1. Introduction

During pregnancy, several important physiological changes occur, maternal thyroid hormones (TH) play a vital role in the development and function of both the fetus and the placenta. Thyroid gland volume usually enlarges during pregnancy, and TH synthesis increases about 50% above the preconception level. These changes are in response to several factors,*(Glinoer.et al,2007).*

The normal pattern of human chorionic gonadotropin (hCG) secretion during pregnancy demonstrates a major increase during the first trimester and a plateau during mid-gestation, where it persists until shortly after delivery,*(Glinoer.et al,2007).* HCG has a much researched thyroid-stimulating hormone (TSH)-like activity secondary to specificity crossover at the TSH receptor (TSHR). As a result, serum thyroxine (T4) and triiodothyronine (T3) levels are elevated, whereas a serum TSH level is reduced. Pregnancy-related hyperestrogenism induces a 100% rise in serum thyroxine-binding globulin (TBG) as a result of changes in TBG half-life secondary to altered glycosylation. As a consequence, by week 10 of gestation, total T4 and T3 serum concentrations increased and plateau at this level until delivery*(Ain et al,2006),* other physiological adjustments also increase TH synthesis, such as elevation in the mater

nal glomerular filtration rate (GFR) and transplacental passage of T4,*(Ain et al,2006).*

There is significant overlap between the symptoms experienced by normal euthyroid pregnant women and those with thyroid dysfunction clinical diagnosis is not always straightforward. Because thyroid physiology is altered in pregnancy it has become clear during the past decade that normative gestational reference ranges for thyroid hormone analyses are necessary. Most clinical laboratory reports only provide non-pregnant reference intervals for the interpretation of laboratory results the range of normal serum total T4 is modified during pregnancy under the influence of a rapid increase in serum TBG levels. The TBG plateau is reached at mid-gestation. If one uses total T4

to estimate thyroid function, therefore the non pregnant reference range (5-12 µg/dl; 50-150 nmol/L) can be multiplied t by 1.5 during pregnancy. However, it should be noted that since total T4 values only reach a plateau around mid-gestation, such adaptation is only fully valid during the 2nd half of gestation. Thus, the use of total T4 does not provide an accurate estimate of thyroid function during early gestation. However, the free thyroxine index appears to be a reliable assay during pregnancy *(Ain et al,2006)*.

The prevalence of Autoimmune Thyroid Disease (AITD) in the pregnant population is comparable to that found in the general female population with a similar age range, between 5-15% ,*(Vanderpump, et al,2009)*. Careful study of women with thyroid antibodies during pregnancy has shown that despite the expected decrease in antibody titers during gestation, thyroid function gradually deteriorated towards hypothyroidism in a significant fraction of such women .In the 1st trimester, serum TSH (albeit within the normal range) was already significantly shifted to higher values in women with AITD, compared with normal pregnant controls. Serum TSH remained higher throughout gestation and at parturition 40% of AITD-positive women had a serum TSH >3mU/L, with almost one-half of them above 4 mU/L. Thus, while women with AITD were able to maintain a normal thyroid function in early gestation (due to sustained thyrotropic stimulation), their mean serum free T4 levels were significantly reduced to (or below) the lower limit of the normal reference range at delivery. Average reduction in serum free T4 reached 30% and almost one half of these women had free T4 values in the hypothyroid range by the time of delivery, confirming that these women have a reduced functional thyroid reserve. The risk of progression to hypothyroidism could be predicted from serum TSH levels and TPO-Ab titers measured in early pregnancy. When serum TSH was already above 2.5 mU/L and/or TPO-Ab titers above 1.250 U/mL before 20 weeks, these markers were predictive for the development of hypothyroidism by the end of pregnancy. Practical use of these markers in early gestation can therefore identify those women who carry the highest risk. Preventive thyroxine treatment administered to avoid the potential

deleteriouseffects of hypothyroxinemia and possibly thyroid antibodies on both maternal and fetal outcomes may then be considered. . There is also evidence from retrospective and some prospective studies that positive thyroid antibodies impacts adversely upon the course of pregnancy in several ways.*(Mariotti, et al, 2008).*

Ovulatory dysfunction is common in hypothyroidism, consequently, hypothyroid women have difficulty conceiving.(NegroR.et al,2010) Overall, the prevalence of hypothyroidism during pregnancy is 2.5%, the majority being subclinical cases.(Mandel SJ,2008)Transiently high serum TSH levels in the first trimester can also occur, however, probably related to iodine deficiency.The most common cause of hypothyroidism in women of reproductive age, in the absence of iodine deficiency, is Autoimmune Thyroid Disease (AITD). A history of past total or subtotal thyroidectomy, radioiodine ablation, or transient thyroiditis accounts for most of the remaining cases of hypothyroidism in the pregnant population,(Mandel SJ,2008).

In some studies, preterm delivery has been found to be 3-fold more common in hypothyroid pregnant women and has also been associated with an increase in spontaneous abortions,*(Casey. et al,2005).*although no adverse outcomes were found in a very large study of mild (subclinical) disease. Another worrying danger associated with maternal hypothyroxinemia (especially when present during the first trimester) is the adverse consequences to child neuropsychointellectual development, as has been demonstrated by several studies.*(Lazarus JH,2006 ,Auso. et al,2008)* Whether very mild hypothyroidism has the same associations remains uncertain.*(Lazarus JH,2006*)

Assessment of hyperthyroidism, laboratory evaluation of hypothyroidism should be made using TSH and an assessment of free hormone values, Total T4 and T3 measurements should be considered unreliable because of the increase in TBG concentrations. Anti-TPO antibodies and anti-thyroglobulin antibodies are increased in most patients with Hashimoto

thyroiditis and therefore may be useful in establishing this diagnosis. It is important to note that the natural course of Hashimoto thyroiditis is altered in pregnancy, with amelioration in the second half of pregnancy and aggravation in the postpartum period.. In addition, pregnant women who are on thyroid replacement therapy require larger doses compared with non-pregnant patients because of increases in the TBG concentration and increased type III deiodinases from the placenta. TSH should be monitored closely, and the dose of thyroid replacement should be adjusted to maintain TSH in the reference interval. Doses of thyroid replacement therapy can be lowered to pre-pregnancy levels at parturition,*(Lazarus ,2006).*

Adequate iodine supplementation is crucial to prevent maternal hypothyroxinemia during pregnancy,(Lazarus,2006). Fertile women with normally functioning thyroid glands should have an average iodine intake of 150 μg/day. During pregnancy and breastfeeding, women should increase their daily iodine intake to 250 μg dailyLT4 therapy is needed if, despite the iodine supplementation, abnormal serum TSH levels are detected,(Becker et al,2006,, Morreale de Escobar et al ,2011).

The incidence of hyperthyroidism in pregnant women has been estimated at 0.2%.(MestmanJ,et al,2005) Most women have symptoms before pregnancy, but some will demonstrate symptoms for the first time during pregnancy. The most common cause is Graves disease, which accounts for85–90% of all cases.*(Mestman , Goodwin ,et al,2005)* Diagnosis of hyperthyroidism during pregnancy is important because untreated or poorly treated hyperthyroidism can lead to adverse obstetrical outcomes. These include first-trimester spontaneous abortions, high rates of still births and neonatal deaths, two- to threefold increases in the frequency of low birth weight infants, preterm delivery, fetal or neonatal hyperthyroidism, and intrauterine growth retardation. Diagnosis of Graves disease can be difficult because healthy pregnant women may exhibit tachycardia, palpitations, mild heat intolerance, emotional lability, diaphoresis, and warm, moist skin. For these reasons, diagnosis of

hyperthyroidism during pregnancy needs to be made on careful clinical observations and well-conceived laboratory testing,(Mestman,et al,2005).

Thyroid anti-microsomal antibodies (also known as thyroid peroxidase antibodies or TPO antibodies) are increased in most (80–90%) patients with Hashimoto disease, and thyroid hormone receptor antibodies(TRAbs) are increased in about 80% or more of patients,(Wilson et al,2009,Skjoldebrand et al,2009).

Therefore, measurement of these antibodies can be useful in establishing a diagnosis of Hashimoto disease. Although the presence of TPO antibodies favors a diagnosis of autoimmune hypothyroidism over other etiologies, the presence of TRAbs is more specific for Graves disease. In addition, the TRAbs have prognostic implications for fetal and neonatal hyperthyroidism,TRAbs can cross the placenta and, at high enough concentrations,can bind to TSH receptors and stimulate the fetal thyroid. High titers of TRAbs in maternal serum during the third trimester are predictive of fetal or neonatal dysfunction. Therefore, it has been suggested that TRAbs be measured early in pregnancy and again in the last trimester. Values >500% of baseline are considered high and are a predictor of fetal or neonatal disease.demonstrated that women with anti-TPO antibodies or anti-thyroglobulin antibodies were fourfold more likely to have spontaneous abortions than healthy controls (13.3% vs 3.3%),(SkjoldebrandL,et al,2009).

The proven or presumed importance of subclinical thyroid dysfunction will have a major effect on the extent to which thyroid function testing is applied in any population. The term ‘subclinical’ is used when the serum concentration of TSH is persistently abnormal, while the concentrations of T4 and T3 remain within their reference intervals. Because results can fluctuate spontaneously, a new diagnosis of subclinical thyroid dysfunction is not warranted on the basis of a single laboratory sample. The five criteria define endogenous subclinical thyroid dysfunction is TSH increased above, or decreased below designated

limits , Normal free T4 concentration (and free T3 for hyperthyroidism), The abnormality is not due to medication , There is no concurrent critical illness or pituitary dysfunction and A sustained abnormality is demonstrated over 3-6 months.

Apart from the situation of impending or early pregnancy, where there is clear consensus that subclinical hypothyroidism should be promptly and fully treated, the approach to subclinical thyroid dysfunction remains uncertain. Various authorities express divergent views on the importance of detecting the mild TSH abnormalities that reflect subclinical thyroid dysfunction. Both hypothyroidism and hyperthyroidism, are disorders that need to be treated in order to avert potential harm. To achieve optimal sensitivity, particularly for the diagnosis of hypothyroidism, some have advocated that the upper limit of the TSH reference interval should be lowered ,*(Gilbert,2008).*because values in the range 2-4 mU/l, usually regarded as normal, are associated with an increased prevalence of future hypothyroidism ,*(Vulsma,et al,2010).*

Active search for subclinical thyroid dysfunction is based on the view that +treatment is usually justified, because of potential adverse outcome, even if proof of benefit is still lacking. At this end of the opinion spectrum, there is support for general community screening for thyroid dysfunction *(AnckaertE,et al,2010)*

Others have taken the view that while there is circumstantial evidence that subclinical thyroid dysfunction can have adverse long-term effects, there is currently a lack of strong evidence that treatment confers benefit *(Gong, Y,et al2008,Marwaha.et al,2008).*

A definitive position on this dilemma may emerge from long-term studies of outcome, but if differences are small, studies may be under-powered and the results may be indeterminate. Other factors to be taken into account in establishing an approach to widespread thyroid testing include ethnic or environmental predisposition to thyroid dysfunction in various communities, balance with other healthcare priorities that may be more compelling, cost of laboratory testing and the extent to which competent clinical assessment and

therapeutic response may be overwhelmed by reliance on laboratory measurements. The special issues that need to be considered in establishing a strategy for the testing of thyroid function before, during and after pregnancy are considered,*(Gilbert, et al, 2008).*

1.2 Rational

In some studies, preterm delivery has been found to be more common in hypothyroid pregnant women and has also been associated with an increase in spontaneous abortions, although no adverse outcomes were found in a very large study of mild (subclinical) disease(SkjoldebrandL,et al,2009).. Another worrying danger associated with maternal hypothyroxinemia (especially when present during the first trimester) is the adverse consequences to child neuropsychointellectual development, as has been demonstrated by several studies,*(Lazarus,2006,Glinoer,2007, Auso.et al,2008, LeBeauet al,2010, Negro et al,2010).* Whether very mild hypothyroidism has the same associations remains uncertain,*(Lazarus,2006, Glinoer,2007, Auso. et al,2008).*

Uncontrolled hyperthyroidism is associated with serious maternal, fetal, and neonatal morbidity, and mortality. Maternal complications include miscarriage, pregnancy-induced hypertension, preterm labour, placental abruption, congestive heart failure, and thyroid storm. Fetal and neonatal complications include stillbirth, low birth weight, goiter, hyperthyroidism, and hypothyroidism these risks can be decreased with the appropriate treatment of maternal hyperthyroidism,*(Ain. Et al,2009, Skjoldebrand.L et al,2009).*

In Sudan there is no data about frequency of anti-TPO among pregnant women.

1.3 Objectives

1.3.1 General objective

This study aims to assess thyroid functions during pregnancy by measuring of Thyroid hormones and anti-thyroid peroxidase antibodies among Sudanese pregnant women.

1.3.2 Specific objectives

1. To measureFT4, FT3and TSH in serum of pregnant women compared to healthy non –pregnant women.
2. To determine anti Thyroid peroxidase antibodies (anti-TPOAbs) in pregnant women compared to healthy non –pregnant women.
3. To get the frequency of anti-TPO Abs and abnormal thyroid function among the pregnant women.
4. To correlate the TPO antibodies to age of pregnant women.
5. To assess the effect of family history of thyroid disease in pregnant women on the level of TSH , FT3, FT4 and anti TPO antibodies.
6. To compare the level of TSH , FT3, FT4 and anti TPO antibodies in pregnant women with and without abortion.

1.4 Literature review

1.4.1 Pregnancy

Pregnancy is a dynamic, anabolic state. Within several weeks of conception, a new endocrine organ, the placenta, is already formed and is secreting hormones that affect the metabolism of all nutrients. These adjustments in nutrient metabolism, in addition to changes in the anatomy and physiology of the mother, support fetal growth and development while maintaining maternal homeostasis and preparing for lactation. Depending on the nutrient, one or more of the following adjustments occur like accretion in new tissue or deposition in maternal stores, redistribution among tissues, and increased turnover or rate of metabolism. To support these adjustments, the use of nutrients from the diet may be altered either by increasing intestinal absorption or by reducing excretion via the kidney or gastrointestinal tract. These adjustments in nutrient metabolism are complex and evolve continuously throughout pregnancy. The changes in nutrient metabolism can be described by several general concepts: adjustments in nutrient metabolism are driven by hormonal changes, fetal demands, and maternal nutrient supply; more than one potential adjustment exists for each nutrient; maternal behavioral changes augment physiologic adjustments; and a limit exists in the physiologic capacity to adjust nutrient metabolism to meet pregnancy needs, which when exceeded, fetal growth and development are impaired,*(Hytten, et al, 2008),*

1.4.2 Hormonal changes during pregnancy

Pregnancy is a continuum of small physiologic adjustments, the changes are often grouped by period of gestation, the first and last halves, the 3 trimesters, or the 4 quarters of pregnancy. provide an excellent summary of the physiologic adjustments in pregnancy in their book, Clinical Physiology in Obstetrics. The first half of pregnancy is primarily a time of preparation for the demands of rapid fetal growth that occur later in pregnancy. The corpus luteumand the placenta secrete hormones that maintain pregnancy and influence metabolism. Human chorionic gonadotropin is detected in the serum

and urine within a few days of implantation. Serum concentrations increase rapidly during early pregnancy to a peak occurring 60 days postconception. Thereafter, serum concentrations decrease as quickly as they increased until a relatively low serum concentration is reached and maintained to term. Human chorionic gonadotropin maintains the corpus luteum in early pregnancy; it has few known effects on non-reproductivetissues.Human placental lactogen increases progressively throughout pregnancy. The precise function of human placental lactogen is not clear; however, because it is biologically similar to growth hormone, it may represent some type of growth factor for the fetus and the placenta. Serum concentrations correlate with placental mass. Human placental lactogen also affects carbohydrate and lipid metabolism,*(Denne, et al,2007).*

1.4.3The Endocrine System and glands

The endocrine system is a control system of ductless glands that secrete hormones within specific organs. Hormones act as "messengers," and are carried by the bloodstream to different cells in the body, which interpret these messages and act on them.It seems like a far fetched idea that a small chemical can enter the bloodstream and cause an action at a distant location in the body. Yet this occurs in our bodies' everyday of our lives. The ability to maintain homeostasis and respond to stimuli is largely due to hormones secreted within the body. Without hormones, you could not grow, maintain a constant temperature, produce offspring, or perform the basic actions and functions that are essential for life.The endocrine system provides an electrochemical connection from the hypothalamus of the brain to all the organs that control the body metabolism, growth and development, and reproduction. There are two types of hormonessecreted in the endocrine system are Steroidal and non-steroidal, (or protein based) hormones. The endocrine system regulates itshormones through negative feedback, except in very specific cases like childbirth. Increases in hormone activity decrease the production of that

hormone. The immune system and other factors contribute as control factors also, altogether maintaining constant levels of hormones,*(Endo, et al, 2007).*

Glands which have no duct and release their secretions directly into the intercellular fluid or into the blood. The collection of endocrine glands makes up the endocrine system,*(Kaushansky,2009).*

The main endocrine glands are the pituitary (anterior and posterior lobes), thyroid, parathyroid, adrenal (cortex and medulla), pancreas and gonads.The pituitary gland is attached to the hypothalamus of the lower forebrain.The thyroid gland consists of two lateral masses, connected by a cross bridge, that are attached to the trachea. They are slightly inferior to the larynx. The parathyroid glands are four masses of tissue, two embedded posterior in each lateral mass of the thyroid gland. One adrenal gland is located on top of each kidney. The cortex is the outer layer of the adrenal gland. The medulla is the inner core. The pancreas is along the lower curvature of the stomach, close to where it meets the first region of the small intestine, the duodenum*),(Walter,et al,2008).*

1.4.4Classification of Hormones

A hormone is a chemical messenger produced by a cell that effects specific change in the cellular activity of other cells (target cells). Unlike exocrine glands (which produce substances such as saliva, milk, stomach acid and digestive enzymes), endocrine glands do not secrete substances into ducts (tubes). Instead, endocrine glands secrete their hormones directly into the surrounding extra cellular space. The hormones then diffuse into nearby capillaries and are transported throughout the body in the blood,*(Endo,et al, 2007).*

The hormonescan be chemically classified into four groups are:

Amino acid-derivedhormones that are modified amino acids.*(Walter.et al ,2008).*

Polypeptide and proteinshormones that are chains of amino acids of less than or more than about 100 amino acids, respectively. Some protein hormones are actually glycoproteins, containing glucose or other carbohydrate groups.*(Walter.et al ,2008).*

Steroidshormones that are lipids synthesized from cholesterol. Steroids are characterized by four interlocking carbohydrate rings,*(Walter.et al ,2008).*

Eicosanoidsare lipids synthesized from the fatty acid chains of phospholipids found in plasma membrane,*(Walter.et al ,2008).*

Steroid hormones and hormones of the thyroid gland diffuse through the cell membranes of target cells. The lipid-soluble hormone then binds to a receptor protein that, in turn, activates a DNA segment that turns on specific genes. The proteins produced as result of the transcription of the genes and subsequent translation of mRNA act as enzymes that regulate specific physiological cell activity, while (polypeptide, protein, and most amino acid hormones) bind to a receptor protein on the plasma membrane of the cell. *(Endo,et al 2005)*

1.4.5Thyroid gland

The Thyroid gland is one of the largest endocrine glands in the body. It is located on the neck just below the Larynx and has two lobes with one on either side of the trachea. It is involved in the production of the hormones T3 (triiodothyronine) and T4 (thyroxine). These hormones increase the metabolic activity of the body‘s cells. The thyroid also produces and releases the hormone calcitonin (thyrocalcitonin) which contributes to the regulation of blood calcium levels. Thyrocalcitonin or calcitonin decreases the concentration of calcium in the blood. Most of the calcium removed from the blood is stored in the bones.The thyroid hormone consists of two components, thyroxine and

iodine. This hormone increases the metabolism of most body cells. A deficiency of iodine in the diet leads to the enlargement of the thyroid gland, known as a simple goiter. Hypothyroidism during early development leads to cretinism. In adults, it produces myxedema, characterized by obesity and lethargy. Hyperthyroidism leads to a condition known as exophthalmic goiter, characterized by weight loss as well as hyperactive and irritable behavior.*(Gharib, et al ,2004, Ain, et al,2006)*

1.4.6Thyroid hormones synthesis and function

The synthesis and storage of thyroid hormones occurs between the follicular cells and the colloid. Different follicles may be in different states of activity. Less active follicles contain cells with a more cuboidal appearance, whilst the active follicles contain columnar cells.The process of thyroid hormone synthesis is complex. Once inside the follicular cell, iodide is oxidized to active iodine by hydrogen peroxide. This reaction is catalyzed by the heme-containing enzyme thyroid peroxidase (TPO). Iodine is then actively transported across the apical surface of the follicular cell by the same active process that occurs at the basal surface,*(Ain. et al,2010).*

At the apical-colloid interface, iodine is immediately incorporated into the tyrosine residues of the large glycoprotein thyroglobulin molecules. Thyroglobulin is synthesized in the follicular cells and has a molecular weight of around 650 000 with about 140 tyrosine residues, depending on the form of thyroglobulin. Approximately one quarter of these residues can be iodinated. Once iodinated, thyroglobulin is taken up into the colloid of the follicle where, still incorporated in the protein, a coupling reaction between pairs of iodinated tyrosine molecules occurs. The coupling of two tyrosine residues each iodinated at two positions (di-iodotyrosine, DIT) produces tetra-iodothyronine or thyroxine (T4) whilst the combination of DIT with mono-iodotyrosine (MIT) produces tri-iodothyronine (T3). Such coupling can occur within a single molecule of thyroglobulin or between dimerized molecules of the

protein. This coupling is catalyzed by TPO. Thyroid hormones are stored in this state and are only released when the thyroglobulin molecule is taken back up into the follicular cells. Stimulated by TSH, thyroglobulin droplets are captured by the follicular cells by a process of pinocytosis. Fusion of the droplets with lysosomes results in hydrolysis of the thyroglobulin molecules and release of T4 and T3. About 10% of T4 undergoes mono-deiodination to T3 before it is secreted and the released iodine is recycled,*(Glinoer,2008,Auso. et al,2007,Ain,et al,2010).*

As blood flows through the gland, iodide is converted to an active form of iodine. This iodine combines with an amino acid called tyrosine. Two molecules of iodinated tyrosine then combine to form thryroxine. Following its formation, the thyroxine becomes bound to a polysaccharide-protein material called thyroglobulin. The normal thyroid gland may store several weeks supply of thyroxine in this bound form. An enzymatic splitting of the thyroxine from the thyroglobulin occurs when a specific hormone is released into the blood. This hormone, produced by the pituitary gland, is known as thyroid-stimulating hormone (TSH). TSH stimulates certain major rate-limiting steps in thyroxine secretion, and thereby alters its rate of release. A variety of bodily defects, either dietary, hereditary, or disease induced, may decrease the amount of thyroxine released into the blood. The most popular of these defects is one that results from dietary iodine deficiency. The thyroid gland enlarges, in the continued presence of TSH from the pituitary, to form a goiter. This is a futile attempt to synthesize thyroid hormones, for iodine levels that are too low. Normally, thyroid hormones act via a negative feedback loop on the pituitary to decrease stimulation of the thyroid. In goiter, the feedback loop cannot be in operation - hence continual stimulation of the thyroid and the inevitable protuberance on the neck. Formerly, the principal source of iodine came from seafood. As a result, goiter was prevalent amongst inland areas far removed from the sea. Today, the incidence of goiter has been drastically reducedby adding iodine to table salt. Thyroxine serves to stimulate oxidative metabolism

in cells; it increases the oxygen consumption and heat production of most body tissues, a notable exception being the brain. Thyroxine is also necessary for normal growth. The most likely explanation being that thyroxine promotes the effects of growth hormone on protein synthesis. The absence of thyroxine significantly reduces the ability of growth hormone to stimulate amino acid uptake and RNA synthesis. Thyroxine also plays a crucial role in the closely related area of organ development, particularly that of the central nervous system,*(Ain,et al,2010).*

The Production of T3 and T4 are regulated by thyroid stimulating hormone (TSH), released by the pituitary gland. TSH Production is increased when T3 and T4 levels are too low. The thyroid hormones are released throughout the body to direct the body's metabolism. They stimulate all cells within the body to work at a better metabolic rate. Without these hormones the body's cells would not be able to regulate the speed at which they performed chemical actions. Their release will be increased under certain situations such as cold temperatures when a higher metabolism is needed to generate heat. When children are born with thyroid hormone deficiency, they have problems with physical growth and developmental problems. Brain development can also be severely impaired,*(Ain. Et al,2010).*

Calcitonin is a 32 amino acid polypeptide hormone. It is an additional hormone produced by the thyroid, and contributes to the regulation of blood calcium levels. Thyroid cells produce calcitonin in response to high calcium levels in the blood. This hormone will stimulate movement of calcium into the bone structure. It can be also used therapeutically for the treatment of hypercalcemia or osteoporosis. Without this hormonecalcium will stay within the blood instead of moving into bones to keep them strong and growing. Its importance in humans has not been as well established as its importance in other animals.*(Beato,et al ,2007).*

1.4.7 Regulation of thyroid hormones levels

TSH stimulates the thyroid gland to secrete the hormones thyroxine (T4) and triiodothyronine (T3).[3] TSH production is controlled by thyrotropin-releasing hormone (TRH), which is manufactured in the hypothalamus and transported to the anterior pituitary gland via the hypothalamo-hypophyseal portal system, where it increases TSH production and release. Somatostatinis also produced by the hypothalamus, and has an opposite effect on the pituitary production of TSH, decreasing or inhibiting its release. The level of thyroid hormones (T3 and T4) in the blood has an effect on the pituitary release of TSH; when the levels of T3 and T4 are low, the production of TSH is increased, and, on the converse, when levels of T3 and T4 are high, TSH production is decreased. This effect creates a regulatory negative feedback loop,*(Beato,et al ,2007, Auso.et al ,2007).*

1.4.8Autoantibody

An autoantibody is an antibody (a type of protein) manufactured by the immune system that is directed against one or more of the individual's own proteins. It is derived from the Greek "auto" which means "self", "anti" which means "against" and "body". Many autoimmune diseases, notably lupus erythematosus, are caused by such autoantibodies.*(Rose, et al,2009).*

1.4.9Production of autoantibody

Antibodies are produced in a process of evolution that is still a subject of scientific research. Briefly, antibodies are produced by B cells in two ways are randomly, or in response to a foreign protein or substance within the body. Initially, one B cell produces one specific kind of antibody. In either case, the B cell is allowed to proliferate or is killed off through a process called clonal deletion. Normally, the immune system is able to recognize and ignore the body's own healthy proteins, cells, and tissues, and to not overreact to non-

threatening substances in the environment, such as foods. Sometimes, however, the immune system ceases to recognize one or more of the body's normal constituents as "self," leading to production of pathological autoantibodies. These autoantibodies attack the body's own healthy cells, tissues, and/or organs, causing inflammation and damage. It should be noted that autoantibodies may also play a nonpathological role; for instance they may help the body to destroy cancers and to eliminate waste products. The role of autoantibodies in normal immune function is also a subject of scientific research.*(Rose, et al,2009).*

1.4.10Cause of autoantibody production

The causes of autoantibody production are varied and not well understood. It is thought that some autoantibody production is due to a genetic predisposition combined with an environmental trigger, such as a viral illness or a prolonged exposure to certain toxic chemicals. There is generally not a direct genetic link however. While families may be susceptible to autoimmune conditions, individual family members may have different autoimmune disorders, or may never develop an autoimmune condition. Researchers believe that there may also be a hormonal component as many of the autoimmune conditions are much more prevalent in women of childbearing age.*(Rose et al,2009).*

1.4.11Diseases of autoantibody

The type of autoimmune disorder or disease that occurs and the amount of destruction done to the body depends on which systems or organs are targeted by the autoantibodies, and how strongly. Disorders caused by organ specific autoantibodies, those that primarily target a single organ, such as the thyroid in Graves' disease and Hashimoto's thyroiditis, are often the easiest to diagnose as they frequently present with organ related symptoms. Disorders due to systemic autoantibodies can be much more elusive. Although the associated autoimmune disorders are rare, the signs and symptoms they cause are

relatively common. Symptoms may include arthritis-type joint pain, fatigue, fever, rashes, cold or allergy-type symptoms, weight loss, and muscular weakness. Associated conditions include vasculitis, which are inflammation of blood vessels and anemia. Even if they are due to a particular systemic autoimmune condition, the symptoms will vary from person to person, vary over time, vary with organ involvement, and they may taper off or flare unexpectedly. Add to this the fact that a person may have more than one autoantibody, and thus have more than one autoimmune disorder, and/or have an autoimmune disorder without a detectable level of an autoantibody, complicating making a diagnosis. The diagnosis of disorders associated with systemic autoantibodies starts with a complete medical history and a thorough physical exam. Based on your signs and symptoms, the doctor may request one or more diagnostic studies that will help to identify a specific disease. These studies include blood tests to detect inflammation, autoantibodies, and organ involvement, x-rays and other imaging scans to detect changes in bones, joints, and organs, biopsies to look for pathologic changes in tissue specimens.As a rule, information is required from multiple sources, rather than a single laboratory test to accurately diagnose disorders associated with systemic autoantibodies.*(Edwards, et al,2009,Rose, et al,2009).*

1.4.12Classification of autoantibody

Autoimmune diseases can be broadly divided into systemic and organ-specific or localized autoimmune disorders, depending on the principal clinico-pathologic features of each disease.Systemic autoimmune diseases include SLE, scleroderma, rheumatoid arthritis, and dermatomyositis. These conditions tend to be associated with autoantibodies to antigens which are not tissue specific. Thus although polymyositis is more or less tissue specific in presentation, it may be included in this group because the autoantigens are often ubiquitous tRNAsynthetases.Local syndromes which affect a specific organ or tissue like endocrinologicdiabetes mellitus type 1, Hashimoto's

thyroiditis and Addison's disease, Gastrointestinal like Coeliac disease, Crohn's disease, Pernicious anemia, Dermatologic pemphigus vulgarisvitiligo ,Haematologicautoimmunehemolyticanemia and idiopathic thrombocytopenic purpura, Neurologicalmyasthenia gravis,*(Edwards, et al,2009).*

1.4.13 Diagnosis of autoantibody

Diagnosis of autoimmune disorders largely rests on accurate history and physical examination of the patient, and high index of suspicion against a backdrop of certain abnormalities in routine laboratory tests (example, elevated C-reactive protein). In several systemic disorders, serological assays which can detect specific autoantibodies can be employed. Localized disorders are best diagnosed by immunofluorescence of biopsy specimens. Autoantibodies are used to diagnose many autoimmune diseases. The levels of autoantibodies are measured to determine the progress of the disease.*(Edwards,et al,2009).*

1.4.14 Anti-Thyroid Peroxidase Antibody anti (TPO)

Thyroid peroxidase or thyroperoxidase (TPO) is the enzyme involved in the biosynthesis of thyroid hormones and is an important autoantigen in autoimmune thyroid disease. Anti-TPO autoantibodies are measured to help diagnose and monitor autoimmune thyroid diseases such as Hashimoto's thyroditis, primary myxedema, Grave's disease and postpartum thyroditis. *(Chardès, et al, 2007, McLachlan Mopo, et al,2008).*

Antibodies against thyroid microsomal antigen are the primary marker of autoimmune thyroid disease. Microsomal antibody levels correlate with lymphocytic infiltration of the thyroid gland and thyroid damage. Historically, microsomal antigen has been technically a cellular membrane preparation that can also contain interfering factors such as thyroglobulin. Characterization of the microsomal antigen as TPO has allowed replacement of the classical qualitative assay with a quantitative anti-TPO autoantibody assay that has

better precision, sensitivity and specificity. This improvement has obviated the need for simultaneous determination of anti-thyroglobulin antibodies in many patients. However, anti-thyroglobulin is still recommended as an adjunct for thyroglobulin measurements and in the prediction of postpartum thyroditis.*(Kessler, et al, 2008).*

The anti-TPO autoantibody assay is most sensitive for detecting Hashimoto's thyroiditis and idiopathic myxedema (sensitivity = 93%) where antibody levels are typically greater than 1000 IU/ml. Patients with Graves' disease are frequently positive (sensitivity = 73%) and often have lower levels of antibodies. Treatment of autoimmune thyroid disease is characterized by a transient increase in antibody levels, followed by a low decline. *(Chardès, et al, 2007).*

1.4.15Stimulation and inhibition of Anti TPO

TPO is stimulated by TSH, which up regulates gene expression. It is inhibited by the thioamide drugs, such as propylthiouracil and methimazole. *(McLachlan, Rapoport,2008).*

1.4.16Clinical significanceof Anti TPO

Thyroid peroxidase is a frequent epitope of autoantibodies in autoimmune thyroid disease, with such antibodies being called anti-thyroid peroxidase antibodies (anti-TPO antibodies). This is most commonly associated with Hashimoto's thyroiditis). Thus, an antibody titercan be used to assess disease activity in patients that have developed such antibodies. *(Kessler, et al, 2008, Edwards, et al ,2010).*

1.4.17Hypothyroidism

It's a condition in which the thyroid gland does not make enough thyroid hormone. (A deficiency of thyroid hormone).Iodine deficiency is the most

common cause of hypothyroidism worldwide but it can be caused by other causes such as several conditions of the thyroid gland or, less commonly, the pituitary gland or hypothalamus. It can result from a lack of a thyroid gland or from iodine-131 treatment, and can also be associatedwith increased stress.Severe hypothyroidism in infants can result in cretinism.*(Simon ,2009).*

1.4.18 Classification of hypothyroidism

Hypothyroidism is often classified by association with the indicated organ dysfunction. *(Simon ,2009).*

Primary it most common forms when the defect from thyroid gland include Hashimoto's thyroiditis (an autoimmune disease) and radioiodine therapy for hyperthyroidism. *(Simon ,2009).*

Secondary when the defect source is Pituitary gland, Occurs if the pituitary gland does not create enough thyroid-stimulating hormone (TSH) to induce the thyroid gland to produce enough thyroxine and triiodothyronine. Although not every case of secondary hypothyroidism has a clear-cut cause, it is usually caused by damage to the pituitary gland, as by a tumor, radiation, or surgery. Secondary hypothyroidism accounts for less than 5% or 10% of hypothyroidism cases.*(Simon ,2009).*

Tertiary if the defect source is hypothalamus gland, Results when the hypothalamus 8fails to produce sufficient thyrotropin-releasing hormone (TRH). TRH prompts the pituitary gland to produce thyroid-stimulating hormone (TSH). Hence may also be termed hypothalamic-pituitary-axis hypothyroidism. It accounts for less than 5% of hypothyroidism cases.*(Simon ,2009).*

1.4.19 Subclinical hypothyroidism

Subclinical hypothyroidism occurs when thyrotropin (TSH) levels are elevated but thyroxine (T4) and triiodothyronine (T3) levels are normal. In primary hypothyroidism, TSH levels are high and T4 and T3 levels are low. TSH usually increases when T4 and T3 levels drop. TSH prompts the thyroid gland to make more hormones. In subclinical hypothyroidism, TSH is elevated but below the limit representing overt hypothyroidism. The levels of the active hormones will be within the laboratory reference ranges. Subclinical hypothyroidism in early pregnancy, compared with normal thyroid function, has been estimated to increase the risk of pre-eclampsia with an odds ratio (OR) of 1.7 and the risk of perinatal mortality with an OR of 2.7.*(Van Den et al, 2011).*

1.4.20 Signs and symptoms of hypothyroidism

Early hypothyroidism is often asymptomatic and can have very mild symptoms. Subclinical hypothyroidism is a state of normal thyroid hormone levels, thyroxine (T4) and triiodothyronine (T3), with mild elevation of thyrotropin, thyroid-stimulating hormone (TSH). With higher TSH levels and low free T4 levels, symptoms become more readily apparent in clinical (or overt) hypothyroidism. Hypothyroidism can be associated with the following symptoms.*(American Thyroid Association (ATA),2003).*

The early signs include poor muscle tone (muscle hypotonia),Fatigue ,Any form of menstrual irregularity and fertility problems ,Hyperprolactinemia and galactorrhea ,Elevated serum cholesterol ,Cold intolerance, increased sensitivity to cold ,Constipation ,Rapid thoughts, Depression, Muscle cramps and joint pain, Thin, brittle fingernails, Coarse hair, Paleness, Decreased sweating,Dry, itchy skin, Weight gain and water retention and bradycardia (low heart rate – fewer than sixty beats per minute).*(Yeum., et al,2008).*

The late signs include goiter, Slow speech and a hoarse, breaking voice – deepening of the voice can also be noticed, caused by Reinke's Edema, Dry puffy skin, especially on the face, Thinning of the outer third of the eyebrows, Abnormal menstrual cyclesand lowbasal body temperature, Thyroid-Related Depression.

1.4.21 Epidemiology of hypothyroidism

About three percent of the general population has hypothyroidism. A 1995 survey in the UK found the mean incidence (with 95% confidence intervals) of spontaneous hypothyroidism in women was 3.5/1000 survivors/year (2.8-4.5) rising to 4.1/1000 survivors/year (3.3-5.0) for all causes of hypothyroidism and in men was 0.6/1000 survivors/year (0.3-1.2*).(Vanderpump, MP; et al,2009)*

Estimates of subclinical hypothyroidism range between 3–8%, increasing with age; incidence is more common in women than in men.*(Fatourechi, , 2009).*

1.4.22 Causes of hypothyroidism

Iodine deficiency is the most common cause of hypothyroidism worldwide. In iodine-replete individuals hypothyroidism is frequently caused by Hashimoto's thyroiditis, or otherwise as a result of either an absent thyroid gland or a deficiency in stimulating hormones from the hypothalamus or pituitary.Factors such as iodine deficiency or exposure to iodine-131 from nuclear fallout, which is absorbed by the thyroid gland like regular iodide and destroys its cells, can increase the risk.Congenital hypothyroidism is very rare accounting for approximately 0.2‰ can have several causes such as thyroid aplasia or defects in the hormone metabolism. Thyroid hormone insensitivity (most often T3 receptor defect) also falls into this category although in this condition the levels of thyroid hormones may be normal or even markedly elevated.*(American Thyroid Association (ATA),2008).*

Hypothyroidism can result from postpartum thyroiditis, a condition that affects about 5% of all women within a year of giving birth.[citation needed] The first phase is typically hyperthyroidism; the thyroid then either returns to normal, or a woman develops hypothyroidism. Of those women who experience hypothyroidism associated with postpartum thyroiditis, one in five will develop permanent hypothyroidism requiring life-long treatment.Hypothyroidism can result from de Quervain's thyroiditis, which, in turn, is often caused by having a bad flu that enters and destroys part, or all, the thyroid. And itcan also result from sporadic inheritance, sometimes autosomal recessive. Hypothyroidism is also a relatively common disease in domestic dogs, with some specific breeds having a definite predisposition.*(Brooks ,2008).*

Temporary hypothyroidism can be due to the Wolff-Chaikoff effect. A very high intake of iodine can be used to temporarily treat hyperthyroidism, especially in an emergency situation. Although iodide is a substrate for thyroid hormones, high levels reduce iodide organification in the thyroid gland, decreasing hormone production. The antiarrhythmic agentamiodarone can cause hyper- or hypothyroidism due to its high iodine content.Hypothyroidism can be caused by lithium-based mood stabilizers, usually used to treat bipolar disorder (previously known as manic depression).(American Thyroid Association *(ATA,2008).*

In fact, lithium has occasionally been used to treat hyperthyroidism.*(Offermanns, et al ,2008).*

Other drugs that may produce hypothyroidism include interferon alpha, interleukin-2, and thalidomide.(American Thyroid Association *(ATA,2008)*

Stress also affects thyroid functioning through the sympathetic nervous system. Refugees from East Germany in a 1994 study who experienced chronic stress were found to have a very high rate of hypothyroidism or subclinical hypothyroidism, although not all refugees displayed clinical or behavioral

symptoms associated with this reduced thyroid functioning. TSH levels correlate positively with physiological stress.*(Peeters, ,2008).*

1.4.23 Laboratory Diagnosis of hypothyroidism

Main article isthyroid function tests,The only validated test to diagnose primary hypothyroidism, is to measure thyroid-stimulating hormone (TSH) and free thyroxine (T4).*(Allahabadia. et al,2009).*

However, these levels can be affected by non-thyroidal illnesses.High levels of TSH indicate that the thyroid is not producing sufficient levels of thyroid hormone (mainly as thyroxine (T4) and smaller amounts of triiodothyronine (T3). However, measuring just TSH fails to diagnose secondary and tertiary hypothyroidism, thus leading to the following suggested blood testing if the TSH is normal and hypothyroidism is still suspected like Anti-thyroidantibodiesfor evidence of autoimmune diseases that may be damaging the thyroid gland like antithyroid peroxidase antibody. Free triiodothyronine (fT3), Freelevothyroxine (fT4) and Total T3 and Total T4.Additionally, the following measurements may be needed like Free T3 from 24-hour urine catch, Serum cholesterol which may be elevated in hypothyroidism, Prolactin is a widely available test of pituitary function and Testing for anemia, including ferritin.*(Baisier, et al, 2008).*

Misdiagnosis is common in hypothyroidism, with many patterns of dysfunction failing to be identified by most laboratory tests: normal TSH, T3 and T4 levels are expected in many types of thyroid dysfunction, especially those associated with increased stress.*(Williams ,2006).*

1.4.24Hyperthyroidism

Is the term for overactive tissue within the thyroid gland causing an overproduction of thyroid hormones (thyroxine or "T4" and/or triiodothyronine or "T3").*(Floyd, J.L,2009)* Hyperthyroidism is thus a cause of thyrotoxicosis

the clinical condition of increased thyroid hormones in the blood. It is important to note that hyperthyroidism and thyrotoxicosis are not synonymous. For instance, thyrotoxicosis could instead be caused by ingestion of exogenous thyroid hormone or inflammation of the thyroid gland, causing it to release its stores of thyroid hormones.*(Floyd. ,2009).*

1.4.25 Causes of hyperthyroidism

There are several causes of hyperthyroidism. Most often, the entire gland is overproducing thyroid hormone. This is calledGraves' disease. Less commonly, a single nodule is responsible for the excess hormone secretion, called a "hot" nodule. Thyroiditis (inflammation of the thyroid) can also cause hyperthyroidism.*(Kittisupamongkol,2009).*

Functional thyroid tissue producing an excess of thyroid hormone occurs in a number of clinical conditions.The major causes in humans areGraves' disease an autoimmune disease (usually, the most common etiology with 50-80% worldwide, although this varies substantially with location .47% in Switzerland to 90% in the USA . due to varying Iodine in the diet.Toxic thyroid adenoma (the most common etiology in Switzerland, 53%, thought to be atypical due to a low level of dietary iodine in this country*)(Floyd,2009).*

Toxic multinodular goiter is a high blood levels of thyroid hormones (most accurately termed hyperthyroxinemia) can occur for a number of other reasons like inflammation of the thyroid was calledthyroiditis. There are several different kinds of thyroiditis including Hashimoto's thyroiditis (immune-mediated). These may be initially associated with secretion of excess thyroid hormone, but usually progress to gland dysfunction and, thus, to hormone deficiency and hypothyroidism.Oral consumption of excess thyroid hormone tablets is possible (surreptitious use of thyroid hormone), as is the rare event of consumption of ground beef contaminated with thyroid tissue, and thus thyroid hormone (termed "hamburger hyperthyroidism").Amiodarone, an anti-

arhythmic drug is structurally similar to thyroxine and may cause either under- or overactivity of the thyroid.Postpartum thyroiditis (PPT) occurs in about 7% of women during the year after they give birth. PPT typically has several phases, the first of which is hyperthyroidism. This form of hyperthyroidism usually corrects itself within weeks or months without the need for treatment.Hyper secretion of thyroid stimulating hormone (TSH), which in turn is almost always caused by a pituitary adenoma, accounts for much less than 1 percent of hyperthyroidism cases.*(EWeissand Samuel Refetoff,2009).*

1.4.26 Symptoms and signs of hyperthyroidism

Hyperthyroidism may be asymptomatic, Major clinical signs include weight loss (often accompanied by an increased appetite), anxiety, intolerance to heat, hair loss, muscle aches, weakness, fatigue, hyperactivity, irritability, hypoglycemia, apathy, polyuria, polydipsia, delirium, tremor, pretibial myxedema, and sweating. In addition, patients may present with a variety of symptoms such as palpitations and arrhythmias (the notable ones being atrial fibrillation), shortness of breath (dyspnea), loss of libido, amenorrhea, nausea, vomiting, diarrhea, gynaecomastia and feminization. Long term untreated hyperthyroidism can lead to osteoporosis. These classical symptoms may not be present often in the elderly.Neurological manifestations can include tremors, chorea, myopathy, and in some susceptible individuals (in particular of Asian descent) periodic paralysis. An association between thyroid disease and myasthenia gravishas been recognized. The thyroid disease, in this condition, is autoimmune in nature and approximately 5% of patients with myasthenia gravis also have hyperthyroidism. Myasthenia gravis rarely improves after thyroid treatment and the relationship between the two entities is not well understood. In Graves' disease, which is the most common form or cause of hyperthyroidism, the eyes may look enlarged because the eye muscles swell and push the eye forward. This can only be resolved surgically by orbital decompression. Sometimes, one or both eyes may bulge. Some patients have

swelling of the front of the neck from an enlarged thyroid gland (a goiter). Because hyperthyroidism, especially Graves' disease, may run in families, examinations of the members of a family may reveal other individuals with thyroid problems. *(EWeissand Samuel Refetoff,2009).*

Minor ocular (eye) signs, which may be present in any type of hyperthyroidism, are eyelid retraction ("stare"), extra-ocular muscle weakness, and lid-lag. In hyperthyroid stare (Dalrymple sign) the eyelids are retracted upward more than normal (the normal position is at the superior corneosclerallimbus, where the "white" of the eye begins at the upper border of the iris). Extra-ocular muscle weakness may present with double vision. In lid-lag (von Graefe's sign), when the patient tracks an object downward with their eyes, the eyelid fails to follow the downward moving iris, and the same type of upper globe exposure which is seen with lid retraction occurs, temporarily. These signs disappear with treatment of the hyperthyroidism.Neither of these ocular signs should be confused with exophthalmos (protrusion of the eyeball), which occurs specifically and uniquely in hyperthyroidism caused by Graves' disease (not all exopthalmos is caused by Graves' disease, but when present with hyperthyroidism is diagnostic of Graves' disease). This forward protrusion of the eyes is due to immune-mediated inflammation in the retro-orbital (eye socket) fat. Exophthalmos, when present, may exacerbate hyperthyroid lid-lag and stare. *(Chan et al ,2009).*

1.4.27 LaboratoryDiagnosisof hyperthyroidism

Measuring the level of thyroid-stimulating hormone (TSH), produced by the pituitary gland (which in turn is also regulated by the hypothalamus's TSH Releasing Hormone) in the blood is typically the initial test for suspected hyperthyroidism. A low TSH level typically indicates that the pituitary gland is being inhibited or "instructed" by the brain to cut back on stimulating the thyroid gland, having sensed increased levels of T4 and/or T3 in the blood. In rare circumstances, a low TSH indicates primary failure of the pituitary, or

temporary inhibition of the pituitary due to another illness (euthyroid sick syndrome) and so checking the T4 and T3 is still clinically useful.Measuring specific antibodies, such as anti-TSH-receptor antibodies in Graves' disease, or anti-thyroid-peroxidase (TPO) in Hashimoto's thyroiditis a common cause of hypothyroidism may also contribute to the diagnosis.The diagnosis of hyperthyroidism is confirmed by blood tests that show a decreased thyroid-stimulating hormone (TSH) level and elevated T4 and T3 levels. TSH is a hormone made by the pituitary gland in the brain that tells the thyroid gland how much hormone to make. When there is too much thyroid hormone, the TSH will be low. A radioactive iodine uptake test and thyroid scan together characterizes or enables radiologists and doctors to determine the cause of hyperthyroidism. The uptake test uses radioactive iodine injected or taken orally on an empty stomach to measure the amount of iodine absorbed by the thyroid gland. Persons with hyperthyroidism absorb too much iodine. A thyroid scan producing images is typically conducted in connection with the uptake test to allow visual examination of the over-functioning gland. In addition to testing the TSH levels, many doctors test for T3, Free T3, T4, and/or Free T4 for more detailed results. Typical adult limits for these hormones are TSH0.45 - 4.50 uIU/mL, T4 Free/Direct (nanograms) is 0.82 - 1.77 ng/dl and T3 (nanograms): 71 - 180 ng/dl. Persons with hyperthyroidism can easily exhibit levels many times these upper limits for T4 and/or T3.*(ChanWB,et al,2009, Kittisupamongkol,2009).*

Chapter Two

2. Material and Methods

2.1 Study design and area

Comparative cross sectional study which done in different hospital in Khartoum state(Khartoum Teaching Hospital, Omdurman Teaching Hospital and Bahrey Teaching Hospital), during the period from Nov 2011 – Jan 2013.

2.2 Study population

Two hundred pregnant women without history of thyroid disease compared with one hundred healthy non-pregnant women used as control group.

2.3 Inclusion criteria

Pregnant women without history of thyroid disease, and pregnant women with family history of thyroid disease.

2.4 Exclusion criteria

Non-pregnant women and pregnant women with thyroid disease or with history of thyroid disease.

2.6 Specimen collection

Five ml of venous blood was be collected in lithium heparin anticoagulant container, and separated in small sterile containers and stored in (-20C) refrigerator.

2.5 Ethical consideration

All individual include in this study were informed about the objectives of this study.

Informed consents were taken from all participant of this study.

2.7 Determination of Anti-thyroidperoxidaseAbs (Anti-TPO)

2.7.1 Principle

Anti-TPO enzyme immunoassay follows a two-step assay. Purified human thyroid peroxidase(TPO) antigen from the thyroid gland is immobilized onto the micro well plate which is react with antibodies of TPO (present in calibrators, control, and serum samples) which bind specifically to the immobilized TPO to make antigen antibody complex.Then the complex isdetected by using an anti-human-IgG-peroxidesconjugate, which can be directly readied spectrophotometric in 540 nm.*(Horworth, et al, 2006)*

2.7.2 Materials and Components (see appendix 2)

2.7.3 Procedure

Reagents were brought to room temperature.

Sample was Diluted 1:99 With Calibrator a before Used (10µl from sample with 990µl from diluents)Working solutionswerePreparedfrom the HRP conjugate and the buffer wash buffer.Required numbers of microwell strips were removed. Reseal the bag and return any unused strips to therefrigerator.100µl of each calibrator, control, and diluted sample were pipetted in duplicate into the correspondingly labeledwells. Micro wells were incubated on a plate shaker (200 rpm) for 30 minutes at room temperature.The wells were washed 3 times with 300 µl of diluted wash buffer per well and tap the plate firmly against absorbentpaper to ensure that it is dry.100 µl of the working HRP conjugate solution was pipetted into each well.Micro wells were incubated on a plate shaker (200 rpm) for 30 minutes at room temperature.Wellswere washed 3 times as in step 5. 100 µl of TMB substratewas pipetted into each well at timed intervals.Micro wells were incubated on a plate shaker for 10-15 minutes at room temperature (or until Calibrator E attains dark bluecolor for desired OD).50µl of stop solutionwaspipetted into each well at the same timed intervals as in step 9.The plate wasreadiedon a microwell plate reader at 450 nm within 20 minutes after addition of the stop solution.

2.7.4 Calculation

The mean was calculated from optical density of each calibrator duplicate.The mean was calculated from optical density of each unknown duplicate.The mean was Subtracted absorbance value of the Zero Calibrator from the mean absorbance values of the calibrators,control, and serum samples. A standardcurve was drowed on log-log paper with the mean optical densities on the Y-axis and the calibratorconcentrations on the X-axis. If immunoassay software is being used, a 4-parameter curve is recommended.The values of the unknownswerereadied directly off the standard curve.

2.8. Free T3

2.8.1 Principle

The purified human T3 antigen from the thyroid gland is immobilized onto the micro well plate which is react with antibodies ofFree T3 (present in calibrators, control, and serum samples) which bind specifically to the immobilized T3 to make antigen antibody complex.Then the complex isdetected by using T3 enzyme conjugate, which can be directly readied spectrophotometrically in 540 nm.*(Tietz,2006).*

2.8.2 Materials and Components: see appendix III

2.8.3 Procedure

All reagents, samples, references and controlswerebroughtto room temperature (18-25℃).

Themicroplates' wellswereformatted for each samples reference, control, and patient specimen to be assayed in duplicate.

0.050 ml (50 µl) of the appropriate samples reference, control, and specimen was pipetted into the assigned well.0.100 ml (100 µl) of T3-Enzyme Conjugate Solution was added to all wells.Themicroplatewasswirled gently for 20-30 seconds to mixed and covered.The microplatewasincubated for 60 minutes at room temperature.The incubation of mixturewas removed by emptying the plate content into a waste container. Themicrotiterplatewas rinsed and emptied

5 times with distilled water. Themicro titer plate was striked sharply onto absorbent paper or paper towels . 0.200 ml (200 µl) of working substrate solutionwas added to all wellsMicroplate was incubated at room temperature in the dark for 20 minutes.The reaction was stopped by add 50µl of 3N HCl to each well. Microplate was mixed gently for 30 seconds. It is important to make sure that all the blue color changes to yellow color completely. Absorbance was readiedat 450 nm with a micro plate reader within 30 minutes.

2.8.4 Calculation

Themeanabsorbance value (A450) was calculated for each set of reference standards, controls and patient samples. A standard curve was constructed by plotted the mean absorbance obtained for each reference standard against its concentration in pg/ml on graph paper, with absorbance values on the vertical or Y axis, and concentrations on the horizontal or X axis. The mean absorbance values was used for each specimen to determine the corresponding concentration of fT3 in pg/ml from the standard curve.

2.9. Free T4

2.9.1 Principle

The purified human T4 antigen from the thyroid gland is immobilized onto the micro well plate which is react with antibodies ofFree T3 (present in calibrators, control, and serum samples) which bind specifically to the immobilized T3 to make antigen antibody complex.Then the complex isdetected by using T4 enzymeconjugate, which can be directly readied spectrophotometrically in 540 nm.*(Selenkow.et al2007).*

2.9.2 Materials and Components (see appendix IV)

2.9.3 Procedure

All reagents, samples references and controlswerebroughtto room temperature (18-25℃).The microplates' wellswereformattedfor each serumreference, control, and patient specimen to be assayed in duplicate.0.050 ml (50 µl) of the

appropriate serum reference, control, and specimen was pipetted into the assigned well. 0.100 ml (100 µl) of T3-Enzyme Conjugate Solution was added to all wells.The microplatewasswirled gently for 20-30 seconds to mixed andcovered.The microplatewasincubated for 60 minutes at room temperature.The incubation mixturewas removed by emptying the plate content into a waste container. Themicrotiter plate was rinsed and emptied 5 times with distilled water. Themicrotiter plate was stroked sharply onto absorbent paper or paper towels

0.200 ml (200 µl) of working substrate solutionwas added to all wellsMicroplate was incubated at room temperature in the dark for 20minutes.The reaction was stopped by add 50µl of 3N HCl to each well. Microplate was mixed gently for 30 seconds. Absorbance was readiedat 450 nm with a micro plate reader within 30 minutes.

2.9.4 Calculation

The meanabsorbance value (A450) was calculated for each set of reference standards, controls and patient samples. A standard curve was constructed by plotted the mean absorbance obtained for each reference standard against its concentration in pg/ml on graph paper, with absorbance values on the vertical or Y axis, and concentrations on the horizontal or X axis. The mean absorbance values were used for each specimen to determine the corresponding concentration of fT3 in pg/ml from the standard curve.

2.10. TSH

2.10.1 Principles

The purified human TSH antigen from the thyroid gland is immobilized onto the micro well plate which is react with antibodies ofTSH (present in calibrators, control, and serum samples) which bind specifically to the immobilized TSH to make antigen antibody complex.Then the complex isdetected by using TSH enzyme conjugate, which can be directly readied spectrophotometrically in 540 nm. *(Ezrin ,et al,2008)*

2.10.2 Materials and Components (see appendix V)

2.10.3 Procedure

The desired number of coated wells was secured in the holder.100 µl of standards, samples, and Controlsweredispensedinto appropriate wells.100 µl of enzyme conjugate reagent was dispensed into each well.Wells were thoroughly mixed for 30 seconds. Wells were incubated at room temperature (18-25℃) for 60 minutes. The incubationmixture was removed by flicking plate contents into a waste container.Themicrotiterwellswere rinsed 5 times with distilled.

The wells were striked sharply onto absorbent paper to remove all residual water droplets.Dispensed 100 µl of TMB reagent into each well.gently mixed for 5 seconds.Themicrotiterwellswere incubated at room temperature for 20 minutes.100 µlof stop solution was added to each well.The microtitewellsweremixed for 30 seconds. Absorbancewas readied at 450 nm with a microtiter plate reader within 15 minutes.

2.10.4 Calculation

The meanabsorbance value (A450) was calculated for each set of reference standards, controls and patient samples. A standard curve was Constructed by plotted the mean absorbance obtained for each reference standard against its concentration in pg/ml on graph paper, with absorbance values on the vertical or Y axis, and concentrations on the horizontal or X axis. The mean absorbance values were used for each specimen to determinethe corresponding concentration of fT3 in pg/ml from the standard curve.

2.11 Quality Control

For quality control we used Quality control material (BioRadLyphochek Control sera).,One positive anti TPO and another one negative, One high level Thyroid hormones , Onelow level Thyroid hormones and onenormal thyroid hormones sample. And all controlsgives excellent results.

2.12. Statistical analysis

The data collected in this study were analyze using spss 17.5 computer program .the mean and standard deviation of anti thyroperoxidaseAbs, TSH, FreeT3 and FreeT4 were obtained ,t test , chi squire and anova test were used for comparison (p value of equal or less than 0.05was considered to significant).

Chapter Three

3. Results

This Study include Two hundred (200) pregnant women as test group, their mean of age were (27.1±7.2) years without thyroid disorders , and one hundred (100) apparently healthy non pregnant women used as control group.

According to trimester of pregnancy, there were 30% of pregnant women in first trimester, 27% in second trimester and 43% in third trimester. There were 15.5% of pregnant women had a family history of thyroid diseases as in fig (3.1), while 28% of pregnant had history of abortion.

The frequency of positive TPO in test group was (0.5%), while (2%) were Equivocal, and (97.5%) were negative as in table (3.1) .

Table (3.2) showed comparison of means between study and control group in TPO(IU/ml) (27.911±6.3718 IU/ml vs 26.300±4.7619 IU/ml), P = (.015) ,TSH(IU/ml) (2.265±1.4679 IU/mL vs 2.164±0.9942 IU/mL),P= (.725), FT3(pg/ml) (2.805±0.7104 pg/ml vs 2.696±0.6488 pg/ml) P=(.185), FT4(ng/dl) (1.416±0.3450 vs 1.385±0.3073 ng/dl) P = (.438).

According to history of abortion table (3.3) showed comparison of meanof TPO(IU/ml) (30.06 ± 8.27 IU/mL vs 27.07 ± 5.25 IU/mL) P =(.009) ,TSH(IU/ml) (2.61 ± 2.15 IU/mL vs 2.06 ± 1.03 IU/ml) P =(.003), FT3(pg/ml) (2.69 ± .84 pg/ml vs 2.85 ± .65 pg/mL) P =(.008), FT4(ng/dl) (1.35 ± .43ng vs1.44 ± .30 ng) P =(.001).

As showed in table (3.4) comparison of mean in test group in presence and absence of family history of thyroid disease in TPO(IU/ml) (35.28 ± 9.26IU/mL vs 26.56 ± 4.57 IU/mL) P =(.000) ,TSH(IU/ml) (3.61 ± 2.56IU/mL vs 1.95 ± .95IU/ml) P =(.001), FT3(pg/ml) (2.50 ± .89pg/ml vs 2.86 ± .66pg/mL) P =(.04), FT4(ng/dl) (1.25 ± .47ng/dl vs1.45 ± .31 ng/dl) P =(.03).

Figure (3.2) showed significant positive weak correlation between TPO(IU/ml) and age in year P =(.000) r =(.406).

Also significant positive strong correlation between TPO (IU/ml) and TSH (IU/ml) (.000) r =(.627) showed in figure (3.3)

Figure (3.4) showed significant negative weak correlation between TPO (IU/ml) and FT3 (pg/ml) P = (.004) r =(-.201).

Finally significant negative weak correlation between TPO (IU/ml) and FT4 (ng/dl) (.002) r =(-216) showed in figure (3.5)

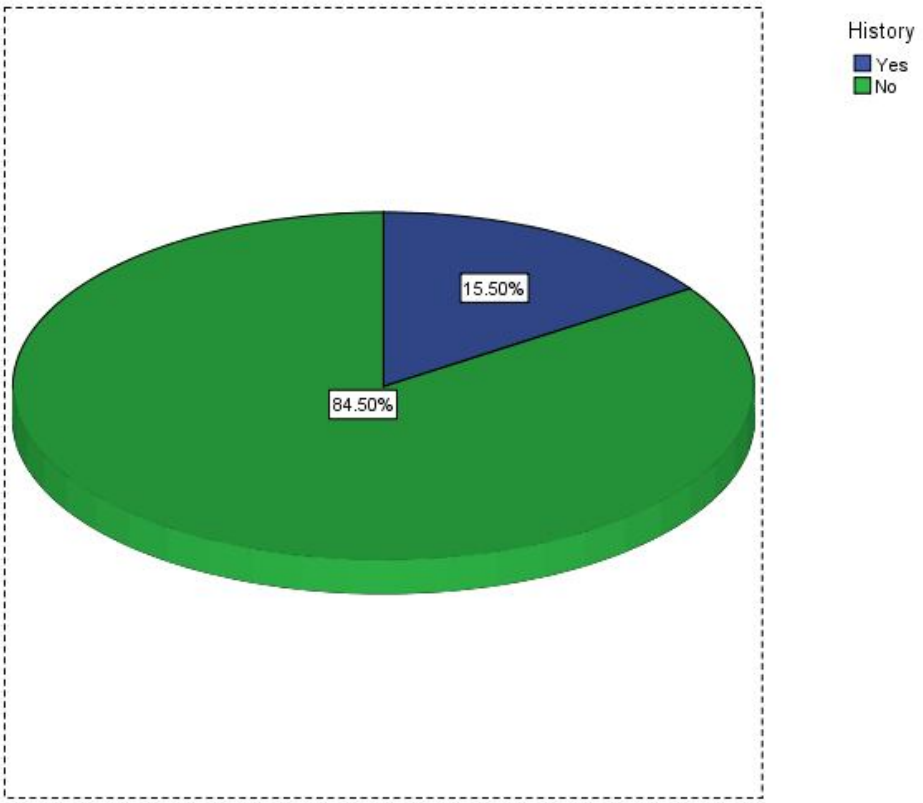

Figure 3.1 Distribution of test group according to family history of thyroid disease . 15.5% of cases had a family history of thyroid disease compared with 84.5% without family history.

Anti-TPO Abs	Frequency	%
Positive	**1**	**0.5%**
Equivocal	**4**	**2.0%**
Negative	**195**	**97.5%**

Table 3.1 Shows the Frequency of TPO among test group

- Positive more than 60 IU/ml
- Equivocal (40 – 59) IU/ml
- Negative Less than 40 IU/ml

Total	**200**	**100%**

Table 3.2comparisons of means of Anti-TPO Abs, TSH, FT3, FT4 in patients and control groups.

Variable	Mean ± Std. Dev	**P value**

Anti-TPO Abs pregnant(n=200) (IU/ml)	27.911±6.3718	.015 *
Control(n=100)	26.300±4.7619	
TSH pregnant(n=200) (IU/ml)	2.265±1.4679	.725
Control(n=100)	2.164±0.9942	
FT3 pregnant (n=200) (pg/ml)	2.805±0.7104	.185
Control(n=100)	2.696±0.6488	
FT4 pregnant (n=200) (ng/dl)	1.416±0.3450	.438
Control(n=100)	1.385±0.3073	

Independent sample t.test was used for comparison.

P.value considered significant at level ≤ 0.05

Table 3.3 comparison of means of Anti-TPO Abs, TSH, FT3, FT4 according to abortion in test group.

Present of abortion	Mean ± Std. Dev	P value
TPO(IU/ml)Yes(n=56)	30.06 ± 8.27	.009 *
No(n=144)	27.07 ± 5.25	
TSH(IU/ml)Yes(n=56)	2.61 ± 2.15	.003 *
No(n=144)	2.06 ± 1.03	
FT3(pg/ml) Yes(n=56)	2.69 ± .84	.008 *
No(n=144)	2.85 ± .65	
FT4(ng/dl) Yes(n=56)	1.35 ± .43	.001 *
No(n=144)	1.44 ± .30	

Independent sample t.test was used for comparison.

P.value considered significant at level ≤ 0.05

Table 3.4 comparison of means of Anti-TPO Abs ,TSH, FT3, FT4 according to family history of thyroid diseases in test group.

Family history	Mean ± Std. Dev	P value
TPO(IU/ml)Yes(n=31)	35.28 ± 9.26	.000*
No(n=169)	26.56 ± 4.57	

TSH(IU/ml)Yes(n=31) No(n=196)	3.61 ± 2.56 1.95 ± .95	.001*
FT3(pg/ml) Yes(n=31) No(n=196)	2.50 ± .89 2.86 ± .66	.040*
FT4(ng/dl) Yes(n=31) No(n=169)	1.25 ± .47 1.45 ± .31	.030*

Independent sample t.test was used for comparison.

P.value considered significant at level ≤ 0.05

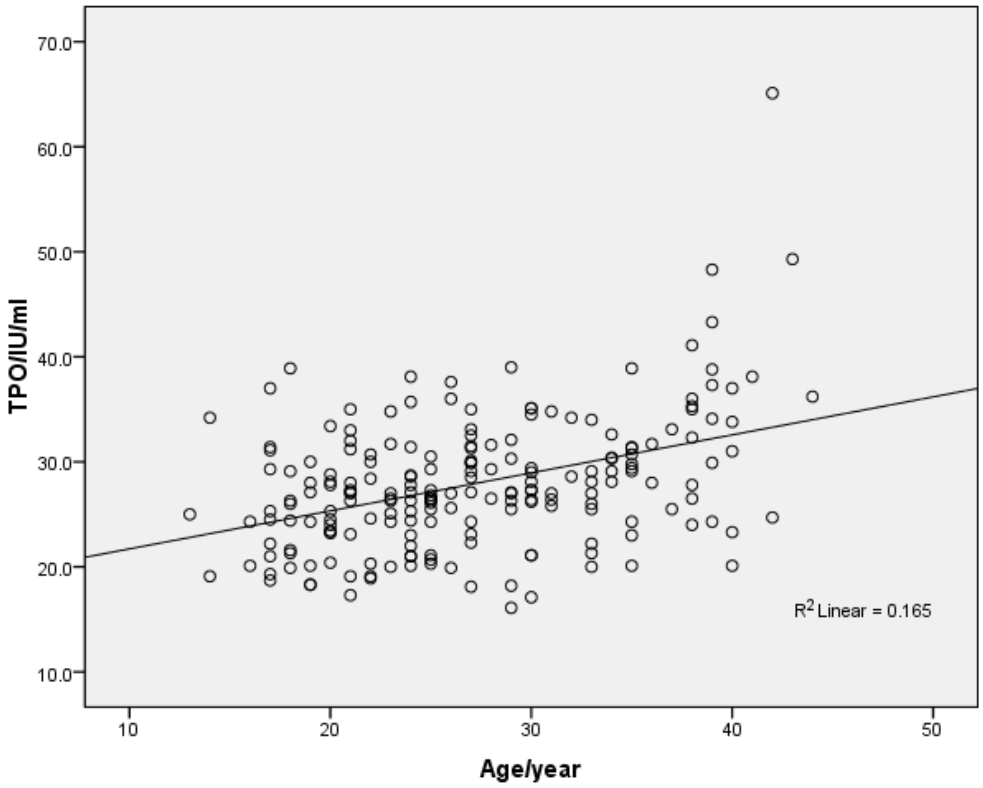

Figure3.2 Scatter plot shows relationship between TPO(IU/ml), and age (Year) in pregnant women, significant positive weak correlation (r = 0.406 , P.value = 0.000)

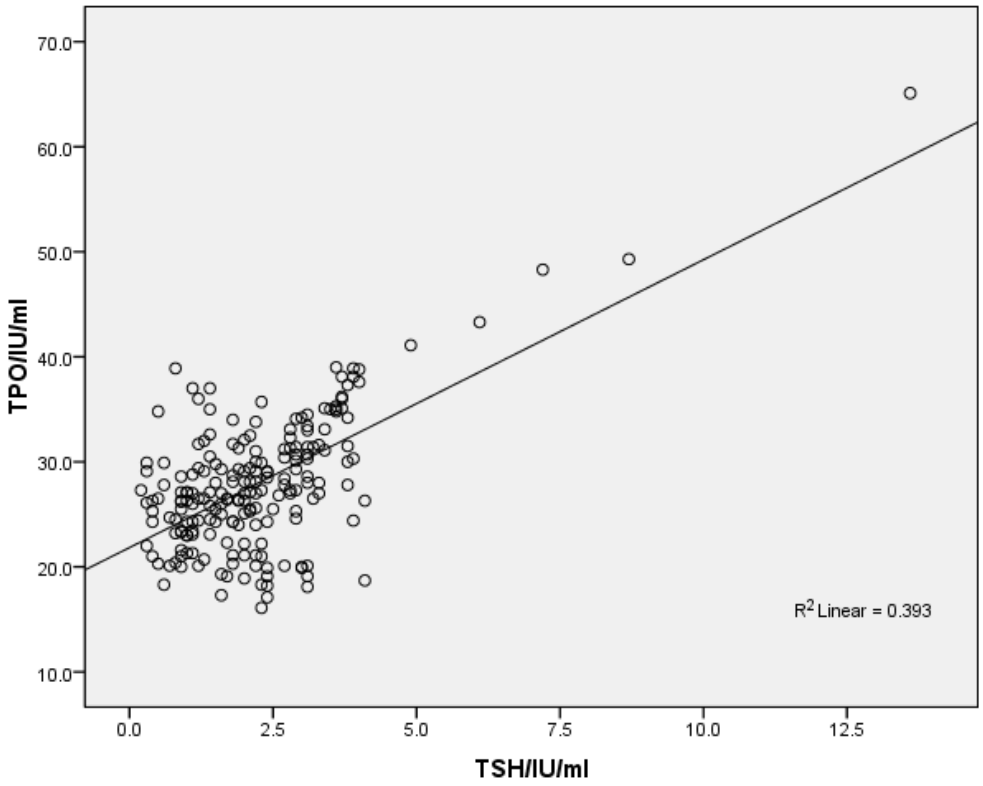

Figure3.3Scatter plot shows relationship between TPO(IU/ml), and TSH(IU/ml) in pregnant women. Significant positive moderate correlation (r=0.627 ,P.value=0.000)

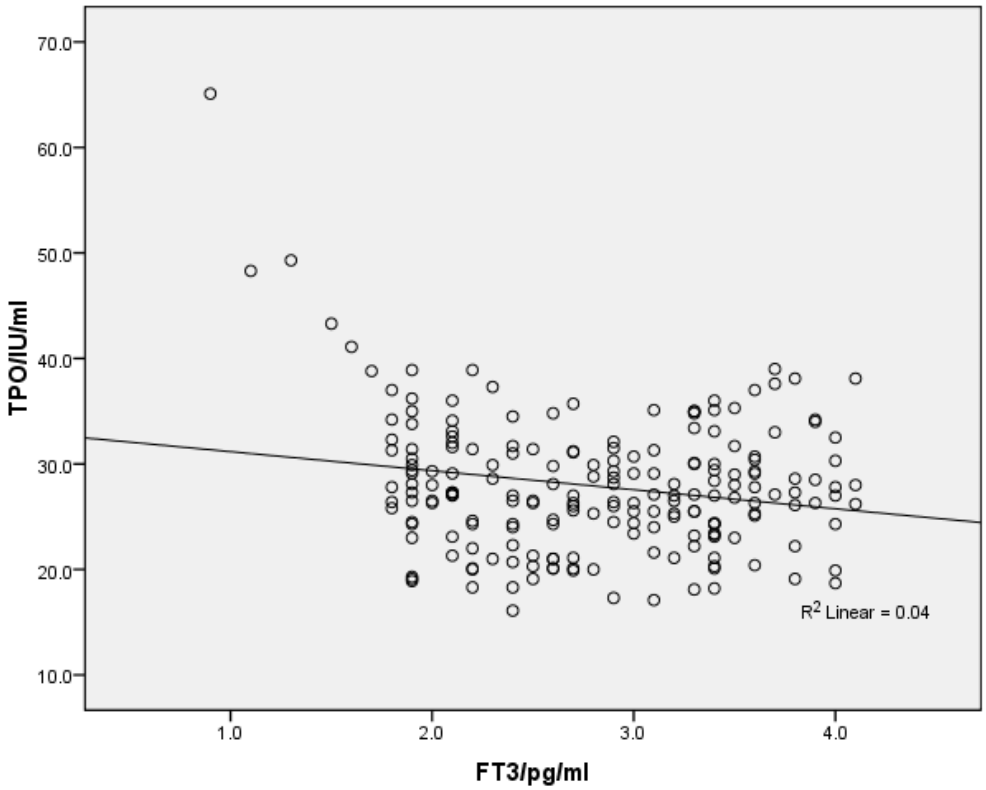

Figure 3.4 Scatter plot shows relationship between TPO(IU/ml), and FT3(pg/ml) in pregnant women, significant negative weak correlation. (r= -0.201 ,P.value=0.004)

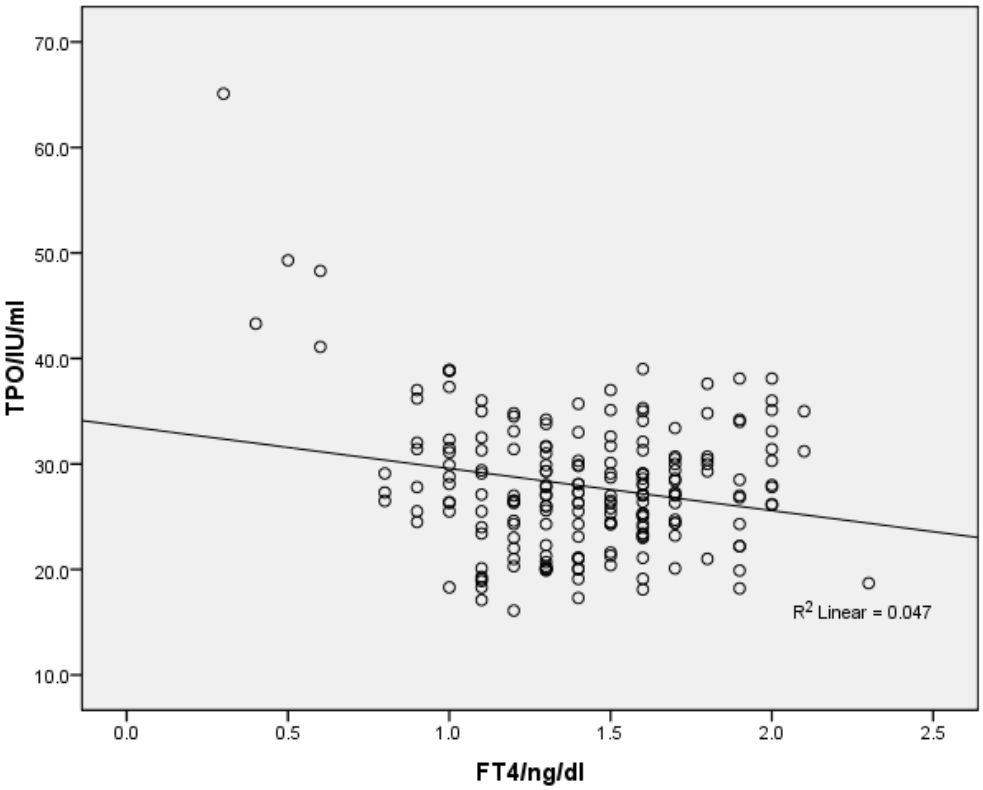

Figure 3.5 Scatter plot shows relationship between TPO (IU/ml) and FT4 (ng/dl) in pregnant women, significant negative weak correlation (r= -0.216 ,P.value =0.002)

Chapter Four

4. Discussion, Conclusion and Recommendation

4.1Discussion

Hyperthyroidism occurs in 1 to 2 of every 1000 pregnant women. The most common cause of hyper-thyroidism (80% to 85%) is Graves disease. Other causes include functioning adenoma, thyroiditis, and excessive thyroid hormone intake. While the incidences of hypothyroidism in pregnant women about (0.3 – 0.7 %) the majority is Hashimoto thyroiditis. *(Glinor,2008)*.uncontrolled thyroid dysfunction is associated with serious maternal, fetal, and neonatal morbidity, and mortality. maternal complications include miscarriage, pregnancy-induced hypertension, preterm labour, placental abruption, heart failure, and thyroid storm. Fetal and neonatal complications include stillbirth, low birth weight, goiter, hyperthyroidism, and hypothyroidism. These risks can be decreased with the appropriate treatment of maternal hyperthyroidism.*(Millar,2004)*

The results of this study showed (0.5%) of pregnant women had positive titter of anti-TPO,(2%) were equivocal titter and (97.5%) were negative titter, this disagree with study of *(Drahomira ,etal, 2009)*which found the prevalence of anti-TPO antibodies in pregnant women is (11.2%). This due to the mean of age of pregnant women was low when anti-TPO antibodies increased with age *(Kessler J, 2008)* who found the incidence of anti-TPO antibodies is five time more frequent in women than men and increased with age.

Also showed there was significant increase in mean titter of anti-TPO antibodies in test group when compared with control group, P.valu = (.015). There was insignificant P.value of TSH in test and control group, P= (.725), insignificant P.value of FT3 in test group and control group P =(.185), insignificant P.value ofFT4 in test group andcontrol group P = (.438)., this disagrees with *(Ain et al,2006)* who sayshCG has a much researched thyroid-stimulating hormone (TSH)-like activity secondary to specificity crossover at the TSH receptor (TSHR). As a result, serum thyroxine (T4) and triiodothyronine (T3) levels are elevated, whereas serum TSH level is

reduced.This due to we estimated Free T3 and Free T4 in our study when estimated Total T3 and Total T4 in *(Ain et al,2006)*.

Significant increase in the mean of TPO in test group who had a positive history of abortion (30.06 ± 8.27) when a negative mean was (27.07 ± 5.25) P =(.009), and significant increase in the mean of TSH in test group who had a positive history of abortion (2.61 ± 2.15) when a negative mean was (2.06 ± 1.03) P =(.003) , and significant decrease in the mean of FT3 in test group who had a positive history of abortion (2.69 ± .84) when a negative mean was (2.85 ± .65) P =(.008),and there was also significant decrease in the mean of FT4 in test group who had a positive history of abortion (1.35 ± .43) when a negative mean was (1.44 ± .30) P =(.001), this agree with *(Skjoldebrandl ,2009)* who found women with anti-TPO antibodies more likely to have spontaneous abortion than healthy control.

Also there was significant increase in the TPO among pregnant women who had a positive family history of thyroid disease (35.28 ± 9.26) when a negative mean was (26.56 ± 4.57) P =(.000), and significant increase in the mean of TSH in pregnant women who had a positive family history of thyroid disease (3.61 ± 2.56) when a negative mean was (1.95 ± .95) P =(.001), and significant decrease in the mean of FT3 in test group which had a positive family history of thyroid disease (2.50 ± .89) when a negative mean was (2.86 ± .66) P =(.04),and there was also significant decrease in the mean of FT4 in test group who had a positive family history of thyroid disease (1.25 ± .47) when a negative mean was (1.45 ± .31) P =(.03), this agrees with *(Rose, 2009)*.

According to age there was significant positive correlation between TPO and age, this agrees with *(Kessler, 2008)* who found the incidence of anti-TPO antibodies is five time more frequent in women than men and increased with age.

Study found there was also significant positive correlationbetween TSH anti-TPO P = (.000) r =(.627),and significant negative correlation between ant-TPO

and FT3 P = (.004) r =(-.201) , and also significant weak negative correlation between ant-TPO and FT4 P. value was (.002) r =(-216), this agree with *(Mariotti S,2008)* which found serum TSH, was already shifted to higher value in women withAutoimmune Thyroid Disease(AITD) compared with normal pregnant control.

4.2Conclusions

1-Frequency of positive anti-TPO is 0.5% among Sudanese pregnant women in Khartoum state.

2-Insignificant differences in mean of TSH, FreeT3 and FreeT4 in test group compared to control.

3-There is significant increase in anti-TPO antibodies in pregnant women who have a family history of thyroid disease.

4-There is significant increase in anti-TPO antibodies in pregnant women who have a history of abortion.

5-Significant positive correlation between anti-TPO and age.

6-Significant negativecorrelationbetweenanti-TPO and FT3 among pregnant women.

7-There is also significant negative correlation betweenanti-TPO and FT4among pregnant women.

4.3Recommendations

1- Screening thyroid function test (TFT) should be done routinely for every pregnant woman.
2- Any pregnant women which have a family history of thyroid disease or a history of abortion serum anti-TPO should be tested.
3- Further study to include other types of thyroid antibodies may givesignificant result.

References

- Abalovich M, Amino N, Barbour LA. (2007)"Management of thyroid dysfunction during pregnancy and postpartum: an Endocrine Society Clinical Practice Guideline". J ClinEndocrinolMetab.; 92(8Suppl):S1-47.

- Ain KB. Mori Y. Refetoff S.(2006) Reduced clearance rate of thyroxine-binding globulin (TBG) with increased sialylation: A mechanism for estrogen-induced elevation of serum TBG concentration. J ClinEndocrinolMetab. ;65:689–696.

- Alexander EK. Marqusee E. Lawrence J. Jarolim P. Fischer GA. Larsen PR. (2008)Timing and magnitude of increases in levothyroxine requirements during pregnancy in women with hypothyroidism. N Engl J Med.;351:241–249.

- Amino N, Tada H, Hidaka Y.(2010) Autoimmune thyroid disease inpregnancy. J EndocrinolInvestig;19:59–69.

- Andersson M, de Benoist B, Delange F, ZupanJ.Public Health Nutr. (2007) "Prevention and control of iodine deficiency in pregnant and lactating women and in children less than 2-years-old: conclusions and recommendations of the Technical Consultation");10(12A):1606-11.

- Anckaert E, Poppe K, Van Uytfanghe K, Schiettecatte J, Foulon W, (2010)Thienpont LMFT4 immunoassays may display a pattern during pregnancy similar to the equilibrium dialysis ID-LC/tandem MS candidate reference measurement procedure in spite of susceptibility towards binding protein alterations ClinChimActa. ; 41:1348-53

- Auso E. Lavado-Autric R. Cuevas E. Del Rey FE.Morreale de Escobar G. BerbelP(2008). A moderate and transient deficiency of maternal thyroid function at the beginning of fetal neocorticogenesis alters neuronal migration. Endocrinology;145:4037–4047.

- Becker DV. Braverman LE. Delange F.(2006) Iodine supplementation for pregnancy and lactation—United States and Canada: Recommendationsof the American Thyroid Associa+tion. Thyroid;16:949–951.

- Berger, S. and Quinn, J. L.(2007) Fund. Clin.Chem., N. W. Tietz (ed.), W. B. Saunders Co., Phila., PA 14,824-848.

- Burger, H. G., Patel, Y. C.(2007), Thyrotropin releasing hormone-TSH Clinic. Endocrinol.andMetab., 6,831-00.

- Casey BM, Dashe JS, Spong CY.(2007) "Perinatal significance of isolated maternal hypothyroxinemia identified in the first half of pregnancy". ObstetGynecol; 109: 1129-35.

- Casey BM, Dashe JS, Wells CE.(2005) "Subclinical hypothyroidism and pregnancy outcomes".ObstetGynecol; 105: 239-45.

- Casey BM. Dashe JS. Wells CE.(2005) Subclinical hypothyroidism and pregnancy outcomes.Obstet Gynecol;105:239.

- Chan WB, Yeung VT, Chow CC, So WY, Cockram CS. (2009). "Gynaecomastia as a presenting feature of thyrotoxicosis". Postgrad Med J. 882 (75): 229–31. PMC 1741202.PMID 10715765.

- Cleary-Goldman J, Malone FD, Lambert-Messerlian G.(2008) "Maternal Thyroid Hypofunction and Pregnancy Outcome" ObstetGynecol112: 85-92

- Cleary-Goldman J. Malone FD.Lambert-Messerlian G,(2008) Maternal thyroid hypofunction and pregnancy outcome.Obstet Gynecol;112:85:92.

- Davis LE, Lucas MJ, Hankins GD, Roark ML, Cunningham FG.Department of Obstetrics and Gynecology, University of Texas Southwestern Medical School, Dallas 75235-9032. National Library of Medicine 8600 Rockville Pike, Bethesda MD, 2010 USA.

- Dussault, j. H., Turcotte, R., and Gieyda, H., ClinEndocrin.Metabol.2008, 43: 232-285.

- Eber O, Buchinger W, Lindner W, et al.(2009) The effect of D-versus L-propranolol in the treatment of hyperthyroidism.ClinEndocrinol 2009;32:363-72.

- Emrich, D., Schondube, H.,-Sehlen, S., and Schreivagel, I., Nuc.(2006) Compact, 16:392.

- Engall, E.(2008) Methods in Enzymology, Vol. 70, VanVunakis, H. &Langone, J.J. (eds.), Acad. Press, NY,419-492.

- Esfahani AF; Kakhki VR; FallahiB (2008). "Comparative evaluation of two fixed doses of 185 and 370 MBq 131I, for the treatment of Graves' disease resistant to antithyroid drugs". Hellenic Journal of Nuclear Medicine 8 (3): 158–61. PMID 16390021.

- Ezrin, C., The Thyroid, S. C. Werner and S. H. lngbar (2007), Harper and Row, Hagerstown, MD, 9,174-178.

- Floyd,J.L.(2009)Thyrotoxicosis.eMedicine.abchttp://next.thyroid.org/patients/patient_brochures/hyperthyroidism.html

- Glinoer D, Fernandez Soto M, Bourdoux P, Lejeune B, Delange F, Lemone M.(2009) "Pregnancy in patients with mild thyroid abnormalities: maternal and neonatal repercussions". J ClinEndocMetab;73:421-427

- Glinoer D. de Nayer P. Bourdoux P,(2007) Regulation of maternal thyroid during pregnancy. J ClinEndocrinolMetab;71:276–287.

- GlinoerD.(2006) Iodine nutrition requirements during pregancy. Thyroid. ;16:947–948.

- Glinoer D. (2003)Management of hypo- and hyperthyroidism during pregnancy. Growth Horm IGF Res;13(Suppl A):S45–54.

- Gong, Y. and B. R. Hoffman (2008). “Free thyroxine reference interval in each trimester of pregnancy determined with the Roche Modular E-170 electrochemiluminescent immunoassay.” ClinBiochem 41(10-11): 902-6.

- Haddow JE, Palomaki GE, Allan WC. (1999)"Maternal thyroid deficiency during pregnancy and subsequent neuropsychological development of the child".NEngl J Med. 19; 341: 549-55.

- Gilbert, R. M., N. C. Hadlow, (2008). “Assessment of thyroid function during pregnancy: first-trimester (weeks 9-13) reference intervals derived from Western Australian women.” Med J Aust 189(5): 250-3.

- Hertz, Barbara, Schuleller, Kristin, Saul Hertz, MD (2010) A Pioneer in the Use of Radioactive Iodine, Endocrine Practice 16,4;713-715.

- Horworth, P.J.N., Ward, RL., J.(1970)ClinPathol.; 25:259-62.

- Ingbar, S.H.J.(2006) Clin. Invest, 44:1679.
- Kamijo K. Saito T. Sato M, et al.(2006) Transient subclinical hypothyroidism in early pregnancy.EndocrinolJpn.;37:397–403.
- Kittisupamongkol W. (2009)Hyperthyroidism or thyrotoxicosis? Cleve Clin J Med;76(3):152.
- Klein RZ, Haddow JE, Faix JD et al. (1991)"Prevalence of thyroid deficiency in pregnant women". ClinEndocrinol (Oxf); 35: 41-6.
- Lao TT. (2007) Thyroid disorders in pregnancy. CurrOpinObstet Gynecol. 2007;17:123–127.
- Lazarus JH. (2006) Epidemiology and prevention of thyroid disease in pregnancy. Thyroid. 2006;12:861–865.
- Lee RH, Spencer CA, Mestman JH, et al.(2009) Free T4 immunoassays are flawed during pregnancy.Am J Obstet Gynecol. 2009 ;200:260.e 1-6
- LeBeau SO. Mandel SJ.(2006) Thyroid disorders during pregnancy. EndocrinolMetabClin North Am.2006;35:117–136.
- Little, Susan (2009)."Feline Hyperthyroidism".Winn Feline Foundation.Retrieved 24 June 2009:13:10-21.
- Lundberg, P.A., Jagenburg, R., Lindstedt, G., Nystrom, E., Clin. Chem. 1982, 28:1241.

- Mandel SJ, Cooper DS.(2001) "The use of antithyroid drugs in pregnancy and lactation". J ClinEndocrinolMetab;86 :23549.

- Mandel SJ, Larsen PR, Seely EW, Brent GA.(2008)Increased need for thyroxine during pregnancy in women with primary hypothyroidism;323:91-6.

- Mandel SJ. (2004)"Hypothyroidism and chronic autoimmune thyroiditis in the pregnant state: maternal aspects." Best Pract Res ClinEndocrinolMetab.2004 ; 18: 213-24.

- Mandel SJ.(2008) Hypothyroidism and chronic autoimmune thyroiditis in the pregnant state: Maternal aspects. Best Pract Res ClinEndocrinolMetab. 2008;18:213–224.

- Mandel SJ. Larsen PR. Seely EW. Brent GA.(2009) Increased need for thyroxine during pregnancy in women with primary hypothyroidism. N EnglJ Med;323:91–96.
- Mandel SJ. Spencer CA. Hollowell JG.(2005) Are detection and treatment of thyroid insufficiency in pregnancy feasible? Thyroid. 2005;15:44–53.

- Markovic V, Eterovic D (September 2007). "Thyroid echogenicity predicts outcome of radioiodine therapy in patients with Graves' disease". The Journal of Clinical Endocrinology and Metabolism 92 (9): 3547–52. doi:10.1210/jc-0879. PMID 17609305.

- Mariotti S, Caturegli P, Piccolo P, Barbesino G, Pinchera A (2008)Antithyroid peroxidase autoantibodies in thyroid diseases. J ClinEndocrinolMetab 71:661-669

- Marx H, Amin P, Lazarus JH.(2008) "Hyperthyroidism and pregnancy". BMJ. 22; 336 (7645): 663-7.

- Melmed, S., Geola, F.L., Reed, A.W., Pekary, A.E., Park, J., Hershmen, J. M., (2005) ClinEndocrin. Metabol.55:441-475

- Mestman JH.(2004) "Hyperthyroidism in pregnancy". Best Pract Res ClinEndocrinolMetab.2004; 18 267-288.

- Millar LK, Wing DA, Leung AS, Koonings PP, Montoro MN, Mestman JH. (2004)Low birth weight and preeclampsia in pregnancies complicated by hyperthyroidism. *Obstet Gynecol.* 2004;84(6):946–9.

- Morreale de Escobar G. Obregon MJ. Escobar del Rey F.(2011) Is neuropsychological development related to maternal hypothyroidism or to maternal hypothyroxinemia? J ClinEndocrinol Metab.2011;85:3975–3987.

- Muller AF, Drexhage HA, Berghout A. (2001)"Postpartum thyroiditis and autoimmune thyroiditis in women of childbearing age: recent insights and consequences for antenatal and postnatal care". Endocr Rev. 2001; 22:605-30.

- Marwaha, R. K., S. Chopra. (2008). “Establishment of reference range for thyroid hormones in normal pregnant Indian women.” Bjog 115(5): 602-6

- Negro R, Formoso G, MangieriT .(2006) "Levothyroxine treatment in euthyroid pregnant women with autoimmune thyroid disease: effects on obstetrical complications". J ClinEndocrinolMetab.2006; 91(7):2587-91.

- Negro R. Mangieri T. Coppola L.(2007) Levothyroxine treatment in thyroid peroxidase antibody-positive women undergoing assisted reproduction technologies: 2010:85:133-164.of endocrine adaptation from physiology to pathology. EndocrRev 2007;18:404–33.

- Okosieme OE, Lazarus JH.(2008) "Management of thyroid dysfunction in pregnancy". Expert opinPharmacother.2008; 9: 2281-2293.

- Oppenheimer, J.H. J.(2006)Clin. Invest. 1962, 42: 1769.Physician;53:215–20.

- Pierce, J. G.(1971), Endocrinology, 89, 1331-1344 .

- Polak M, Le Gac I, Vuillard E (2004). "Fetal and neonatal thyroid function in relation to maternal Graves' disease". Best Pract Res ClinEndocrinolMetab.;18: 289-302.

- Pop VJ, Brouwers EP, Vader HL (2003). "Maternal hypothyroxinaemia during early pregnancy and subsequent child development: a 3-year follow-up study". ClinEndocrinol (Oxf). 2003;59 :282-8.

- Pop VJ, de Vries E, van Baar AL (2005). "Maternal thyroid peroxidase antibodies during pregnancy: a marker of impaired child development?" J ClinEndocrinolMetab. 1995; 80: 3561-6. pregnancy. EndocrinolMetabClin N Am;24:41–71.

- Roy E Weiss and Samuel R (2009)effectoff.Thyrotropin (TSH)-secreting pituitary adenomas.Last literature review version 19.1: January 2011. This topic last updated: July 2, 2009

- Sati, C., Chattor, A.J., Watts, N. (2006) Fundamentals of Clinical Chemistry.Ed.Tietz, N.W. 3rd Ed., pg. 586.

- Selenkow, H.A., and Robin, N.I., J.(1970) Maine Med. Assoc. 1970, 61:199.

- Skjoldebrand L, Brundin J, Carlstrom A, Pettersson T. (2009)Thyroid associated components in serum during normal pregnancy. ActaEndocrinol 2009;100:504-511.

- Smyth PP, Hetherton AM, Smith DF (1997). "Maternal iodine status and thyroid volume during pregnancy: correlation with neonatal iodine intake". J ClinEndocrinolMetab; 82: 2840-3.

- Snyder, P. J. &Utiger, R. D., J(2007). Clin. Endo.Metab: 34.

- Soos, M. and Siddle, K., J. Immun.(1982) Methods, 51, 57-68.

- Stagnaro-Green A, Chen X, Bogden JD (2005). "The thyroid and pregnancy: a novel risk factor for very preterm delivery". Thyroid; 15: 351-7.

- Stagnaro-Green A, Roman SH, Cobin RH, el-Harazy E, Alvarez-Marfany M, Davies TF(2009) Detection of at-risk pregnancy by means of highly sensitive assays for thyroid autoantibodies..Department of Medicine, Mount Sinai School of Medicine, New York, NY 10029.National Center for Biotechnology Information, National Center for Biotechnology Information, U.S. National Library of Medicine 8600 Rockville Pike, Bethesda MD, 2009 USA.

- Stagnaro-Green A. Glinoer D.(2008) Thyroid autoimmunity and the risk of miscarriage.BestPract Res ClinEndocrinolMetab;18:167â€“181.

- Tarnoky, A.L. Advan(1981). Clin. Chem, 21:101-146.

- Tietz, N.W(1976)., Fundamentals of Clinical Chemistry, 2nd Ed., pg. 602, Sauders Press, Phila.

- Tintinalli, Judith (2008). Emergency Medicine: A Comprehensive Study Guide, Sixth edition.McGraw-Hill Professional.p. 1312.ISBN 0071388753.

- ToftA(2004). Increased levothyroxine requirements in pregnancy—Why, when, and how much? N Engl J Med;351:292–294.

- Uotila, M., Ruoslahti, E. and Engvall, E., J(1981). lmmunol. Methods, 42, 11-15.

- Utiger, R. D.(1978) The Thyroid, S.C. Werner and S. H. Ingbar (eds.), Harper and Row, Hagerstown, MD, 9,196-205.

- Vulsma T, Gons MH, De VijlderJMM(2009). Maternal fetal transfer of thyroxine in congenital hypothyroidism due to a total organification defect of thyroid dysgenesis. N Engl J Med 321:13, 3007

- Vanderpump MP, Tunbridge WM.(2009) French JM: The incidence of thyroid disorders in a community: a twenty-year follow-up of the Whickam survey. ClinEndocrinol 43:55.

- Vincent Marks,DušanMeško(2005). Differential diagnosis by laboratory medicine: a quick reference for physicians;, page 156

- Wada, H. G., Danisch, R. J., Baxter, S. R., (1982), Clin. Chem.,28, 1862-1866.

- Wilson JD Foster DW Kronenberg HM Larsen PR eds.(2009) Williams textbook of endocrinology 9th ed:389-515 WB Saunders Philadelphia.

- Zimmerman D.(1999) "Fetal and neonatal hyperthyroidism".Thyroid;9: 727-33

Appendixes

Appendix I **Questionnaire**

Name **:**.....**NO:** ( )

Age **:** ( ) Years

No of Pregnancy:( ) Times

Trimester **:** First ( ) Second ( ) Third ( )

Abortion **:**Yes ( ) No ( )

If Yes Frequency: ( )Times.

History of thyroid disease:Family

Yes( ) NO ( )

Laboratory investigations:

Test	F.T3	F.T4	TSH	Anti-TPO Abs
Result				

Appendix II **Materials and Components**

•Pipette capable of delivering 50 µl volumes with a precision of better than 1.5%.

• Dispenser(s) for repetitive deliveries of 50, 100, and 200 µl volumes with a precision ofbetter than 1.5%.

•Microplate Reader with 450 nm wavelength absorbance capability.

• Test tubes for preparing Working Substrate Solution from Color Reagents A and B.

• Absorbent paper for blotting the microplate wells.

• Timer.

• Quality control material (e.g., BioRadLyphochek Control sera).

• Distilled or deionized water.

• Linear graph paper or appropriate computer program.