بسم الله الرحمن الرحيم

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Title:

Estimation of Thyroid Hormone and TSH in Type II DiabeticPatientsAttending Ribat University Hospital, Khartoum State, Sudan

A Thesis Submitted in Partial Fulfillment of the Requirements of Master Degree in Medical Laboratory Sciences (Clinical Chemistry)

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الأية

بسم الله الرحمن الرحيم

قال تعالى:

(إن الله لايستحيي أن يضرب مثلا مابعوضة فما فوقها فأما الذين آمنوا فيعلمون أنه الحق من ربهم وأما الذين كفروا فيقولون ماذا أراد الله بهذا مثلا يضل به كثيرا ويهدي به كثيرا ومايضل به إلا الفاسقين

سورة البقرة

رقم الاية (26)

صدق الله العظيم

Dedication

To my mother……

Who is one of the most inspiring and strong women I ever know. She gave me and taught me unconditional love and acceptance.

To the soul of my father……

Who have offered me everything without taking anything!

To my family……

Who offered me unconditional love and support throughout the course of this thesis.

Acknowledgements

All thanks to Allah from the start to the end…..

And pray for Prophet Mohammed peace be upon him

I would like to acknowledge the contribution of my

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Doctor: Mosab Omer Khalid

Who guide me throughout my way and helped me to make this research as accurate and useful as possible.

And I'm grateful to my friends and all those who contributed their time and helped me.

My thanks also extend to my college and my teachers

مستخلص الدراسة

هدفت هذه الدراسة الي تحديد مستوى هرمونات الغدة الدرقية والهرمون المحفز للغدة الدرقية في بلازما مر ضى السكري النوع الثاني لدي السودانيين.
اجريت هذه الدراسة (دراسة الحالة والحالة الضابطة) في مستشفي الرباط الجامعي بالخرطوم ، تم الفحص علي (40) مريض يعانون من السكري النوع الثاني ، و (30) شخص غير مريض كمجموعة ضابطة في الفترة من مارس الي يونيو 2018 ، وقد تم قياس مستوى هرمونات الغدة الدرقية والهرمون المحفز للغدة الدرقية بواسطة استخدام جهاز توشو (AIA 360).

فقد وضح من هذه الدراسة زيادة ($p<0.05$) في مستوى الثيرونين رباعي الايودين في مرضي السكري النوع الثاني مقارنة مع المجموعة الضابطة . متوسط مستوى الهرمون لدى المرضى 15,53 وفي العينة الضابطة 8,10 والقيمة الاحتمالية كانت 0.000 ولا يوجد اختلاف في مستوى الثيرونين ثلاثي الايودين والهرمون المحفز للغده الدرقية ($p>0.05$)

كما اوضحت هذة الدراسة ايضا عدم تأثر في مستوى هرمونات الغدة الدرقية والهرمون المحفز للغدة الدرقية بالعمر وجنس المريض ، وايضا لم يتاثرا بالفترة الزمنية للمرض.

Abstract

The aim of this study was to determine the serum levels of thyroid hormone and thyroid stimulating hormone in Sudanese patient with type2 DM.

A case control study was carried out in Ribat University Hospital in Khartoum state, during period from March to June 2018; (40) patients male and females with DM type 2 attended Ribat University Hospital and (30) healthy individuals as control group, these samples were evaluated for the serum levels of thyroid hormones and thyroid stimulating hormone determined by Tosoh AIA 360.

The study results showed that serum level of T4 was statistically significant increased (p-value<0.05) in diabetic patients when compared with control group (mean of case=15.53 and control=8.10) and the serum levels of T3 and TSH were insignificantly (P-value>0.05).

Also the statistically study showed the age of the patient and the duration of the disease have no effect on the serum level of T3, T4 and TSH.

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List of Abbreviations

DM	Diabetes mellitus
DIT	Diiodotyrosines
GFR	Glomerular filtration rate
HLA	Human leukocyte antigen
IDDM	Insulin dependent diabetes mellitus
LADA	Latent Autoimmune Diabetes of Adulthood
MIT	Mono iodotyrosines
NIDDM	None Insulin dependent diabetes mellitus
rT3	Reverse triiodothyronine
T4	Thyroxin
T3	Triiothyronine
TSH	Thyroid stimulating hormones
TBG	Thyroxin-binding globulin
WHO	World health organization

1.1Introduction

DM 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 butter fly .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: thyroxin (T4) and triiothyronine (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 *etal*, 2008).

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

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In type 1 and 2 diabetes, the metabolism of food stuff is altered (Briscoe *etal*, 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 *etal*, 2006, Guyton and Hall, 2006).

Diabetic patients have a higher prevalence of thyroid disorders than the normal population. (Wu, 2000). Thyroid disease is found in both type 1 and 2 DM. People with type 1 DM 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 *etal*, 1983; Wu, 2000).

1.2 Rationale

The incidences of DM are increasing in Sudan.

Study conducted in Bangladesh by (Sufial *etal* 2008) reported that diabetic patients have a higher prevalence of thyroid disorders.

The incidences of thyroid disorder in diabetic population was reported to be (13.4%) with higher prevalence (31%) in female DM patients as compared to (69%) in male DM (Perros *etal*, 1995).

This study aimed to answer the question if there is any significant changes in the concentration of thyroid hormones in Sudanese patients with DM.

1.3. Objectives

1.3.1 General objective

To measure the levels of thyroid hormones and TSH in diabetic patients

1.3.2 Specific objectives

- To find out levels of T3, T4 and TSH with duration of diabetes mellitus.
- To assess levels of T3, T4 and TSH with age of patients.
- To study levels of T3, T4 and TSH with gender of patients.

2 Literature Review

2.1 Diabetes mellitus

DM 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 *etal* 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 (200mg/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

2.1.1.1 Primary diabetes mellitus

Which classify to two types: 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 *etal*, 2008).

Causes of type 1 DM include: Genetics: Susceptibility to type1diabetes is inherited, but the mode of inheritance is complex and has not been completely

defined. It is a mutagenic trait, and the major locus is the major histocompatibility complex on chromosome (Lawrence *etal*, 2008).

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

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 cow's milk (Carl *etal*, 2008).

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 *etal*, 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 DM 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 DM over the age of 25 have Latent Autoimmune Diabetes of Adulthood (LADA) patient with LADA may be misclassified as having type 2 DM (Marshall, 2004).

Causes of type 2 DM include: Genetics: Genetic factor contribute to the development of type 2 DM. For example, the concordance rate for type 2 diabetes in identical twins approaches (100%). In addition, type 2 DM it is 10 times more likely to occur in obese individual without a diabetic family history. The mode of inheritance however is unknown (Carl *etal*, 2008).

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

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 *etal*, 2008).

Other factors such as Family history of type 2 diabetes, the duration of obesity and the distribution of fats (Carl *etal*, 2008).

2.1.1.2 Secondary DM

May be caused by Absolute insulin deficiency due to pancreatic disease (chronic pancreatitis, hemochromatosis, cystic fibrosis) or 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).

2.1.1.3 Gestational DM

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

2.1.2 Pathophysiology of diabetes mellitus

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

As hepatic glucose overproduction continues, the plasma glucose concentration 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 *etal*, 2005).

The individual with type 1 DM has a higher tendency to produce ketones. Patient with type 2 DM seldom generate ketones, but instead have a greater tendency to develop hyperosmolar nonketotic states (Bishop *etal*, 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 DM, there is an absence of insulin with an excess of glucagons. This permits gluconeogenesis and lipolysis to occur (Bishop *etal*, 2005).

In type 2 DM, 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 *etal*, 2005).

2.1.3 Complications of DM

2.1.3.1 Acute metabolic complications

Patients with diabetes mellitus may develop one of several metabolic complications. These include: Diabetic ketoacidosis and Hyperosmolar nonketotic coma (Mayne, 1998).

2.1.3.2 Late complications

Vascular disease is a common complication of DM which include Macro vascular 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).

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).

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

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 uremia (Allan *etal*, 2004).

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 *etal*, 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 *etal*, 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 *etal*, 2005).

2.1.4.1 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 *etal*, 2005).

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

2.1.4.2 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 is 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 butter fly .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 *etal*, 2005).

2.2.1 Thyroid anatomy

The thyroid gland is positioned in the lower anterior neck and is shaped like a butterfly. It is made up of two lobes that rest on each side of the trachea, with a band of thyroid tissue called the isthmus running anterior to the trachea and bridging the lobes. Posterior to the thyroid gland are the parathyroid glands that regulate serum calcium levels and the recurrent laryngeal nerves that innervate the vocal cords. These posterior structures become important during thyroid surgery, when care must be exercised to avoid injury that could lead to hypocalcemia or permanent hoarse voice (Bishop *etal*, 2010).

2.2.2 Thyroid physiology

Once released from the thyroid gland, thyroid hormone circulates in the bloodstream where free T4 and T3 are available to travel across the cell membrane. In the cytoplasm, T4 is deiodinated into T3, the active form of thyroid hormone. T3 combines with its nuclear receptor on thyroid hormone-responsive genes,

leading to production of messenger RNA that, in turn, leads to production of proteins that influence metabolism and development. Effects of thyroid hormone include tissue growth, brain maturation, increased heat production, increased oxygen consumption, and an increased number of β -adrenergic receptors. Clinically, individuals who have excess thyroid hormone (thyrotoxicosis) will have symptoms of increased metabolism such as tachycardia and tremor, while individuals with hypothyroidism note symptoms of lowered metabolism like edema and constipation (Morley JE, 1990).

2.2.3 Thyroid hormones

Thyroid gland secretes three hormones: thyroxine (T4) and triiothyronine (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 *etal*, 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 *etal*, 2008).

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 *etal*, 2008).

2.2.4 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 - ATPase involved in ion transport and increasing the availability of energy substrates (Stands bury JB, 2000).

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 (Stands bury JB, 2000).

2.2.5 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 reutilized. A small amount of thyroglobulin also reaches the bloodstream (William *etal*, 2008).

2.2.6 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 (Morley JE, 1990).

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 (Morley JE, 1990).

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 bindings 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 (Bishop *etal*, 2010).

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 (Bishop *etal*, 2010).

2.2.7 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 goiter can be the presenting feature of thyroid cancer (William *etal*, 2008).

2.2.7.1 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 (Lavin L, 2009).

2.2.7.2 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 (Lavin L, 2009).

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 *etal*, 2008).

2.2.7.3 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 (Lavin L, 2009).

Hashimoto s thyroiditis, an autoimmune condition, has been mentioned as a cause of hypothyroidism. Autoantibodies are present in high titer, 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 *etal*, 2008).

2.2.7.4 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 (Lavin L, 2009).

3. Materials and Methods

3.1 Study approach

A quantitative method was used to measure thyroid hormones T3, T4 and TSH in diabetic Sudanese patients during the period from March to June 2018.

3.2 Study design

This is analytical laboratory based case control study.

3.3 Study area

The study was conducted in Ribat University Hospital in Khartoum State.

3.4 Study population

The study included patients with diabetes mellitus.

3.5 Sample size

This study included (40) patients with diabetes mellitus and (30) apparently healthy subjects serve as control without any diseases.

3.6 Selection criteria

3.6.1 Inclusion criteria

Sudanese patients with DM and apparently healthy volunteers were included in this study.

3.6.2 Exclusion criteria

Any individual have disease that affect the result.

3.7 Ethical consideration

Ethical approval will be obtained from ethical committee of Shendi University and Informed consent will be taken from all the participants prior to their inclusion in the study. All the procedures will be informed to the patients in their native language and informed written consent will be taken from them.

3.8 Ethical committee

Clearance from Shendi University ethical committee.

3.9 Data collection

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

3.10 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 mins) at 3000 RPM to obtain serum for thyroid hormones, then they obtained sample were kept at -20c till the time of analysis.

3.11 Quality control

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

3.12 Statistical analysis

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

3.13 Estimation of thyroid hormone using TOSOH BIOSCIENCE

AIA-360 Automated immunoassay Analyzer

Sophisticated diagnostics in a compact analyzer

The AIA-360 size and affordability make it an excellent fit for POLs and small hospitals as well as for specialty testing or for use as a back-up analyzer.

System features: 36 tests per hour, First result ~ 20 minutes, Full ST test menu, Random access, Continuous processing, Simple touch screen operation and Bar-coded primary tube sampling

Principle of T3, T4 and TSH see the appendix

4.1 Result

This study includes (70) blood samples, a (40) from these sample were collected from patient with diabetes mellitus and samples (30) were collected from apparently healthy subjects as control without any diseases. A total of 40 blood samples from patient with diabetes mellitus the males were 15 with 38% while the rests 25 were females with (62%), the age of population are matched.

The results of the study were presented in tables and figures.

Table 4.1 Mean and Std. deviation of T3 among case and control group

Study group	Number of samples	Mean	Std.Deviation	P.value
Case	**40**	**1.29**	**1.29**	**0.507**
Control	**30**	**1.35**	**1.46**	

P.value less than 0.05 indicate significant change.

P.value more than 0.05 indicate insignificant change.

Table 4.2 Mean and Std. deviation of T4 among case and control group

Study group	Number of samples	Mean	Std.Deviation	P.value
Case	40	15.53	2.71	0.000
Control	30	8.10	0.85	

Table 4.3 Mean and Std. deviation of TSH among case and control group

Study group	Number of samples	Mean	Std.Deviation	P.value
Case	40	2.10	0.94	0.099
Control	30	1.98	0.77	

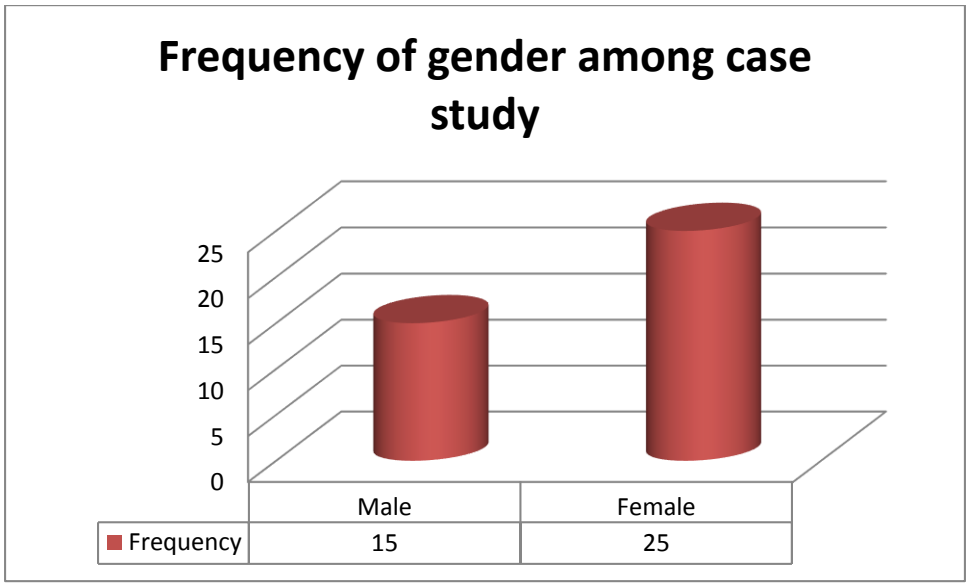

Figure 4.1 Distribution of gender among case group

Table 4.4 Relation between Hormones and gender among case study

Hormones	Gender				P.value
	Male	Mean	Female	Mean	
T3	15	0.92	25	1.26	0.134
T4	15	15.46	25	15.4	0.505
TSH	15	1.96	25	2.18	0.089

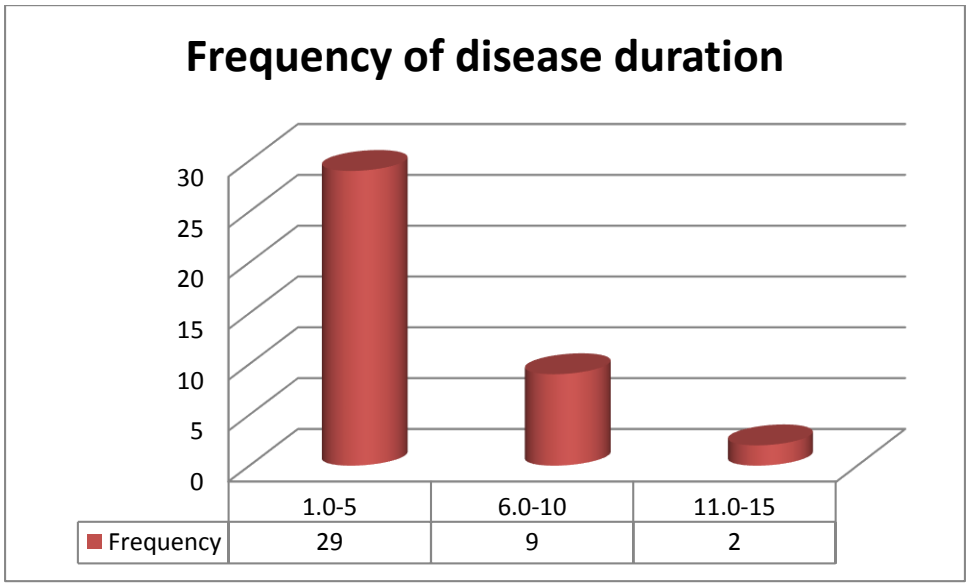

Figure 4.2 Distribution of disease duration among case group

Table 4.5 Relation between Hormones and disease duration among case study

Hormones	Duration						P.value
	1-5 years	Mean	6-10 years	Mean	11-15 years	Mean	
T3	29	1.24	9	1.23	2	1.25	0.196
T4	29	15.4	9	15.4	2	17.5	0.694
TSH	29	2.21	9	1.88	2	1.3	0.342

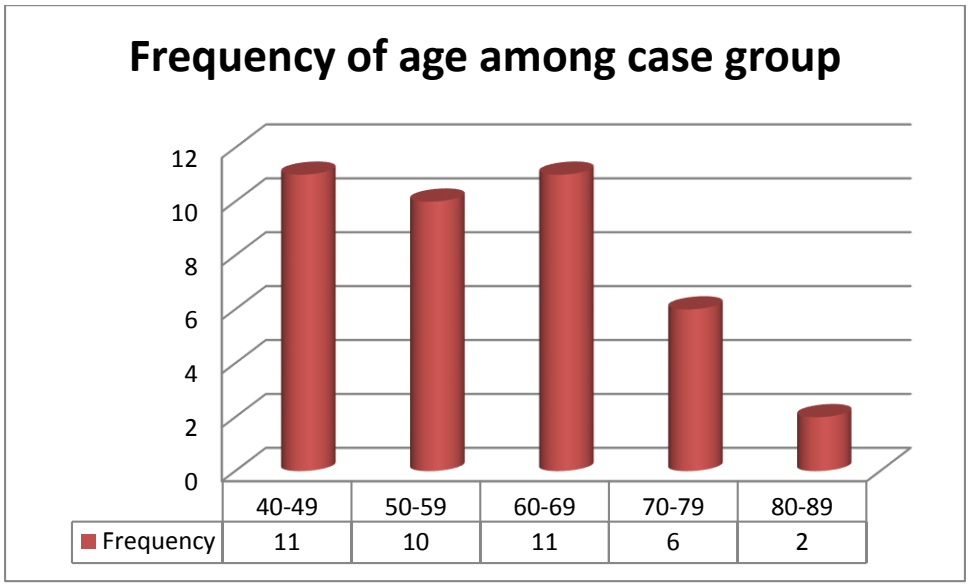

Figure 4.3 Distribution of age among case group

Table 4.6 Relation between Hormones and age groups among case study

Hormones	Age groups										P.value
	40-49	Mean	50-59	Mean	60-69	Mean	70-79	Mean	80-89	Mean	
T3	11	1.26	10	1.30	11	1.3	6	1.36	2	1.17	0.252
T4	11	13.8	10	15.4	11	16	6	16	2	18	0.619
TSH	11	2.1	10	2.21	11	1.9	6	2	2	2.8	0.064

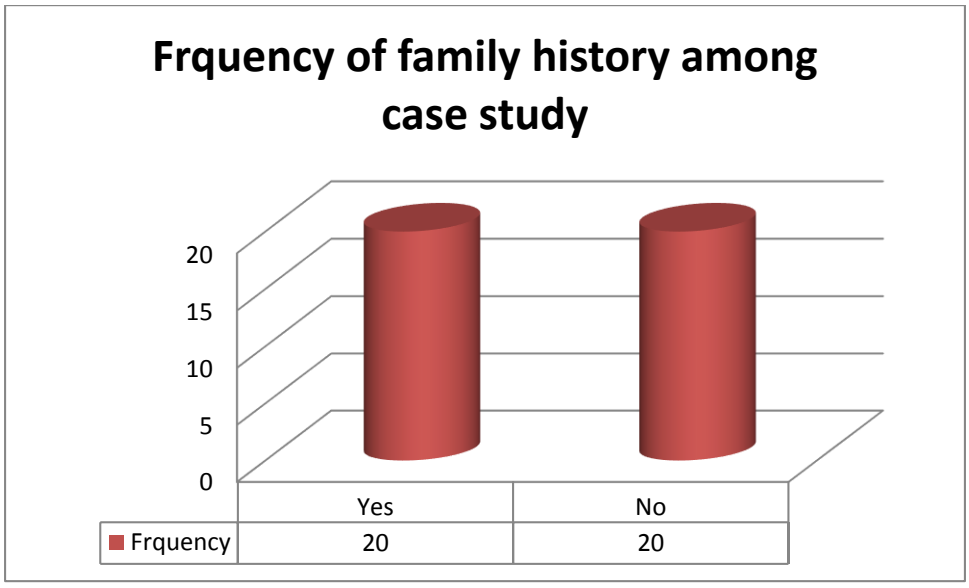

Figure 4.4 Distribution of family history among case group

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).

The present study was carried out to investigate T3, T4 and TSH among diabetes mellitus in Ribat University Hospital, this hospital is especially for police and student, in Khartoum state in the Sudan during period from March to July 2018;(70) blood samples were collected, a (40) of these samples were collected from patient with diabetes mellitus as test group, and (30) samples were collected from health individual as control group.

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).

The present study showed statically significant difference between the mean of the serum levels of T4 (mean=15.53) of the test group when compared with that of the control group (mean=8.10), with (P-value = 0.000). the serum levels of T3 and TSH were insignificantly at (p-value=0.507) and (P-value=0.099) respectively,. This result agreed with another result of study carried by (Saunders *etal*, 1978), showed a significantly increase in T4 level and there is no different in TSH and T3 in patients group when compared with control group.

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

And 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 (P-value=0.196), T4 (p-value= 0.694) and TSH (P-value=0.342) . 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 patients according to age (p-value$>$0.05), and gender (p-value$>$0.05), the result of gender disagreed with another study carried by (Michalek et al, 2000) which showed a significant increase 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 type2 diabetes mellitus.
2. No difference in the levels of T3 and TSH in patients with type 2 DM.
3. No correlation between thyroid hormones and duration of DM, also the age and gender of patients.

5.3 Recommendations

From the findings of this study it is recommended that:

1. Farther research include 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.

6.1 References

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Appendix (1) **Shendi University**

Faculty of Graduate Studies and Scientific Research

Research questionnaire

Estimation of Thyroid Hormones and TSH in Diabetic Patients in Khartoum State.

The patient's questionnaire includes the following sections:

Section 0 – Questionnaire identification data (6) codes
Section 1 – Background characteristics (6) Questions

0 QUESTIONNAIRE IDENTIFICATION DATA

001 QUESTIONNAIRE IDENTIFICATION NUMBER |___|___|___|

002 CITY----------

003 HOSPITAL----------

004 NAMES ----------

005 DATE OF INTERVIEW: __\ ____ \ _____

006 CHECKED BY SUPERVISOR: Signature _____ Date _____

Section 1: Background characteristics

No.	Questions and filters	Coding categories
Q101	Duration	□ less than 6 years □ more than 6 years □ No Response
Q102	Type	□ type I □ type II
Q103	Age	□ ……years □ No Response
Q104	Gender	□ male □ female
Q105	History of disease	□ Yes □ No

If no escape the following questions; but if yes, go to the following questions:

Q106	Other disease	□ Thyroid disease □ any disease that affect thyroid hormone

If there is positive history of any diseases we excluded this patient from our study.

Investigation:

Serum T3: …… ng/ml Serum T4: …… microg/dl Serum TSH: …… miu/m