)	0.808	0.098	0.09
		Number	50	50	50
		Strength	Weak	Weak	Weak
		Direction	Positive	Positive	Positive
Healthy individuals	Age (Years)	Pearson Correlation	0.081	0.14	-0.053
		Sig. (2-tailed)	0.577	0.334	0.715
		Number	50	50	50
		Strength	Weak	Weak	Weak
		Direction	Positive	Positive	Negative
Correlation is insignificant at 0.05 level					

Chapter five

Discussion, Conclusion and Recommendations

5. Discussion, conclusion and recommendations

5.1. Discussion:

Thyroid antibody positivity precedes a clinical diagnosis of Hashimoto thyroiditis by several years.

Our study showed that most of participants were female which agree with Susan 2011 ؛ Fatima 2013. This study showed that significant increase in the mean of TSH level in negative tpo hypothyroidism and positive tpo hypothyroidism , this study in agreement with Mathias, 2008.

This study showed significant increase in TSH level in positive tpo hypothyroidism and negative tpo hypothyroidism with p. value: 0.00, and significantly decrease in FT4 level in positive tpo hypothyroidism and negative tpo hypothyroidism with p value 0.00,

Our findings are consistent with studies of the relationship between antibodies and thyroid function carried by Syedet al., 2006. agree with our study which aim to asses the relationship between serum levels of anti-TPO antibody and thyroid function test parameters (T4 and TSH) in patients with hypothyroidism disease among different Saudi Arabia patients in range of age from 20 to 40 years around 66% almost were female also with same study around20 to 39 years his study confirmed correlation between TSH , ft4 level and abnormality TPO abs was detected.

Anti-TPO antibodies are more likely to be of pathogenic importance than other antibodies as they fix complement and may directly damage thyroid cells, agree with Susan 2011 and Fatima 2013 both were significantly TPO abs elevated in 66% of Hashimoto thyroiditis.

Anti-TPO antibodies may exaggerate or perpetuate thyroid injury but probably do not initiate it.Therefore, the evaluation of serum anti-TPO antibody levels with respect to serum concentration of thyroid hormones would help in elucidating its probable pathogenic role in induction of hypo- or hyperthyroidism.

for TPO abs also show significantly increase in positive tpo hypothyroidism with p value 0.00 .our study agree with Mariotti 1990 study , his study about measure TPO abs in thyroid disease , detect in 99.3% of patients with Hashimoto thyroiditis, his result showed that the highest TPO abs concentration were found in un treated Hashimoto thyroiditis.

5.2. Conclusion

This study concluded that, there was significantly elevated TPO abs in positive tpo hypothyrodism. while both positive and negative hypothyroidism had significantly increase in serum TSH and significantly decrease in serum FT4.

5.3 Recommendations

1- Suggesting checking TPO abs as a hypothyroid panel to avoid any miss diagnoses in mid age specificly with women .

2- Results confirm the correlation between thyroid function test and anti-TPO antibody values, emphasizing the clinical significance of this antibody and suggesting a through clinical examination and follow up of individuals with high anti-TPO antibody titer.

3-Further studies on a relation between TPO abs and other auto immune thyroid disease to avoid any discrepancies or interferences.

4-the communities need more awareness about thyroid disorders and auto immune disease and how to deal with it.

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Appendix

(APPENDIX 1)

Sudan University of Science and Technology

College Of Graduate Studies

Thyroid stimulating hormone, Free Thyroxin and Thyroid Peroxidase Antibodies for Discrimination of Hashimoto's Thyroiditis from Hypothyroidism

(Study In Saudi Arabia)

Questionnaire No ( )

Participant name .....address ……

Contact phone number …….

Age ……..years

Nationality ……

Male ( ) Female ( )

Do you have history with hypo thyroid disease yes ( ) No( )

Do you have history of Hashimoto thyroiditis yes ( ) No ( )

Do you requested to check TPO abs before yes ( ) No ( )

Do you accept to use your information and your sample to do this research yes( ) No( )

Laboratory Investigations:

TSH ……mIU/L

Ft4……ng/dL

TPO abs……IU/MlParticipant signature

VITROS® Immunodiagnostic Products

INSTRUCTIONS FOR USE

TSH

VITROS Immunodiagnostic Products TSH Reagent Pack REF 191 2997

VITROS Immunodiagnostic Products TSH Calibrators REF 148 7289

Intended Use

For *in vitro* diagnostic use only.

VITROS Immunodiagnostic Products TSH Reagent Pack

For the quantitative measurement of thyroid stimulating hormone (TSH) in human serum and plasma (EDTA or heparin) using the VITROS ECi/ECiQ Immunodiagnostic Systems, the VITROS 3600 Immunodiagnostic System and the VITROS 5600 Integrated System to aid in the differential diagnosis of thyroid disease.

VITROS Immunodiagnostic Products TSH Calibrators

For use in the calibration of the VITROS ECi/ECiQ Immunodiagnostic Systems, the VITROS 3600 Immunodiagnostic System and the VITROS 5600 Integrated System for the quantitative measurement of thyroid stimulating hormone (TSH) in human serum and plasma (EDTA or heparin).

Summary and Explanation of the Test

TSH secretion by the anterior pituitary is controlled by thyrotropin releasing hormone, a tripeptide produced by the hypothalamus. TSH stimulates the production of thyroxine (T4) and triiodothyronine (T3) by the thyroid gland. The circulating free fractions of T4 and T3 in turn regulate the secretion of TSH by a negative feedback mechanism at the pituitary and possibly the hypothalamus.[1] The diagnosis of overt hypothyroidism by the finding of a low total T4 or free T4 concentration is readily confirmed by a raised TSH concentration.[2]

Measurement of low or undetectable TSH concentrations may assist the diagnosis of hyperthyroidism,[3, 4] where concentrations of T4 and T3 are elevated and TSH secretion is suppressed. TSH tests with high levels of precision and functional sensitivity claims of 0.01–0.02 mIU/L have been termed "third generation" tests.[5] These have the advantage of discriminating between the concentrations of TSH observed in thyrotoxicosis, compared with the low, but detectable, concentrations that occur in subclinical hyperthyroidism.[6]

Principles of the Procedure

The VITROS TSH test is performed using the VITROS TSH Reagent Pack and the VITROS TSH Calibrators on the VITROS ECi/ECiQ Immunodiagnostic Systems, VITROS 3600 Immunodiagnostic System and VITROS 5600 Integrated System using Intellicheck® Technology. An immunometric immunoassay technique is used, which involves the simultaneous reaction of TSH present in the sample with a biotinylated antibody (mouse monoclonal anti-whole TSH) and a horseradish peroxidase (HRP)-labeled antibody conjugate (mouse monoclonal anti-TSH β-subunit). The antigen-antibody complex is captured by streptavidin on the wells. Unbound materials are removed by washing.

The bound HRP conjugate is measured by a luminescent reaction.[7] A reagent containing luminogenic substrates (a luminol derivative and a peracid salt) and an electron transfer agent, is added to the wells. The HRP in the bound conjugate catalyzes the oxidation of the luminol derivative, producing light. The electron transfer agent (a substituted acetanilide) increases the level of light produced and prolongs its emission. The light signals are read by the system. The amount of HRP conjugate bound is directly proportional to the concentration of TSH present.

Test Type	System*	Incubation Time	Time to first result	Test Temperature	Reaction Sample Volume
Immunometric	ECi/ECiQ, 3600, 5600	29 minutes	37 minutes	37 °C	80 µL

* Not all products and systems are available in all countries.

INSTRUCTIONS FOR USE

FT4

VITROS Immunodiagnostic Products Free T4 Reagent Pack REF 138 7000

VITROS Immunodiagnostic Products Free T4 Calibrators REF 172 8872

Intended Use

For *in vitro* diagnostic use only.

VITROS Immunodiagnostic Products Free T4 Reagent Pack

For the quantitative measurement of free thyroxine (FT4) in human serum using the VITROS ECi/ECiQ Immunodiagnostic Systems, the VITROS 3600 Immunodiagnostic System and the VITROS 5600 Integrated System, to aid in the differential diagnosis of thyroid disease.

VITROS Immunodiagnostic Products Free T4 Calibrators

For use in the calibration of the VITROS ECi/ECiQ Immunodiagnostic Systems, the VITROS 3600 Immunodiagnostic System and the VITROS 5600 Integrated System for the quantitative measurement of free thyroxine (FT4) in human serum.

Summary and Explanation of the Test

The free fraction of the circulating thyroxine (T4) is considered to exert the main influence on metabolic control.[1] Consequently, the FT4 concentration is believed to be the most direct indicator of an individual's thyroid status. FT4 concentrations are generally depressed in hypothyroidism and raised in hyperthyroidism. Measurement of FT4 thus provides an aid to the differential diagnosis of thyroid disease.

FT4 concentrations are independent of the concentration of thyroid hormone binding proteins[2,3] and may therefore be measured in patients with elevated or reduced binding protein concentrations without the need for additional tests of binding capacity.[2,3] In borderline cases of suspected thyroid malfunction, additional tests such as free T3 or TSH may be necessary.

Principles of the Procedure

The VITROS Free T4 test is performed using the VITROS Free T4 Reagent Pack and the VITROS Free T4 Calibrators on the VITROS ECi/ECiQ Immunodiagnostic Systems, the VITROS 3600 Immunodiagnostic System and the VITROS 5600 Integrated System using Intellicheck® Technology. A direct, labeled antibody, competitive immunoassay technique is used. FT4 present in the sample competes with ligand on the modified well surface for a limited number of binding sites on a horseradish peroxidase (HRP)-labeled antibody conjugate (sheep anti-T4). The well surface has been modified to act as a ligand for uncombined conjugate. Unbound materials are removed by washing. The test design, with optimal reagent concentrations, ensures that disturbance of the T4/binding protein equilibrium is so small as to be negligible.

The bound HRP conjugate is measured by a luminescent reaction.[4] A reagent containing luminogenic substrates (a luminol derivative and a peracid salt) and an electron transfer agent, is added to the wells. The HRP in the bound conjugate catalyzes the oxidation of the luminol derivative, producing light. The electron transfer agent (a substituted acetanilide) increases the level of light produced and prolongs its emission. The light signals are read by the system. The amount of HRP conjugate bound is indirectly proportional to the concentration of FT4 present.

Test Type	System*	Incubation Time	Time to first result	Test Temperature	Reaction Sample Volume
Competitive immunoassay	ECi/ECiQ, 3600, 5600	16 minutes	24 minutes	37 °C	25 µL

* Not all products and systems are available in all countries.

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Anti-TPO

Antibodies to thyroid peroxidase

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11820818 122 **100 tests**

• Indicates analyzers on which the kit can be used

Elecsys 1010	Elecsys 2010	MODULAR ANALYTICS E170	**cobas e** 411	**cobas e** 601
•	•	•	•	•

English

Intended use

Immunoassay for the in vitro quantitative determination of antibodies to thyroid peroxidase in human serum and plasma. The anti-TPO determination is used as an aid in the diagnosis of autoimmune thyroid diseases.
The **e**lectro**c**hemi**l**uminescence **i**mmuno**a**ssay "ECLIA" is intended for use on Elecsys and **cobas e** immunoassay analyzers.

Summary[1,2,3,4,5,6]

Thyroid-specific peroxidase (TPO) is present on the microsomes of thyrocytes and is expressed at its apical cell surface. In synergy with thyroglobulin (Tg) this enzyme has an essential function in the iodination of L-tyrosine and the chemical coupling of the resulting mono- and di-iodotyrosine to form the thyroid hormones T4, T3, and rT3.
TPO is a potential autoantigen. Elevated serum titers of antibodies to TPO are found in several forms of thyroiditis caused by autoimmunity. The still freqently found term "microsomal antibody" originates from the time when TPO had not yet been identified as an antigen in autoimmunity caused by microsomes. In the clinical sense the two terms anti-TPO and microsomal antibody can be used synonymously; there are differences, however, with regard to the test methods. High anti-TPO titers are found in up to 90% of patients with chronic Hashimoto's thyroiditis. In Graves' disease, 70% of the patients have an elevated titer. Although the sensitivity of the procedure can be increased by simultaneously determining other thyroid antibodies (anti-Tg, TSH-receptor-antibody - TRAb), a negative finding does not rule out the possibility of an autoimmune disease. The magnitude of the antibody titer does not correlate with the clinical activity of the disease. Initially elevated titers can become negative after lengthy periods of illness or during remission. If antibodies reappear following remission, then a relapse is probable.
Whereas the usual microsomal antibody tests employ unpurified microsomes as an antigen preparation, the anti-TPO tests use a purified peroxidase. The two procedures are of comparable performance in terms of clinical sensitivity, but better lot-to-lot consistency and higher clinical specificity can be expected from anti-TPO tests due to the higher quality of the antigen used. Recombinant antigen and polyclonal anti-TPO antibodies are used in the Elecsys Anti-TPO assay.

Test principle

Competition principle. Total duration of assay: 18 minutes.

- 1st incubation: 20 µL of sample are incubated with anti-TPO-antibodies labeled with a ruthenium complex[a].
- 2nd incubation: After addition of biotinylated TPO and streptavidin-coated microparticles, the anti-TPO antibodies in the sample compete with the ruthenium-labeled anti-TPO antibodies for the biotinylated TPO antigen. The entire complex becomes bound to the solid phase via interaction of biotin and streptavidin.
- The reaction mixture is aspirated into the measuring cell where the microparticles are magnetically captured onto the surface of the electrode. Unbound substances are then removed with ProCell. Application of a voltage to the electrode then induces chemiluminescent emission which is measured by a photomultiplier.
- Results are determined via a calibration curve which is instrument-specifically generated by 2-point calibration and a master curve provided via the reagent barcode.

a) Tris(2,2'-bipyridyl)ruthenium(II)-complex ($Ru(bpy)_3^{2+}$)

Reagents - working solutions

M Streptavidin-coated microparticles (transparent cap), 1 bottle, 6.5 mL: Streptavidin-coated microparticles 0.72 mg/mL; preservative.

R1 Anti-TPO-Ab~$Ru(bpy)_3^{2+}$ (gray cap), 1 bottle, 10 mL: Polyclonal anti-TPO antibody (sheep) labeled with ruthenium complex 1.0 mg/L; TRIS buffer 100 mmol/L, pH 7.2; preservative.

R2 TPO~biotin (black cap), 1 bottle, 10 mL: Biotinylated TPO (recombinant) 0.15 mg/L; TRIS buffer 30 mmol/L, pH 7.0; preservative.

Cal1 A-TPO calibrator 1 (white cap), 1 bottle, 1.5 mL: Polyclonal anti-TPO antibody (sheep) approx. 35 IU/mL in human serum matrix; preservative.

Cal2 A-TPO calibrator 2 (black cap), 1 bottle, 1.5 mL: Polyclonal anti-TPO antibody (sheep) approx. 350 IU/mL in human serum matrix; preservative.

PC A-TPO1 PreciControl A-TPO 1 (beige cap), 1 bottle, 1.5 mL: Anti-TPO antibodies (human) approx. 35 IU/mL in human serum matrix; preservative.

PC A-TPO2 PreciControl A-TPO 2 (brown cap), 1 bottle, 1.5 mL: Anti-TPO antibodies (human) approx. 100 IU/mL in human serum matrix; preservative.

Calibrators: The exact lot-specific calibrator values are encoded in the barcoded labels of the test-specific reagent.
Controls: The exact lot-specific target values and ranges are encoded in the barcodes as well as printed on the enclosed (or electronically available) value sheet.

Precautions and warnings

For in vitro diagnostic use.
Exercise the normal precautions required for handling all laboratory reagents.
Disposal of all waste material should be in accordance with local guidelines.
Safety data sheet available for professional user on request.
All human material should be considered potentially infectious.
All products derived from human blood are prepared exclusively from the blood of donors tested individually and shown to be free from HBsAg and antibodies to HCV and HIV.
The testing methods applied were FDA-approved or cleared in compliance with the European Directive 98/79/EC, Annex II, List A.
The materials of human origin used for the controls were tested for HIV, HBV, and HCV infection. The findings were negative.
However, as no testing method can rule out the potential risk of infection with absolute certainty, the material should be treated just as carefully as a patient specimen. In the event of exposure the directives of the responsible health authorities should be followed.[7,8]
Avoid the formation of foam with all reagents and sample types (specimens, calibrators, and controls).

Reagent handling

The reagents in the kit are ready for use and are supplied in bottles compatible with the system.

Elecsys 1010/2010 and **cobas e** 411 analyzers: The Elecsys calibrators Cal1 and Cal2 and the Elecsys controls PC A-TPO1 and PC A-TPO2 should only be left on the analyzers at 20-25°C during calibration/quality control. After use, close the bottles as soon as possible and store at 2-8°C.
Ensure that no calibration and control solution is trapped in the opened snap-cap. Because of possible evaporation effects, not more than 5 calibration/quality control procedures per bottle set should be performed.

MODULAR ANALYTICS E170 and **cobas e** 601 analyzers: Unless the entire volume is necessary for calibration and quality control on the analyzer, transfer aliquots of the ready-for-use calibrators and controls into empty snap-cap bottles (CalSet Vials/ControlSet Vials). Attach the supplied labels to these additional bottles. Store the aliquots for later use at 2-8°C. Perform **only one** calibration or control procedure per aliquot.
All information required for correct operation is read in via the respective reagent barcodes.

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Storage and stability

Store at 2-8°C.

Store the Elecsys Anti-TPO reagent kit (M, R1, R2) **upright** in order to ensure complete availability of the microparticles during automatic mixing prior to use.

Stability:

unopened at 2-8°C:	up to the stated expiration date
after opening at 2-8°C:	6 weeks
on MODULAR ANALYTICS E170 and **cobas e** 601:	2 weeks
on Elecsys 2010 and **cobas e** 411:	2 weeks
on Elecsys 1010:	1 week (stored alternately in the refrigerator and on the analyzer - ambient temperature 20-25°C; up to 20 hours opened in total)
Cal1, Cal2 after opening:	6 weeks at 2-8°C
PC A-TPO1, PC A-TPO2 after opening:	6 weeks at 2-8°C
on Elecsys 1010/2010 and **cobas e** 411 at 20-25°C:	up to 5 hours
on MODULAR ANALYTICS E170 and **cobas e** 601:	use only once

Store the calibrators and controls **upright**! Do not freeze! Ensure that no calibration solution is trapped in the opened snap-cap.

Specimen collection and preparation

Only the specimens listed below were tested and found acceptable.

Serum collected using standard sampling tubes or tubes containing separating gel.

Li-, Na-, NH_4^+-heparin, K_3-EDTA, sodium citrate, and sodium fluoride/potassium oxalate plasma.

Criterion: Recovery within 90-110% of serum value or slope 0.9-1.1 + intercept within <± 2 x analytical sensitivity (LDL) + coefficient of correlation > 0.95.

Stable for 3 days at 2-8°C, at least 1 month at -20°C. Freeze only once.[9]

The sample types listed were tested with a selection of sample collection tubes that were commercially available at the time of testing, i.e. not all available tubes of all manufacturers were tested. Sample collection systems from various manufacturers may contain differing materials which could affect the test results in some cases. When processing samples in primary tubes (sample collection systems), follow the instructions of the tube manufacturer.

Centrifuge samples containing precipitates before performing the assay. Do not use heat-inactivated samples. Do not use samples and controls stabilized with azide.

Ensure the patients' samples, calibrators, and controls are at ambient temperature (20-25°C) before measurement.

Because of possible evaporation effects, samples, calibrators, and controls on the analyzers should be measured within 2 hours.

Materials provided

See "Reagents - working solutions" section for reagents.

- 2 barcode cards
- control barcode sheet
- 4 x 4 bottle labels

Materials required (but not provided)

- Cat. No. 11776576, CalSet Vials, 2 x 56 empty snap-cap bottles
- Cat. No. 03142949, ControlSet Vials, 2 x 56 empty snap-cap bottles
- General laboratory equipment
- Elecsys 1010/2010, MODULAR ANALYTICS E170 or **cobas e** analyzer

Accessories for Elecsys 1010/2010 and **cobas e** 411 analyzers:

- Cat. No. 11662988, ProCell, 6 x 380 mL system buffer
- Cat. No. 11662970, CleanCell, 6 x 380 mL measuring cell cleaning solution
- Cat. No. 11930346, Elecsys SysWash, 1 x 500 mL washwater additive
- Cat. No. 11933159, Adapter for SysClean
- Cat. No. 11706829, Elecsys 1010 AssayCup, 12 x 32 reaction vessels or Cat. No. 11706802, Elecsys 2010 AssayCup, 60 x 60 reaction vessels
- Cat. No. 11706799, Elecsys 2010 AssayTip, 30 x 120 pipette tips

Accessories for MODULAR ANALYTICS E170 and **cobas e** 601 analyzers:

- Cat. No. 04880340, ProCell M, 2 x 2 L system buffer
- Cat. No. 04880293, CleanCell M, 2 x 2 L measuring cell cleaning solution
- Cat. No. 12135027, CleanCell M, 1 x 2 L measuring cell cleaning solution (for USA)
- Cat. No. 03023141, PC/CC-Cups, 12 cups to prewarm ProCell M and CleanCell M before use
- Cat. No. 03005712, ProbeWash M, 12 x 70 mL cleaning solution for run finalization and rinsing during reagent change
- Cat. No. 12102137, AssayTip/AssayCup Combimagazine M, 48 magazines x 84 reaction vessels or pipette tips, waste bags
- Cat. No. 03023150, WasteLiner, waste bags
- Cat. No. 03027651, SysClean Adapter M

Accessories for all analyzers:

- Cat. No. 11298500, Elecsys SysClean, 5 x 100 mL system cleaning solution

Only available in the USA:

- Cat. No. 11820923, Elecsys Anti-TPO CalCheck, 3 concentration ranges

Assay

For optimum performance of the assay follow the directions given in this document for the analyzer concerned. Refer to the appropriate operator manual for analyzer-specific assay instructions.

Resuspension of the microparticles takes place automatically before use.

Read in the test-specific parameters via the reagent barcode. If in exceptional cases the barcode cannot be read, enter the 15-digit sequence of numbers.

MODULAR ANALYTICS E170, Elecsys 2010 and **cobas e** analyzers: Bring the cooled reagents (M, R1, R2) to approx. 20°C and place on the reagent disk (20°C) of the analyzer. Avoid the formation of foam. The system **automatically** regulates the temperature of the reagents and the opening/closing of the bottles.

Elecsys 1010 analyzer: Bring the cooled reagents (M, R1, R2) to approx. 20-25°C and place on the sample/reagent disk of the analyzer (ambient temperature 20-25°C). Avoid the formation of foam. **Open** bottle caps **manually** before use and **close manually** after use. Store at 2-8°C after use.

Place the Elecsys calibrators A-TPO Cal1 and Cal2 in the sample zone of the analyzer. Only keep open during calibration. All information necessary for calibration is encoded on the barcoded bottle labels and is read in automatically. After calibration has been performed, store Cal1 and Cal2 at 2-8°C or discard (MODULAR ANALYTICS E170 and **cobas e** 601 analyzers).

Analyze the Elecsys controls PC A-TPO1 and PC A-TPO2. The information on the barcoded label of the control serum bottle is read in automatically. After the control procedure has been performed, store the controls at 2-8°C or discard (MODULAR ANALYTICS E170 and **cobas e** 601 analyzers).

Calibration

Traceability: This method has been standardized against the NIBSC (National Institute for Biological Standards and Control) 66/387 Standard.

Every Elecsys Anti-TPO reagent set (M, R1, R2) has a barcoded label containing the specific information for calibration of the particular reagent lot. The predefined master curve is adapted to the analyzer by the use of Elecsys Anti-TPO Cal1 and Cal2.

Calibration frequency: Perform calibration on all analyzers as follows:

- with every reagent kit

Renewed calibration on all analyzers:

- daily: when using the same reagent kit on the analyzers (ambient temperature for Elecsys 1010 analyzer: 20-32°C)
- as required: e.g. quality control findings outside the specified limits

Quality control

For quality control, use Elecsys PreciControl A-TPO 1 and 2.

Other suitable control material can be used in addition.

Controls for the various concentration ranges should be run as single determinations at least once every 24 hours when the test is in use, once per reagent kit, and after every calibration. The control intervals and limits should be adapted to each laboratory's individual requirements. Values obtained should fall within the defined limits.

Each laboratory should establish corrective measures to be taken if values fall outside the limits.

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Note: When using two reagent kits with different lots in the same run, the controls will be measured with both reagent lots.

Calculation
The analyzer automatically calculates the analyte concentration of each sample (either in IU/mL or kIU/L).

Limitations - interference
The assay is unaffected by icterus (bilirubin < 1129 µmol/L or < 66 mg/dL), hemolysis (Hb < 0.93 mmol/L or < 1.5 g/dL), lipemia (triglycerides < 23.9 mmol/L or < 2100 mg/dL), and biotin < 40.9 nmol/L or < 10 ng/mL. Criterion: Recovery within ± 10% of initial value.
In patients receiving therapy with high biotin doses (i.e. > 5 mg/day), no sample should be taken until at least 8 hours after the last biotin administration.
No interference was observed from rheumatoid factors up to a concentration of 1500 IU/mL.
In vitro tests were performed on 23 commonly used pharmaceuticals. No interference with the assay was found.
The risk of interference from potential immunological interactions between test components and rare sera has been minimized by the inclusion of suitable additives.
In rare cases, interference due to extremely high titers of antibodies to streptavidin and ruthenium can occur.
The test contains additives which minimize these effects.
For diagnostic purposes, the results should always be assessed in conjunction with the patient's medical history, clinical examination and other findings.

Measuring range
5-600 IU/mL (defined by the lower detection limit and the maximum of the master curve). Values below the detection limit are reported as < 5 IU/mL. Values above the measuring range are reported as > 600 IU/mL.

Dilution
Sample dilution is not possible. The autoantibodies are heterogeneous and this gives rise to non-linear dilution phenomena.

Expected values
Extended studies with the Elecsys Anti-TPO assay performed on samples from 208 healthy test subjects in 3 clinical centers in Austria and Germany showed a borderline value of 34 IU/mL for 95% of the results.

For detailed information about reference intervals in children, adolescents and pregnant women, refer to the brochure "Reference Intervals for Children and Adults", Cat. No. English: 04640292, German: 04625889.
This booklet also contains results of a detailed study about influencing factors on thyroid parameters in a well characterized reference group of adults. Different inclusion and exclusion criteria were applied (e.g. sonographic results (thyroid volume and density) as well as criteria according to the guidelines of the National Academy of Clinical Biochemistry - NACB).

Each laboratory should investigate the transferability of the expected values to its own patient population and if necessary determine its own reference ranges.

Specific performance data
Representative performance data on the analyzers are given below. Results obtained in individual laboratories may differ.

Precision
Reproducibility was determined using Elecsys reagents, pooled human sera, and controls (within-run: n = 21, between-run: n = 21); total precision on MODULAR ANALYTICS E170 analyzer was determined in a modified protocol (EP5-A) of the NCCLS (National Committee for Clinical Laboratory Standards): 6 times daily for 10 days (n = 60). The following results were obtained:

Elecsys 1010/2010 and **cobas e** 411 analyzers						
	Within-run precision			**Between-run precision**		
Sample	Mean IU/mL	SD IU/mL	CV %	Mean IU/mL	SD IU/mL	CV %
Human serum 1	15.3	1.07	7.0	12.4	3.02	24.4
Human serum 2	113	2.88	2.5	109	10.1	9.2
Human serum 3	269	11.4	4.2	308	21.9	7.1
PC A-TPO1	25.9	1.45	5.6	25.4	3.35	13.2
PC A-TPO2	112	3.78	3.4	116	8.83	7.6

MODULAR ANALYTICS E170 and **cobas e** 601 analyzers						
	Within-run precision			**Total precision**		
Sample	Mean IU/mL	SD IU/mL	CV %	Mean IU/mL	SD IU/mL	CV %
Human serum 1	21.3	1.34	6.3	20.8	1.97	9.5
Human serum 2	51.2	2.61	5.1	53.1	3.25	6.1
Human serum 3	473	12.7	2.7	455	19.1	4.2
PC A-TPO1	19.4	1.07	5.5	22.0	2.02	9.2
PC A-TPO2	100	2.57	2.6	103	4.39	4.3

Analytical sensitivity (lower detection limit)
< 5.0 IU/mL
The detection limit represents the lowest analyte level that can be distinguished from zero. It is calculated as the value lying two standard deviations above that of the lowest standard (master calibrator, standard 1 + 2 SD, within-run precision, n = 21).

Method comparison
A comparison of the Elecsys Anti-TPO assay (y) with a commercially available anti-TPO test (x) using clinical samples gave the following correlations:
Number of samples measured: 50

Passing/Bablok[10]	Linear regression
y = 0.77x + 2.94	y = 0.63x + 17.1
τ = 0.785	r = 0.899

The sample concentrations were between approx. 12 and 460 IU/mL.

Analytical specificity
No influence with human autoantibodies to thyroglobulin (< 394 IU/mL) was detectable.

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Anti-TPO

Antibodies to thyroid peroxidase

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For further information, please refer to the appropriate operator's manual for the analyzer concerned, the respective application sheets, the product information, and the package inserts of all necessary components.

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