1.1. Introduction

Diabetes mellitus is a metabolic disorder characterized by chronic hyperglycemia with disturbances in carbohydrate, fat and protein metabolism, arising from a defect in insulin secretion or action or both (Marshall, 2004).

Insulin enables cells to absorb glucose in order to turn it into energy this causes glucose to accumulate in blood leading to various potential complications (Rother, 2007- Tierney et al 2002).

The thyroid gland is positioned in the lower anterior neck and has a shape similar to a butterfly. It is divided into two lobes, one on either side of the trachea. A band of thyroid tissue, called the isthmus, bridges the lobes. Underneath the thyroid gland are the parathyroid glands (responsible for calcium balance) and the recurrent laryngeal nerves (innervations for the vocal cords) (Bishop et al, 2005).

Thyroid gland secretes three hormones: thyroxine (T4) and triiodothyronine (T3) both of which are iodinated derivatives of tyrosine, and calcitonin a poly peptide hormone. T3 and T4 are produced by the follicular cells but calcitonin is secreted by C cells which are separate of embryological origin and is functionally unrelated to the other thyroid hormones and has a minor role in calcium homeostasis and disorders of it is secretion are rare and thyroid disorders in which there is either over production or under secretion of T3 and T4 are however common (William et al, 2008).

Thyroid disease is common in the general population and the prevalence increases with age (Hegedus et al, 1983).

Hypothyroidism is the most common thyroid disorder in the adult population, especially in older woman. It is usually autoimmune in origin, presenting as either primary atrophic hypothyroidism or Hashimotos thyroiditis (kamel, 1999).

Thyroid disease is common in the general population and the prevalence increases with age (Hegedus et al, 1983).
Hypothyroidism is the most common thyroid disorder in the adult population, especially in older woman. It is usually autoimmune in origin, presenting as either primary atrophic hypothyroidism or Hashimotos thyroiditis (kamel, 1999).

In type 1 and 2 diabetes, the metabolism of food stuff is altered(Briscoe et al, 2006).

Lack of insulin or insulin resistance prevents the efficient uptake and utilization of glucose by most cells of the body, except those of the brain. As a result, blood glucose concentration increases, cell utilization of glucose decreases and utilization of fats and proteins increases (Briscoe et al, 2006, Guyton and Hall, 2006).

Diabetes patients have a higher prevalence of thyroid disorders than the normal population. (Wu,2000). Thyroid disease is found in both type 1 and 2 diabetes. People with type 1 diabetes and underlying autoimmune disease may have associated thyroid disease (Johnson, 2006).

Since thyroid hormones regulate metabolism and diabetes can alter metabolism of foodstuff, the metabolism of the organism may be further affected by the combination of thyroid disease and diabetes (Bernal and Refeloff, 1977; Notarbartolo et al, 1983; Wu, 2000).
1.2. Rationale:

The incidence and prevalence of type 2 diabetes mellitus is seemed to be increasing in Sudan.

Study conducted in Bangladesh by Dr Sufial et al 2008 reported that diabetic patients have a higher prevalence of thyroid disorders than the general population this may influence diabetic management.

The prevalence of thyroid disorder in diabetic population was reported to be 13.4% with higher prevalence 31.4% in female type 2DM patients as compared to 6.9% in male type 2DM (Perros et al, 1995).

This study aimed to answer the question if there any significant change in the concentration of thyroid hormones in Sudanese patient with type 2 diabetes mellitus.
1.3. Objectives:

1.3.1. General objectives:

To study the levels of thyroid hormones and TSH in type 2 diabetic patients

1.3.2. Specific objectives:

1- To measure and compare the level of T3, T4 and TSH in type 2 diabetic patients and control group

2- To correlate the levels of T3, T4 and TSH with duration of diabetes, BMI, age, family history and gender.
2. Literature Review

2.1. Diabetes mellitus:

Diabetes mellitus is a metabolic disorder characterized by chronic hyperglycemia with disturbance in carbohydrate, fat and protein metabolism, arising from a defect in insulin secretion or action or both (Marshall, 2004).

Insulin enables cells to absorb glucose in order to turn it into energy. This causes glucose to accumulate in the blood leading to various potential complications (Rother, 2007; Tierney et al, 2002).

Diabetes mellitus has been defined by the WHO, on basis of laboratory findings, as a fasting venous plasma glucose concentration greater than 7.8 mmol/L (140 mg/dl) or greater than 11.1 mmol/L (200 mg/dl) two hours after a carbohydrate meal or two hours after the oral ingestion of the equivalent of 75 g of glucose even if the fasting concentration is normal (Mayne, 1998).

2.1.1. Classification of diabetes mellitus:

(A) Primary diabetes mellitus:

Which classify to two types:

I-Type 1 DM:

Insulin dependent diabetes mellitus (IDDM) is characterized by loss of beta cells functions which leading to insulin deficiency also can be classified as immune mediated or idiopathic. The majority of type 1 is the immune mediated nature, where beta cell loss is T-cell mediated autoimmune attack (Rother, 2007).

There is no known preventive measure against type 1 which causes approximately 10% of DM in North America and Europe, type 1 can affect children or adult but
traditionally termed juvenile diabetes because it represents a majority of the diabetes cases in children (Lawrence et al, 2008).

**Causes of type 1 DM:**

(i) *Genetics:-*

Susceptibility to type 1 diabetes is inherited, but the mode of inheritance is complex and has not been completely defined. It is a multigenic trait, and the major locus is the major histocompatibility complex on chromosome (Lawrence et al, 2008).

Subjects most at risk are those with HLA- types DR3 and DR4 of the major histocompatibility complex (Mayne, 1998).

(ii) *Environmental factors:-*

Environmental factors are thought to be involved in initiating diabetes, for example, viruses, such as: rubella, mumps and coxsackie virus B, have been implicated others environmental factors that have been suggested include chemicals and cows milk (Carl et al, 2008).

**2-Type 2 DM:**

Formerly called non-insulin-dependent diabetes mellitus (NIDDM) or adult onset diabetes is a disorder that is characterized by high blood glucose in the context of insulin resistance and relative insulin deficiency (Wild et al, 2004).

Type 2 constitutes the majority of diabetes cases, and represent more chronically in the middle aged and elderly with symptoms developing over months or even longer (Marshall, 2004).

The prevalence of type 2 diabetes increases with increasing age and reaches over 10% in people over the age of 75 years. It has become apparent that some young patients with diabetes are not insulin dependent, while approximately 10% of patients developing diabetes over the age of 25 have Latent Autoimmune Diabetes of
Adulthood (LADA) patient with LADA may be misclassified as having type 2 diabetes (Marshall, 2004).

**Causes of type 2 DM:**

(i) **Genetics:**

Genetic factors contribute to the development of type 2 diabetes. For example, the concordance rate for type 2 diabetes in identical twins approaches 100%. In addition, type 2 diabetes is 10 times more likely to occur in obese individuals without a diabetic family history. The mode of inheritance is unknown (Carl et al, 2008).

A variety of approaches have identified several genes that are associated with type 2 diabetes. Therefore, the gene or genes causing the common forms of type 2 diabetes remain unknown (Carl et al, 2008).

(ii) **Environmental factors** such as diet and exercise are important determinants in the pathogenesis of type 2 diabetes. Although 60% to 80% of those with type 2 diabetes are obese, diabetes develops in fewer than 15% of obese individuals. In contrast, virtually all obese people, even those with normal carbohydrate tolerance, have hyperinsulinemia and are insulin resistant (Carl et al, 2008).

**Other factors such as:**

- Family history of type 2 diabetes.
- The duration of obesity
- The distribution of fats (Carl et al, 2008).

(B) **Secondary DM:**

May be caused by:
- Absolute insulin deficiency due to pancreatic disease (chronic pancreatitis, haemochromatosis, cystic fibrosis).

- Relative insulin deficiency due to excessive growth hormone (acromegaly), glucocorticoid secretion (Cushing syndrome), or increased plasma glucocorticoid concentrations due to administration of steroids.

- Drugs such as thiazide diuretics (Mayne, 1998).

(C) Gestational DM:

Gestational DM is any degree of glucose intolerance with onset or first recognition during pregnancy (Bishop et al, 2005).

2.1.2. Pathophysiology of diabetes mellitus:

In both type 1 and type 2 diabetes, the individual will be hyperglycemic which can be severe, glucosuria can also occur after the renal tubular transporter system for glucose becomes saturated (Bishop et al, 2005).

As hepatic glucose overproduction continues, the plasma glucose concentration reaches a plateau around 300-500 mg/dl (17-28mmol/L) provided output is maintained, glucose excretion will match the overproduction, causing the plateau (Bishop et al, 2005).

The individual with type 1 diabetes has a higher tendency to produce ketones. Patient with type 2 diabetes seldom generate ketones, but instead have a greater tendency to develop hyperosmolar nonketotic states (Bishop et al, 2005).

The difference in glucagons and insulin concentrations in these two groups appears to be responsible for the generation of ketones through increased B-oxidation. In type 1, there is an absence of insulin with an excess of glucagons. This permits gluconeogenesis and lipolysis to occur (Bishop et al, 2005).

In type 2, insulin is present as (at times) hyperinsulinemia, therefore, glucagons is attenuated. Fatty acid oxidation is inhibited in type 2. This causes fatty acids to be
incorporated into triglycerides for release as very low-density lipoprotein (Bishop et al, 2005).

2.1.3. Complications of DM:

(A) Acute metabolic complications:

Patients with diabetes mellitus may develop one of several metabolic complications. These include:

- Diabetic ketoacidosis.
- Hyperosmolar nonketotic coma (Mayne, 1998).

(B) Late complications:

Vascular disease is a common complication of DM:

1. Macrovacular disease:

Is due to abnormalities of large vessels, may present as coronary artery, cerebrovascular or peripheral vascular insufficiency. The condition is probably related to alterations in lipid metabolism (Mayne, 1998).

2. Microvascular disease:

Is due to abnormalities of small blood vessels, particularly affects the retina and the kidney, the incidence of both may be related to inadequate glucose control (Mayne, 1998).

(i) Retinopathy: may lead to blindness because of vitreous haemorrhage from proliferating retinal vessels, and maculopathy as a result of exudates from vessels or oedema affecting the macula (Allan et al, 2004).

(ii) Nephropathy: leads ultimately to renal failure. In the early stage there is kidney hyperfunction, associated with an increased glomerular filtration rate (GFR), increased glomerular size and microalbuminuria.
In the late stage there is increasing proteinuria and a marked decline in renal function, resulting in ureamia (Allan et al, 2004).

(iii) Neuropathy may become evident as diarrhea, postural hypotension, impotence, neurogenic bladder and neuropathic food ulcers due to microangiopathy of nerve blood vessels and abnormal glucose metabolism in nerve cells (Allan et al, 2004).

2.1.4. Control of blood glucose:

The liver, pancreas and other endocrine glands are all involved in controlling the blood glucose concentration within a narrow range (Bishop et al, 2005).

Control of blood glucose is under two major hormones:

Insulin: is the only hormone responsible for the entry of glucose into the cells and synthesized by the Beta cells of islets of langerhans in the pancreas. The release of insulin causes an increased movement of glucose into the cells and increase glucose metabolism (Bishop et al, 2005).

1- Insulin is normally released when glucose level are high and is not released when glucose levels are decreased. It also regulate glucose by increasing glycogenesis, lipogenesis and glycolysis and inhibiting glycogenolysis (Bishop et al, 2005).

Insulin is the only hormone that decrease glucose levels and can be referred to as a hypoglycemic agent (Bishop et al, 2005).

2- Glucagon: is the primary hormone responsible for increasing glucose level which is a polypeptide synthesized by the alpha-cells of the pancreatic islets and it's secretion stimulated by hypoglycemia. When plasma insulin are low like during fasting glucagon enhances hepatic glyconeogenesis. The hyperglycemic actions of other hormones such as growth hormone, glucocorticoides and adrenaline become apparent even if there is no increase in secretion rates (Mayne, 1998).

2.2. The Thyroid glands:
The thyroid gland is positioned in the lower anterior neck and has a shape similar to a butterfly. It is divided into two lobes, one on either side of the trachea. A band of thyroid tissue, called the isthmus, bridges the lobes. Underneath the thyroid gland are the parathyroid glands (responsible for calcium balance) and the recurrent laryngeal nerves (innervation for the vocal cords) (Bishop et al, 2005).

2.2.1. thyroid hormones:

Thyroid gland secretes three hormones: thyroxine (T4) and triiodothyronine (T3) both of which are iodinated derivatives of tyrosine, and calcitonin a poly peptide hormone. T3 and T4 are produced by the follicular cells but calcitonin is secreted by C cells which are separate of embryological origin and is functionally unrelated to the other thyroid hormones and has a minor role in calcium homeostasis and disorders of it is secretion are rare and thyroid disorders in which there is either over production or under secretion of T3 and T4 are however common (William et al, 2008).

Thyroxine synthesis and release are stimulated by the pituitary trophic hormone, thyroid stimulating hormone (TSH). The secretion of TSH is controlled by negative feedback by the thyroid hormones, which modulate the response of the pituitary to the hypothalamic hormone, thyrotrophin-releasing hormone (TRH) (William et al, 2008).

This feedback is mediated primarily by T3 produced by the action of iodothyronine deiodinase on T4 in the thyrotroph cells of the anterior pituitary. Glucocorticoids, dopamine and somatostatin inhibit TSH secretion. The physiological significance of this is not known but it may be relevant to the disturbances of the thyroid hormones that can occur in non-thyroidal illness. The feedback mechanisms result in the maintenance of steady plasma concentrations of thyroid hormones.

The major product of the thyroid gland is T4. Ten times less T3 is produced (the proportion may be greater in thyroid disease), most T3 (approximately 80%) being derived from T4 by deiodination in peripheral tissues, particularly the liver, kidneys, and muscle, catalyzed by selenium-containing iodothyronine deiodinases. T3
is 3-4 times more potent than T4. In tissues, most of the effect of T4 results from this conversion to T3, so that T4 itself is essentially a prohormone. Deiodination can also produce reverse triiodothyronine (rT3), which is physiologically inactive. It is produced instead of T3 in starvation and many non-thyroidal illnesses, and the formation of either the active or inactive metabolite of T4 appears to play an important part in the control of energy metabolism (William et al., 2008).

2.2.2. Thyroid hormones actions:

Thyroid hormones are essential for normal growth and development and have many effects on metabolic processes. They act by entering cells and binding to specific receptors in the nuclei, where they stimulate the synthesis of a variety of species of mRNA, thus stimulating the synthesis of polypeptides, including hormones and enzymes. Among the latter are key enzymes involved in energy metabolism, including cytochrome oxidase. Their most obvious overall effect on metabolism is to stimulate the basal metabolic rate, oxygen consumption and heat production, through the actions that include stimulating sodium, potassium-ATPases involved in ion transport and increasing the availability of energy substrates (William et al., 2008).

Overall, the effect of thyroid hormones is to increase net catabolism: weight loss and muscle wasting are typical features of excessive secretion of thyroid hormones. Thyroid hormones also increase the sensitivity of the cardiovascular and nervous systems to catecholamines, the former leading to increases in heart rate and cardiac output, and the latter to increased arousal (William et al., 2008).

2.2.3. Thyroid hormone synthesis:

Thyroid hormone synthesis involves a number of specific enzyme-catalyzed reactions, beginning with the uptake of iodide by the gland and culminating in the iodination of tyrosine residues in the protein thyroglobulin, these reactions are all stimulated by TSH. Rare, congenital forms of hypothyroidism caused by inherited deficiencies of each of the various enzymes concerned have been described. Thyroglobulin is stored within the thyroid gland in colloid follicles. These are
accumulations of thyroglobulin-containing colloid surrounded by thyroid follicular cells. Release of thyroid hormones (stimulated by TSH) involves pinocytosis of colloid by follicular cells, fusion with lysosomes to form phagocytic vacuoles, and proteolysis. Thyroid hormones are thence released into the bloodstream. Proteolysis also results in the liberation of mono and diiodotyrosines (MIT and DIT); these are usually degraded within thyroid follicular cells and their iodine is retained and re-utilized. A small amount of thyroglobulin also reaches the bloodstream (William et al, 2008).

2.2.4. Thyroid hormones in blood:

The normal plasma concentrations of T4 and T3 are 60-150 nmol/L and 1.0-2.9 nmol/L, respectively. Both hormone are extensively protein bound: some 99.98% of T4 and 99.66% of T3 are bound, principally to a specific thyroxine –binding globulin(TBG) and, to a lesser extent, to prealbumin and albumin. TBG is approximately one-third saturated at normal concentrations of thyroid hormones. It is generally accepted that only the free, non-protein-bound, thyroid hormones are physiologically active. Although the total T4 concentration is normally 50 times that of T3, the different extents to which these hormones are bound to protein mean that the free T4 concentration is only 2-3 times that of free T3 (William et al, 2008).

The precise physiological function of TBG is unknown; individuals who have a genetically determined deficiency of the protein show no clinical abnormality. It has, however, been suggested that the extensive binding of thyroid hormones to TBG provides a buffer that maintains the free hormones concentrations constant in the face of any tendency to change. Protein binding also reduces the amount of thyroid hormones that would otherwise be lost by glomerular filtration and subsequent renal excretion (William et al, 2008).

Total (free and bound) thyroid hormones concentrations in plasma are dependent not only on thyroid function but also on the concentrations of binding proteins. If these were to increase, the temporary fall in free hormone concentration caused by
increased protein binding would stimulate TSH release and this would restore the free hormone concentrations to normal: if binding protein concentrations were to fall, the reverse would occur. In either situation, there would be a change in the concentrations of total hormones, but the free hormone concentrations would remain normal. This is a matter of considerable practical importance, since changes in the concentrations of the binding proteins occur in many circumstances, causing changes in total hormone concentrations but not necessarily in those of the free hormones. Further, certain drugs, for example salicylates and phenytoin, displace thyroid hormones from their binding proteins, thus reducing total, but not free, hormone concentrations once a new steady state is attained. If an attempt is made to assess thyroid status in a patient who is not in a steady state, the results may be bizarre and misleading (William et al, 2008).

Only small amounts of T4 and T3 are excreted by the kidneys owing to the extensive protein binding. The measure route of thyroid hormone degradation is by deiodination and metabolism in tissues, but they are also conjugated in the liver and excreted in bile (William et al, 2008).

2.2.5. Disorders of the thyroid:

The metabolic manifestations of thyroid disease relate to either excessive or inadequate production of thyroid hormones (hyperthyroidism and hypothyroidism, respectively). The clinical syndrome that results from hyperthyroidism is thyrotoxicosis. The term myxoedema is often used to describe the entire clinical syndrome of hypothyroidism but strictly refers specifically to the dryness of the skin, coarsening of the features and subcutaneous swelling characteristic of severe hypothyroidism. Patients with thyroid disease may present with a thyroid swelling or goitre. Investigation may reveal hypo or hyperthyroidism but there may be no functional abnormality. A goitre can be the presenting feature of thyroid cancer (William et al, 2008).
A- Hyperthyroidism:

Primary hyperthyroidism is far more than secondary hyperthyroidism. The commonest single cause is graves disease, an autoimmune disease characterized by the presence of thyroid stimulating antibodies in the blood. These autoantibodies bind to TSH receptors in the thyroid and stimulate them in the same way as TSH, through activation of adenylate cyclase and the formation of cyclic AMP. Although thyrotoxicosis is usually the result of hyperthyroidism, it can also occur as a result of release of pre-formed thyroid hormones from a damaged gland (e.g in thyroiditis ) and from excessive intake of thyroid hormones (William et al,2008).

B- Hypothyroidism:

There are many causes of primary hypothyroidism but can also occur secondarily to decreased trophic stimulation both in hypopituitarism and hypothalamic disease. It is, however, uncommon for patients with pituitary failure to present with clinical features of hypothyroidism alone. The commonest cause of hypothyroidism in developed countries is atrophic myxoedema, the end result of autoimmune destruction of the gland. Iodine deficiency is a major cause of hypothyroidism in undeveloped countries, particularly in mountainous areas, although it is incidence has been reduced by supplementation programmes. Affected individuals usually have a goitre as a result of the increased secretion of TSH. The increased drive to the thyroid may be sufficient to prevent the development of frank hypothyroidism in borderline deficiency (William et al, 2008).

Subclinical hypothyroidism it is unusual to find patients whose plasma TSH concentration is elevated though with free thyroxine within the reference range. This may be associated with a history of treated hyperthyroidism but can occur de novo, particularly in the elderly. In the absence of clinical features of hypothyroidism, this is termed subclinical or compensated hypothyroidism (William et al,2008).
C- Thyroiditis:

Inflammation of the thyroid, or thyroiditis, may be a result of infection (usually viral) or autoimmune disease. In viral thyroiditis, associated with coxsackie, mumps and adenovirus, the inflammation results in a release of preformed colloid and there is an increase in the concentration of thyroid hormones in the blood. Patients may become transiently, and usually only mildly, thyrotoxic. This phase persists for up to six weeks and is followed by a similar period in which thyroid hormone output may be decreased, although not sufficiently to cause symptoms. Thereafter, normal function is regained (William et al, 2008).

Hashimoto’s thyroiditis, an autoimmune condition, has been mentioned as a cause of hypothyroidism. Autoantibodies are present in high titre, and the disease is associated with the presence of other organ-specific autoimmune diseases. Very occasionally, transient hyperthyroidism may occur early in the course of the disease due, as in viral thyroiditis, to increased release of preformed colloid (William et al, 2008).

D- Goitre and thyroid cancer:

Goitre or thyroid enlargement of the thyroid, can occur in patients with hyperthyroidism (e.g in Graves disease, toxic multinodular goitre or thyroid adenoma), hypothyroidism (e.g in Hashimotos disease or iodine deficiency) and in euthyroid individuals with benign or malignant tumours of the glands. Physiological enlargement of the thyroid may occur during adolescence, unaccompanied by any change in function, but, otherwise, thyroid function tests should be performed even in apparently euthyroid patients presenting with a goitre since the results may provide a clue to the cause. The biochemistry has no part to play in the diagnosis of thyroid cancer, with the exception of calcitonin-secreting medullary carcinoma. When patients with other thyroid cancers are treated by ablative doses of radioactive iodine and put on replacement thyroxine, the efficacy of the treatment can be assessed by measuring plasma thyroglobulin concentrations. Since small amounts of thyroglobulin are normally released from the gland together with thyroid hormones,
persistent thyroid activity can be inferred if thyroglobulin is present in the plasma (William et al., 2008).
3. Materials and Methods

3.1. Materials:

3.1.1. Study approach:

A quantitative method was used to measure thyroid hormones T3, T4 and TSH in type 2 diabetic Sudanese patients during the period from February to March 2016.

3.1.2. Study design:

This is case control study.

3.1.3. Study area:

The study was conducted in Jabber Abo Aleiz hospital in Khartoum State.

3.1.4. Study population:

The study included patients with diabetes mellitus.

3.1.5. Sample size:

This study included 50 patients with diabetes mellitus type 2 and 25 apparently healthy subjects serve as control without any diseases.

Inclusion criteria:

Sudanese patients with diabetes mellitus type 2 and apparently healthy volunteers were included in this study.

Exclusion criteria:

Lipemic, haemolytic and ecteric sample should be excluded.
3.1.6. **Ethical consideration:**

Consent was taken regarding acceptance to participate in the study and reassurance of confidentiality. Before the specimen was collected, the donors knew that this specimen was collected for research purpose.

3.1.7. **Data collection:**

Data were collected using a structural interviewing questionnaire, which was designed to collect and maintain all valuable information concerning each case examined.

3.1.8. **Sample collection and processing:**

About 5 ml of venous blood were collected from each participant (both case and control). The sample collected under aseptic conditions and placed in sterile plain containers and centrifuged for 5 minutes at 3000 RPM to obtain serum for thyroid hormones, then the obtained sample were kept at -20℃ till the time of analysis.

3.2. **Methods:**

3.2.1. **Estimation of T3 level using competitive using ELISA method:**

3.2.1.1. **Principle of method:**

Competitive Enzyme Immunoassay

The essential reagents required for a solid phase enzyme immunoassay include antibody, conjugate and native antigen.

Upon mixing antibody, enzyme-antigen conjugate and a serum containing the native antigen, a competition reaction results between the native antigen and the enzyme antigen conjugate for a limited number of binding sites. The interaction is illustrated by the followed:
equation:

$$\text{Enz Ag} + \text{ag} + \text{Abc.w} \xleftrightarrow{\frac{\text{Ka}}{\text{K-a}}} \text{AgAbC.W} + \text{Enz AgAbc.w}$$

**Ka** = Rate Constant of Association  
**K-a** = Rate Constant of Disassociation  
**K** = **Ka** / **K-a** = Equilibrium Constant

A simultaneous reaction between the biotin attached to the antibody and the streptavidin immobilized on the microwell occurs. This effects the separation of the antibody bound fraction after decantation or aspiration. The enzyme activity in the antibody bound fraction is measured by reaction with a suitable substrate to produce colour, which is inversely proportional to the native antigen concentration. By utilizing several different serum references of known antigen concentration, a dose response curve can be generated from which the antigen concentration of an unknown can be ascertained.  
(Appendix II)

**3.2.2. Estimation of T4 level using ELISA:**

**3.2.2.1. Principle of method:**

Competitive Enzyme Immunoassay

The essential reagents required for a solid phase enzyme immunoassay include immobilized antibody, enzyme antigen conjugate and native antigen.
Upon mixing immobilized antibody, enzyme-antigen conjugate and a serum containing the native antigen, a competition reaction results between the native antigen and the enzyme-antigen conjugate for a limited number of insolubulized binding sites. The interaction is illustrated by the followed equation:

$$\text{Enz Ag} + \text{ag} + \text{Abc.w} \xrightleftharpoons[k_a]{K_a} \text{AgAbC.W} + \text{Enz AgAbc.w}$$

- $\text{Abc.w}=\text{Monospecific immobilized Antibody (Constant)}$
- $\text{Ag}=\text{Native Antigen (Variable Quantity)}$
- $\text{EnzAg}=\text{Enzyme-antigen conjugate (Constant Quantity)}$
- $\text{AgAbc.w}=\text{Antigen-Antibody Complex}$
- $\text{Enz AgAbc.w}=\text{Enzyme-antigen Conjugate-Antibody Complex}$
- $K_a=\text{Rate Constant of Association}$
- $K_a=\text{Rate Constant of Disassociation}$
- $K=K_a/K_a=\text{Equilibrium Constant}$

After equilibrium is attained, the antibody-bound fraction is separated from unbound antigen by decantation or aspiration. The enzyme activity in the antibody-bound fraction is inversely proportional to the native antigen concentration. By utilizing several different serum references of known antigen concentration, a dose response curve can be generated from which the antigen concentration of an unknown can be ascertained. (Appendix III)

**3.2.3. Estimation of TSH level using ELISA:**

**3.2.3.1. Principle of method:**

**Immuno enzymometric assay:**

The essential reagents required for an immune enzymometric assay include high affinity and specificity antibodies (enzyme conjugated and immobilized), with different and distinct epitope recognition, in excess, and native antigen. In this procedure, the immobilization takes place during the assay at the surface of a
microplate well through the interaction of streptavidin coated on the well and exogenously added biotinylated monoclonal anti-TSH antibody

Upon mixing monoclonal biotinylated antibody, the enzyme-labeled antibody and a serum containing the native antigen, reaction results between the native antigen and the antibodies, without competition or steric hindrance, to form a soluble sandwich complex. The interaction is illustrated by the following equation

\[
\text{EnzAb(p) + AgTSH + BtnAb(m) } \xrightleftharpoons{K\text{-a}}^{Ka} \text{EnzAb(p)-AgTSH-BtnAb(m)}
\]

BtnAb(m) = Biotinylated Monoclonal Antibody (Excess Quantity)
AgTSH = Native Antigen (Variable Quantity)
EnzAb(p) = Enzyme - Polyclonal Antibody (Excess Quantity)
EnzAb(p)-AgTSH-BtnAb(m) = Antigen-Antibodies Sandwich Complex
ka = Rate Constant of Association
k-a = Rate Constant of Dissociation

Simultaneously, the complex is deposited to the well through the high affinity reaction of streptavidin and biotinylated antibody. This interaction is illustrated below:

\[
\text{EnzAb(p)-AgTSH-BtnAb(m)+StreptavidinC.W. } \Rightarrow \text{immobilized complex}
\]

StreptavidinC.W. = Streptavidin immobilized on well

After equilibrium is attained, the antibody-bound fraction is separated from unbound antigen by decantation or aspiration. The enzyme activity in the antibody-bound fraction is directly proportional to the native antigen concentration. By utilizing several different serum references of known antigen values, a dose response curve can be generated from which the antigen concentration of an unknown can be ascertained. (Appendix IV)

3.3. BMI calculation:

BMI expressed as body weight (Kg) per height (m²).
3.4. **Quality control:**

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

3.5. **Statistical analysis:**

Data obtained from this study was analyzed using statistical package for the social science (SPSS).
4. Results

The result of the biochemical determinant of serum of T3, T4 and TSH in patients with type 2 diabetes mellitus are given in tables and figures:

Table (4-1): Illustrate the age, sex, and family history of patients with diabetes mellitus. The results showed that the number of patients whose ages over sixty years was 31(62%) while the number of patients with ages below sixty years was 19(38%).

The numbers of male patients was 32(64%) While the numbers of female patients was 18(36%).

Patients who had family history disease constitute 34% while those who has no family history of disease constitute 66%.

Table (4-2): Represent the mean of T4:T3 ratio in both the study groups. Indicated that the patients with type 2 diabetes mellitus have more T4:T3 ratio(76.5±9.81) while the mean of control group is (66.12±17.71).

Table (4-3): Represent the mean of the levels of serum T3, T4 and TSH in both of the study groups.

The level of T4 was significantly increased (p-value =.000) in diabetic patients, and the serum levels of T3 and TSH were insignificantly differences (p-value=.229) (p-value=.211) respectively.

Mean ±SD for cases versus controls:

(1.25±0.32 versus 1.16±0.25) for T3
(8.99±1.06 versus 7.59±1.23) for T4
(1.96±0.80 versus 1.71±0.79) for TSH
Table (4-4): Represent the mean of the levels of serum T3, T4 and TSH in male and female group.

Mean ± SD for males versus females:

(1.187± 0.215 versus 1.272±0.405) for T3
(8.376±1.177 versus 8.769±1.464) for T4
(1.686±0.771 versus 2.009±0.834) for TSH

Figure (4-1): a Scatter plot shows the correlation between T3 level and duration of DM. The scatter showed that no correlation between T3 levels and duration of DM (r=.038, p-value=.792).

Figure (4-2): a Scatter plot shows the correlation between T4 level and duration of DM. The scatter showed that no correlation between T4 levels and duration of DM (r=.083, p-value=.568).

Figure (4-3): a Scatter plot shows the correlation between TSH level and duration of DM. The scatter showed that no correlation between TSH levels and duration of DM (r=.063, p-value=.662).
### Table (4-1)

Age, gender and family history of patients with diabetes mellitus:

<table>
<thead>
<tr>
<th>Variable</th>
<th>Number</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ages 40-60 years</td>
<td>19</td>
<td>38</td>
</tr>
<tr>
<td>Ages 61-80 years</td>
<td>31</td>
<td>62</td>
</tr>
<tr>
<td>Sex male</td>
<td>32</td>
<td>64</td>
</tr>
<tr>
<td>Sex female</td>
<td>18</td>
<td>36</td>
</tr>
<tr>
<td>Family history disease</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>17</td>
<td>34</td>
</tr>
<tr>
<td>NO</td>
<td>33</td>
<td>66</td>
</tr>
</tbody>
</table>

### Table (4-2)

Mean of T4:T3 ratio of patients with diabetes mellitus and control group:

<table>
<thead>
<tr>
<th>Variable</th>
<th>Patients group N=50</th>
<th>Control group N=25</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>T4:T3 ratio</td>
<td>76.5±9.81</td>
<td>66.12±17.71</td>
<td>0.002</td>
</tr>
</tbody>
</table>

- Results given in mean ±SD.
- P-value ≤ 0.05 consider significant.
Table (4-3)

The mean of serum T3, T4 and TSH in patients groups and control group:

<table>
<thead>
<tr>
<th>Variable</th>
<th>Case Mean ±SD</th>
<th>Control Mean±SD</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>T3(ng/ml)</td>
<td>1.25±0.32</td>
<td>1.16±0.25</td>
<td>.229</td>
</tr>
<tr>
<td>T4(micro g/dl)</td>
<td>8.99±1.06</td>
<td>7.59±1.23</td>
<td>.000</td>
</tr>
<tr>
<td>TSH(miu/ml)</td>
<td>1.96±0.80</td>
<td>1.71±0.79</td>
<td>.211</td>
</tr>
</tbody>
</table>

-Result given in mean±SD.

- p-value ≤ 0.05 consider significant.

Table(4-4)

The mean of T3, T4 and TSH in male and female group:

<table>
<thead>
<tr>
<th>Variable</th>
<th>Male Mean ± SD</th>
<th>Female Mean ± SD</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>T3</td>
<td>1.187 ± 0.215</td>
<td>1.272 ± 0.405</td>
<td>.238</td>
</tr>
<tr>
<td>T4</td>
<td>8.376 ± 1.177</td>
<td>8.769 ± 1.464</td>
<td>.205</td>
</tr>
<tr>
<td>TSH</td>
<td>1.686 ± 0.771</td>
<td>2.009 ± 0.834</td>
<td>.093</td>
</tr>
</tbody>
</table>

-Result given in mean±SD.

-p-value ≤ 0.05 consider significant.
Figure (4-1)

Scatter plot of correlation between T3 level and duration of DM ($r = 0.038$, p-value = 0.792)
Figure (4-2)

Scatter plot of correlation between T4 level and duration of DM ($r=0.083$, $p$-value $=0.568$)
Figure (4-3)

Scatter plot of correlation between TSH level and duration of DM (r=.063, P-value=.662).
5.1. Discussion

Diabetes mellitus and thyroid diseases are the two common endocrine diseases. On one hand, thyroid hormones contribute to the regulation of carbohydrate metabolism and pancreatic function, and on the other hand, diabetes affects thyroid function to variable extents. The association between diabetes mellitus and thyroid disorders is widely known, with the first studies published in 1979. (Feely et al, 1678)

This study conducted to study the effect of diabetes mellitus on levels of T3, T4 and TSH. They were chosen for assessment the effect of DM on the levels of parameters.

Preliminary investigated and findings obtained from specially designed questionnaire revealed that the majority of patients with DM participated in this study were in the average ages of about 60 years. This agreed with previous result confirm that thyroid disease is common in the general population and the prevalence increases with age (Hegedus et a, 1983).

From the findings of this study it appears that serum level of T4(8.99±1.06 versus 7.59±1.23) was significantly increased at (p-value=.000) in the Sudanese diabetic patients versus control subjects and the serum levels of T3 (1.25±.32 versus 1.16±.25) and TSH(1.96±.80 versus 1.71±.79) were insignificantly different at (p-value=.229) and (p-value=.211) respectively in patients with DM group compared to control group. This result agreed with another result of study carried by (Saunders et al,1978), showed a significantly increased inT4:T3 ratio in patients group when compared with control group.

Also this study disagreed with another study carried by (Schlienger et al, 1982) who showed a significant decreased in T3 levels in type 2 diabetic patients.

Also this result disagreed with another study carried by (Gurjeet et al, 2011) which finding confirmed that Significant decreased of T3and T4 and higher level of TSH in diabetic group.
The findings of this study showed that there were no correlation between duration of DM and concentration of T3($r=.038$, p-value=.792), T4($r=.083$, p-value=.568) and TSH($r=.063$, p-value=.662) as appeared in figure (4-1) (4-2) (4-3).

This result agree with another study carried by (Diez et al, 2011) which showed no correlation between thyroid hormones and duration of diabetes.

In this study there were no significant difference between the mean of level of thyroid hormones in diabetic females compared to diabetic males, this results disagreed with another study carried by (Michalek et al, 2000) which showed a significant increased in the mean of thyroid hormones in diabetic females compared to diabetic males.
5.2. Conclusion:

According to the results of this study it is concluded that:

1- T4 is increased in patients with type 2 diabetes mellitus.

2- No difference in the levels of T3 and TSH in patients with type 2 diabetes mellitus.

3- No correlation between thyroid hormones and duration of diabetes mellitus.
5.3. Recommendations:

From the findings of this study it is recommended that:

1- Estimation and comparison between free T3 and free T4 in study and control group.

2- Diabetic patients should be monitored regularly for thyroid hormones to avoid complication of disease.


References


Appendix I

Questionnaire

Sudan University of Sciences and Technology
Collage of graduate studies

Estimation of Thyroid Hormones and TSH in type 2 Diabetic Patients in Khartoum State

Number ( )

A: General information:-

1- Name: ____________________________  2- Age: ____________________________

3- Gender: ____________________________

B: Duration of disease (in years): ____________________________

C: Family history of diabetes mellitus:

1- Yes ( )  2- No ( )

D: Height: ____________________________  weight: ____________________________

F: History of other disease:-

G: Investigation:-

1- Serum T3: ____________________________ ng/ml

2- Serum T4: ____________________________ microg/dl

3- Serum TSH: ____________________________ miu/ml