Chapter One

1.1 Introduction

Diabetes mellitus (DM) is a group of metabolic diseases characterized by hyperglycemia resulting from defects of insulin secretion and/or increased cellular resistance to insulin (American Diabetes Association, 1998, American Diabetes Association, 2007). Chronic hyperglycemia and other metabolic disturbances of DM lead to long-term tissue and organ damage as well as dysfunction involving the eyes, kidneys, nervous and vascular systems (World Health Organization Study Group, 1994), (International Expert Committee, 2009). The definitions and categories of DM used in this document are based on the most recent classifications reported by the American Diabetes Association. (American Diabetes Association, 2009), (Fagot-Campagna et al, 2000).

The prevalence and incidence of DM in Sudan, as in many others low-income countries, are increasing to epidemics proportion, leading to the emergence of a public health problem of major socio-economic impact (Beran, 2006). Diabetes Mellitus in Sudan is associated with poor glycemic control, a high prevalence of complications, a low quality of life and particularly with morbidity. Patients with a median duration of diabetes of 9 years showed a high prevalence of micro and macro vascular complications. (Elbagir et al, 1996).

The thyroid hormones, thyroxine (T4) and triiodothyronine (T3), are tyrosine-based hormones produced by the thyroid gland primarily responsible for regulation of metabolism. Iodine is important for the production of T3 and T4. A deficiency of iodine leads to decreased production of T3 and T4, enlarges the thyroid tissue and will cause the disease known as goitre. The major form of thyroid hormone in the blood is thyroxine (T4), which has a longer half-life than T3. The ratio of T4 to
T3 released into the blood is roughly 20 to 1. Thyroxine is converted to the active T3 (three to four times more potent than T4) within cells by deiodinases.

Most of the thyroid hormones circulating in the blood are bound to transport proteins (thyroxine-binding globulin (TBG), transthyretin or "thyroxine-binding prealbumin" (TTR or TBPA), and paraalbumin). Only a very small fraction of the circulating hormones is free (unbound) and biologically active (T4 0.03% and T3 0.3%), hence measuring concentrations of free thyroid hormones is of great diagnostic value. (Dietrich, 2008).

Thyroid-stimulating hormone (TSH or thyrotropin) is a hormone that stimulates the thyroid gland to produce thyroxine (T4), and then triiodothyronine (T3) which stimulates the metabolism of almost every tissue in the body. It is a glycoprotein hormone synthesized and secreted by thyrotrope cells in the anterior pituitary gland, which regulates the endocrine function of the thyroid gland (Baskin et. al, 2002).

HbA1c, or glycated haemoglobin, is a term used to describe a series of stable minor haemoglobin component formed none enzymatically from haemoglobin and glucose. The level of HbA1c in a blood sample provides a glycemic history of the previous 120 days, the average erythrocyte life span. The optimal use of HbA1c testing requires standardization of HbA1c assays. The national glycohaemoglobin standardization program has established standard assays for HbA1c based on the results of the Diabetes Control and Complications Trial (DCCT). (American Diabetes Association, 2001).
1.2 Rationale:

Diabetes mellitus (DM) is a chronic disease with long-term complications, including ischemic heart disease, diabetic nephropathy, neuropathy and retinopathy. Thyroid disorders are common and second to diabetes mellitus, are the most common endocrine diseases. People with diabetes have an increased risk of developing thyroid disorders.

Since people with one form of autoimmune disorder have an increased chance of developing other autoimmune disorders, people with Type 1 diabetes have a higher risk of autoimmune thyroid disorders. (Patricia, 2000).

In Sudan, there is no previous study about the prevalence of thyroid disorders in Sudanese diabetic patients. Although thyroid disorders are common in patients with type 1, regular assessments of thyroid hormones and thyroid stimulating hormone are rarely done in Sudan for patients with diabetes.

This study will help authorities to evaluate the problem more objectively and implement appropriate measures to reduce morbidity and mortality in patients with type 1 diabetes.
1.3 Objectives:

1.3.1 General objective:
To assess the serum levels of Thyroid Hormones (T3, T4), Thyroid Stimulating Hormone (TSH) and Haemoglobin A1c% (HbA1c%) among Sudanese with type 1 Diabetes (as a test group) in comparison with apparently healthy non-diabetics (as a control group).

1.3.2 Specific objectives:
1. To measure the serum levels of T3, T4, TSH and HbA1c% among Sudanese with type 1 diabetes and in apparently healthy non-diabetic controls.
2. To assess the relationship of the serum levels of T3, T4 and TSH to HbA1c% (as a marker of glycemic control) and the duration of diabetes.
3. To assess female : male ratio of thyroid disorders in Sudanese diabetic Patients.
Chapter Two

2. Literature review

2.1 Diabetes mellitus:

2.1.1 Definition:

The term diabetes mellitus describes a metabolic disorder of multiple aetiology characterized by chronic hyperglycemia with disturbances of carbohydrate, fat, and protein metabolism resulting from defects in insulin secretion, insulin action, or both. The effects of diabetes mellitus include long–term damage, dysfunction and failure of various organs. Diabetes mellitus may present with characteristic symptoms such as thirst, polyuria, blurring of vision, and weight loss. In its most severe forms, ketoacidosis or a non–ketotic hyperosmolar state may develop and lead to stupor, coma and, in absence of effective treatment, death. Often symptoms are not severe, or may be absent, and consequently hyperglycaemia sufficient to cause pathological and functional changes may be present for a long time before the diagnosis is made. The long–term effects of diabetes mellitus include progressive development of the specific complications of retinopathy with potential blindness, nephropathy that may lead to renal failure, and/or neuropathy with risk of foot ulcers, amputation, charcot joints, and features of autonomic dysfunction, including sexual dysfunction. People with diabetes are at increased risk of cardiovascular, peripheral vascular and cerebrovascular disease (McCance et al, 1994).

Several pathogenetic processes are involved in the development of diabetes. These include processes which destroy the beta cells of the pancreas with consequent insulin deficiency, and others that result in resistance to insulin action. The
abnormalities of carbohydrate, fat and protein metabolism are due to deficient action of insulin on target tissues resulting from insensitivity or lack of insulin. (McCance et al, 1994).

### 2.1.2 Classification of diabetes mellitus:

Diabetes mellitus is classified into:

#### 2.1.2.1 Type 1:

Type 1 indicates the processes of beta–cell destruction that may ultimately lead to diabetes mellitus in which “insulin is required for survival” to prevent the development of ketoacidosis, coma and death. It can be associated with other autoimmune endocrine disorders as well as autoimmune impairment of non-endocrine tissue. The associated autoimmune disease may influence the control of diabetes by impairing function of the respective organ.

An individual with a Type 1 process may be metabolically normal before the disease is clinically manifest, but the process of beta–cell destruction can be detected. Type 1 is usually characterized by the presence of anti–GAD, islet cell or insulin antibodies which identify the autoimmune processes that lead to beta–cell destruction. In some subjects with this clinical form of diabetes, particularly non–Caucasians, no evidence of an autoimmune disorder is demonstrable and these are classified as “Type 1 idiopathic”. Aetiological classification may be possible in some circumstances and not in others. Thus, the aetiological Type 1 process can be identified and sub–categorized if appropriate antibody determinations are performed. It is recognized that such measurements may be available only in certain centers at the present time. If these measurements are performed, then the classification of individual patients should reflect this (National Diabetes Data Group, 1995).
2.1.2.2 Type 2:
Type 2 is the most common form of diabetes and is characterized by disorders of insulin action and insulin secretion, either of which may be the predominant feature. Both are usually present at the time that this form of diabetes is clinically manifest. By definition, the specific reasons for the development of these abnormalities are not yet known (National Diabetes Data Group, 1995).

2.1.2.3 Secondary diabetes:
Also known as other specific types, this form of hyperglycemia may be secondary result of non-insulin related events. Blood glucose levels are increased in endocrine disorders, such as Cushing's syndrome; in exocrine disorders, such as cystic fibrosis; and as a response to specific drugs, such as protease inhibitors and glucocorticoids. Other causes of this form of diabetes are the results of genetic defects that affect pancreatic beta cells or the action of insulin (National Diabetes Data Group, 1995).

2.1.2.4 Gestational diabetes mellitus:
Gestational diabetes is similar in etiology to type 2 diabetes; however, it’s defined as diabetes that diagnosed in pregnancy. Pregnancy is associated with increased tissue cell resistance to insulin. Most pregnant women will compensate with increased secretion of insulin; those individuals who are unable to compensate may develop Gestational diabetes. The hyperglycemia of Gestational diabetes diminish after delivery; however, the individual who has developed Gestational diabetes is at higher risk for the development of type 2 diabetes thereafter (National Diabetes Data Group, 1995).

2.1.3 Description of aetiological types:
Patients with any form of diabetes may require insulin treatment at some stage of their disease. Such use of insulin does not, of itself, define the aetiological class.
2.1.3.1 Type 1 (beta–cell destruction, usually leading to absolute insulin deficiency); this could be:

(i) Autoimmune Diabetes Mellitus:

This form of diabetes previously encompassed by the terms insulin–dependent diabetes, Type 1 diabetes, or juvenile–onset diabetes, results from autoimmune mediated destruction of the beta cells of the pancreas. The rate of destruction is quite variable, being rapid in some individuals and slow in others (Zimmet et al, 1994). The rapidly progressive form is commonly observed in children, but also may occur in adults (Humphrey et al, 1998). The slowly progressive form generally occurs in adults and is sometimes referred to as latent autoimmune diabetes in adults (LADA). Some patients, particularly children and adolescents, may present with ketoacidosis as the first manifestation of the disease (Japan and Pittsburgh Childhood Diabetes Research Groups, 1985).

Others have modest fasting hyperglycaemia that can rapidly change to severe hyperglycaemia and/or ketoacidosis in the presence of infection or other stress. Still others, particularly adults, may retain residual beta–cell function, sufficient to prevent ketoacidosis, for many years (Zimmet, 1995).

Individuals with this form of Type 1 diabetes often become dependent on insulin for survival eventually and are at risk for ketoacidosis (Willis et al, 1996). At this stage of the disease, there is little or no insulin secretion as manifested by low or undetectable levels of plasma C–peptide (Hother–Nielsen et al, 1988).

Markers of immune destruction, including islet cell autoantibodies, and/or autoantibodies to insulin, and autoantibodies to glutamic acid decarboxylase (GAD) are present in 85–90 % of individuals with Type 1 diabetes mellitus when fasting diabetic hyperglycaemia is initially detected (Verge et al, 1996). The peak
incidence of this form of Type 1 diabetes occurs in childhood and adolescence, but
the onset may occur at any age, ranging from childhood to the ninth decade of life
(Mølbak et al, 1994). There is a genetic predisposition to autoimmune destruction
of beta cells, and it is also related to environmental factors that are still poorly
defined. Although patients are usually not obese when they present with this type
of diabetes, the presence of obesity is not incompatible with the diagnosis. These
patients may also have other autoimmune disorders such as Graves’ disease,
Hashimoto’s thyroiditis, and Addison’s disease (Betterle et al, 1983).

(ii) Idiopathic:

There are some forms of Type 1 diabetes which have no known aetiology. Some of
these patients have permanent insulinopenia and are prone to ketoacidosis, but
have no evidence of autoimmunity (McLarty et al, 1990). This form of diabetes is
more common among individuals of African and Asian origin. In another form
found in Africans an absolute requirement for insulin replacement therapy in
affected patients may come and go, and patients periodically develop ketoacidosis
(Ahrén et al, 1984).

2.1.3.2 Type 2 (predominantly insulin resistance with relative insulin
deficiency or predominantly an insulin secretary defect with/without insulin
resistance):

Diabetes mellitus of this type previously encompassed non–insulin–dependent
diabetes, or adult–onset diabetes. It is a term used for individuals who have relative
(rather than absolute) insulin deficiency. People with this type of diabetes
frequently are resistant to the action of insulin (DeFronzo et al, 1997), (Lillioja et
al, 1993). At least initially, and often throughout their lifetime, these individuals do
not need insulin treatment to survive. This form of diabetes is frequently
undiagnosed for many years because the hyperglycaemia is often not severe enough to provoke noticeable symptoms of diabetes (Mooy et al., 1995), (Harris, 1993).

Nevertheless, such patients are at increased risk of developing macrovascular and microvascular complications. There are probably several different mechanisms which result in this form of diabetes, and it is likely that the number of people in this category will decrease in the future as identification of specific pathogenetic processes and genetic defects permits better differentiation and a more definitive classification with movement into “Other types”. Although the specific aetiologies of this form of diabetes are not known, by definition autoimmune destruction of the pancreas does not occur and patients do not have other known specific causes of diabetes. The majority of patients with this form of diabetes are obese, and obesity itself causes or aggravates insulin resistance (Campbell et al., 1993), (Banerji et al., 1994). Many of those who are not obese by traditional weight criteria may have an increased percentage of body fat distributed predominantly in the abdominal region (Umpierrez et al., 1995).

Ketoacidosis is infrequent in this type of diabetes; when seen it usually arises in association with the stress of another illness such as infection (Polonsky et al., 1996), (Simonson et al., 1984). Whereas patients with this form of diabetes may have insulin levels that appear normal or elevated, the high blood glucose levels in these diabetic patients would be expected to result in even higher insulin values had their beta-cell function been normal. Thus, insulin secretion is defective and insufficient to compensate for the insulin resistance. On the other hand, some individuals have essentially normal insulin action, but markedly impaired insulin secretion. Insulin sensitivity may be increased by weight reduction, increased physical activity, and/or pharmacological treatment of hyperglycaemia but is not
restored to normal (Zimmet, 1992), (Harris et al, 1995). The risk of developing Type 2 diabetes increases with age, obesity, and lack of physical activity. It occurs more frequently in women with prior GDM and in individuals with hypertension or dyslipidaemia. Its frequency varies in different racial/ethnic subgroups (Valle et al, 1997). It is often associated with strong familial, likely genetic, predisposition (Knowler et al, 1993). However, the genetics of this form of diabetes are complex and not clearly defined. Some patients who present with a clinical picture consistent with Type 2 diabetes have autoantibodies similar to those found in Type 1 diabetes, and may masquerade as Type 2 diabetes if antibody determinations are not made. Patients who are non-obese or who have relatives with Type 1 diabetes and who are of Northern European origin may be suspected of having late onset Type 1 diabetes (Knowler et al, 1993).

2.1.4 Screening and diagnosis of diabetes mellitus:

Laboratory tests used for screening and diagnosis of diabetes mellitus includes the following:

(i) Random plasma glucose:
Random or “casual”, plasma glucose (RBG) measurement is inexpensive and easily accomplished but not always diagnostic. In the absence of unequivocal hyperglycemia or metabolic decompensation, a patient with a random plasma glucose level of 200 mg/dL (11.1 m.mol/L) or higher should have a second confirmatory test for the diagnosis of diabetes to be established (Report of the expert committee on the diagnosis and classification of diabetes mellitus, 1997).

(ii) Fasting plasma glucose:
Fasting plasma glucose (FPG) requires and overnight fast of at least 8 hours. It is inexpensive and risk free and, thus, is the test of choice. The result of FPG are more reproducible over a short term than those of OGTT. Studies that have
compared the two tests found coefficients of variation (CVs) almost two times higher in OGTT than in FPG (Ko et al, 1998).

(iii) Oral glucose tolerance test:
In compliance with the most recent American Diabetes Association (ADA) recommendations, OGTT is not indicated for routine use with the exception of pregnancy. One reason is the reduced overall test-retest reproducibility (some studies report only 65.6% reproducibility). In addition, FPG is easier to perform and more cost-effective. However, renewed emphasis of the importance of the postprandial hyperglycemia (PPG) OGTT has been given with the recently published results of the Diabetes Program, in which diet/exercise, as well as drug therapy (metformin and troglitazone), was shown to slow/prevent the progression of IGT to overt diabetes mellitus. It has thus been proposed that performing an OGTT could be considered in selected individuals (e.g., obese patients, or patient who share the features of metabolic syndrome) in order to identify diabetes or any degree of glucose intolerance at nearly stage (Wang et al, 2002).

2.1.5 Laboratory test used for monitoring glycaemic control:

These include the following:

(i) Glycosylated hemoglobin (HbA1c):
HbA1c, or glycated hemoglobin, glycohemoglobin, or glycoslyted hemoglobin, is a term used to describe a serious of stable minor hemoglobin components formed none enzymatically from hemoglobin and glucose. The level of HbA1c in a blood sample provides a glycemic history of the previous 120 days, the average erythrocyte lifespan. The optimal use of HbA1c testing requires standardization of HbA1c assays. The National Glycohemoglobin Standardization Program has established standard assays for HbA1c based on the results of the Diabetes Control and Complication Trial (DCCT) (American Diabetes Association, 2001).
(ii) Glycosylated serum proteins:
The degree of glycation of serum proteins (mainly albumin and fructose amine) provides an index of glycemic status over the preceding 1 to 2 weeks (the half life of albumin is 14-20 days). This test is useful especially in a situation in which HbA1c assay is subject to interference and also in order to document relatively short-term changes in glycemic status, such as in diabetic pregnancy or after major changes in therapy (Flier et al, 2005).

2.1.6 Complications of diabetes mellitus:

Diabetes mellitus affects many organ system and the morbidity and motility associated with diabetes are related to the short (acute) and long term complications which include the following:

(A) Short term (acute) complications:

(i) Diabetic ketoacidosis:

Diabetic ketoacidosis is the most common, serious and demanding medical emergency within the field of diabetology and endocrinology. There is no generally accepted definition of DKA and, in particular, very mild cases may be difficult to diagnose. At a minimum, it’s reasonable to require that pH is below the normal range and that the levels of ketoacids (ketone bodies) in the blood or urine are markedly elevated (Holt et al, 2010).

(ii) Hypoglycemia:

Hypoglycemia, simply defined as a low blood glucose level, is a laboratory finding that may or may not be associated with significant pathology. Pathologic hypoglycemia may be caused by a large spectrum of entities, which vary
depending on the age at onset and the presence of other, related symptoms (United Kingdom Prospective Diabetes study (UKPDS) Group, 1998).

(B) Long term complications:
The major long term complications in diabetic patients are:

(i) Cardiovascular disease (CVD):
Diabetes mellitus is a major independent risk factor for cardiovascular disease (CVD). The increase prevalence of CVD in diabetes has been attributed in large part to the acceleration of coronary atherosclerosis, which occurs at an earlier age and advances more rapidly to clinical cardiovascular events in individuals with diabetes than in those without diabetes. Patients with diabetes are also prone to arterial thrombosis due to persistency activated thrombogenic pathways and impaired fibrinolysis. This combination of increased arterial disease and prothrombotic milieu in diabetes is a major underlying cause of acute ischemic coronary heart disease (CHD) (Grundy et al, 1999).

Type 2 diabetes increases relative risk of cardiovascular disease two to fourfold compared with the risk in the general population. Traditional risk factors play an important role in the development of atherosclerosis in subjects with diabetes, the rate of cardiovascular mortality and morbidity in persons with diabetes exceeds by 50% the rate predicted by these risk factors. Several other risk factors may account for this discrepancy. Possible nontraditional risk factors include insulin resistance, insulin levels and hyperglycemia (Best et al, 2000).

(ii) Diabetic retinopathy:
is a highly specific vascular complication of both type 1 and type 2 diabetes, and the duration of diabetes is a significant risk factor for development of retinopathy. After 20 years of diabetes, nearly all patients with type 1 diabetes and more than
60% of those with type 2 diabetes have some degree of retinopathy (Spranger et al, 2001).

(iii) Diabetic nephropathy:
Diabetic kidney disease takes years to develop. It’s rare to see diabetic nephropathy earlier than 3 years after the diagnosis of diabetes; it’s usually seen after 5 to 15 years in patients with type 1 diabetes. For patients with type 1 diabetes this time course is well understood because these patients present with very clear symptoms at diagnosis. The natural history of kidney disease in type 2 diabetes is less well understood, as patients can have relatively mild symptoms for quite some time before a diagnosis is made. It’s likely, however, that in type 2 diabetes, as in type 1 diabetes, kidney disease develops only after a number of years of diabetes (Ruggenenti et al, 2000).

Diabetic nephropathy occurs in 30% to 50% of patients with type 1 or type 2 diabetes. In the past, except in ethnic populations, the incidence and prevalence of nephropathy have been lower in patients with type 2 diabetes than in those with type 1 diabetes. In recent years, however, the incidence and prevalence of diabetic nephropathy have been steadily increasing, so that the percentage of patients with type 2 diabetes who have nephropathy is approaching that seen in patients with type 1 diabetes (Ruggenenti et al, 2000). The increasing incidence of diabetic nephropathy in patients with type 2 diabetes is in part the result of the greater success in decreasing mortality due to type 2 diabetes. Improved management of the cardiovascular complication of type 2 diabetes through better control of lipids and improved more stringed blood pressure control has significantly increased life expectancy and has allowed time for other complications to develop. The increased prevalence of diabetic nephropathy is due in part to the current worldwide epidemic of diabetes (Parving et al, 2001).
(iv) **Diabetic neuropathy:**
Most recognized neurologic complication associated with diabetes involve the peripheral nervous system. The diabetic neuropathies include several distinctive clinical syndromes with differing clinical manifestations, anatomic distributions, clinical courses and possibly underlying pathophysiology (American Diabetes Association, 1996).

2.2 **The thyroid gland:**

2.2.1 **Anatomy:**
The thyroid gland is a butterfly-shaped organ and is composed of two cone-like lobes or wings, lobus dexter (right lobe) and lobus sinister (left lobe), connected via the isthmus. The organ is situated on the anterior side of the neck, lying against and around the larynx and trachea, reaching posteriorly the oesophagus and carotid sheath. It starts cranially at the oblique line on the thyroid cartilage (just below the laryngeal prominence, or 'Adam's Apple'), and extends inferiorly to approximately the fifth or sixth tracheal ring. It is difficult to demarcate the gland's upper and lower border with vertebral levels because it moves position in relation to these during swallowing.

The thyroid gland is covered by a thin fibrous sheath, the capsula glandulæ thyroidea, composed of an internal and external layer. The external layer is anteriorly continuous with the lamina pretrachealis fasciae cervicalis and posteriorrolaterally continuous with the carotid sheath. The gland is covered anteriorly with infrahyoid muscles and laterally with the sternocleidomastoid muscle also known as sternomastoid muscle. On the posterior side, the gland is fixed to the cricoid and tracheal cartilage and cricopharyngeus muscle by a thickening of the fascia to form the posterior suspensory ligament of Berry (Eugster et al, 2004). The thyroid gland's firm attachment to the underlying trachea
is the reason behind its movement with swallowing. In variable extent, Lalouette's Pyramid, a pyramidal extension of the thyroid lobe, is present at the most anterior side of the lobe. In this region, the recurrent laryngeal nerve and the inferior thyroid artery pass next to or in the ligament and tubercle (Stephen Nussey et al, 2001).

Between the two layers of the capsule and on the posterior side of the lobes, there are on each side two parathyroid glands.

The thyroid isthmus is variable in presence and size, can change shape and size, and can encompass a cranially extending pyramid lobe (lobus pyramidalis or processus pyramidalis), remnant of the thyroglossal duct. The thyroid is one of the larger endocrine glands, weighing 2-3 grams in neonates and 18-60 grams in adults, and is increased in pregnancy.

The thyroid is supplied with arterial blood from the superior thyroid artery, a branch of the external carotid artery, and the inferior thyroid artery, a branch of the thyrocervical trunk, and sometimes by the thyroid ima artery, branching directly from the brachiocephalic trunk. The venous blood is drained via superior thyroid veins, draining in the internal jugular vein, and via inferior thyroid veins, draining via the plexus thyroideus impar in the left brachiocephalic vein.

Lymphatic drainage passes frequently the lateral deep cervical lymph nodes and the pre- and paratracheal lymph nodes. The gland is supplied by parasympathetic nerve input from the superior laryngeal nerve and the recurrent laryngeal nerve (Stephen Nussey et al, 2001).
2.2.2 Histology:
The thyroid is composed of spherical follicles that selectively absorb iodine (as iodide ions, I⁻) from the blood for production of thyroid hormones, but also for storage of iodine in thyroglobulin. Twenty-five percent of all the body's iodide ions are in the thyroid gland. Inside the follicles, in a region called the follicular lumen, colloid serves as a reservoir of materials for thyroid hormone production and, to a lesser extent, acts as a reservoir for the hormones themselves. Colloid is rich in a protein called thyroglobulin. The follicles are surrounded by a single layer of thyroid epithelial cells, which secrete T3 and T4. When the gland is not secreting T3/T4 (inactive), the epithelial cells range from low columnar to cuboidal cells. When active, the epithelial cells become tall columnar cells.
Scattered among follicular cells and in spaces between the spherical follicles are another type of thyroid cell, parafollicular cells, which secrete calcitonin (Stephen Nussey et al, 2001).

2.2.3 Thyroid Hormones:
2.2.3.1 Triiodothyronine(T3):
Is tyrosine-based hormones produced by the thyroid gland which responsible for regulation of metabolism, growth, body temperature and heart rate.
As the true hormone, the effects of T3 on target tissues are roughly four times more potent than those of T4. T3 is about 20% of the thyroid hormone that is produced, whereas 80% is produced as T4. Roughly 85% of the circulating T3 is later formed in the thyroid by removal of the iodine atom from the carbon atom number five of the outer ring of T4. In any case, the concentration of T3 in the human blood plasma is about one-fortieth that of T4. This is observed in fact
because of the short half-life of T3, which is only 2.5 days. This compares with the half-life of T4, which is about 6.5 days.

It circulated in the blood, bound to plasma proteins, Thyronine-binding globulin (TBG, Tranthyretin and serum albumin (Jansen et al, 2005).

**2.2.3.2 Thyroxine (T4):**

Is tyrosine-based hormones produced by the thyroid gland which responsible for regulation of metabolism, growth, body temperature and heart rate (Jansen et al, 2005).

**2.2.3.3. Regulation of Thyroid Hormones:**

The production of thyroxine and triiodothyronine is regulated by thyroid-stimulating hormone (TSH), released by the anterior pituitary. The thyroid and thyrotropes form a negative feedback loop: TSH production is suppressed when the T4 levels are high. The TSH production itself is modulated by thyrotropin-releasing hormone (TRH), which is produced by the hypothalamus and secreted at an increased rate in situations such as cold exposure (to stimulate thermogenesis). TSH production is blunted by somatostatin (SRIH), rising levels of glucocorticoids and sex hormones (estrogen and testosterone), and excessively high blood iodide concentration(Kester et al, 2004).

**2.2.3.4 Calcitonin:**

An additional hormone produced by the thyroid contributes to the regulation of blood calcium levels. Parafollicular cells produce calcitonin in response to hypercalcemia. Calcitonin stimulates movement of calcium into bone, in opposition to the effects of parathyroid hormone (PTH). However, calcitonin seems far less essential than PTH, as calcium metabolism remains clinically normal after removal of the thyroid (thyroidectomy), but not the parathyroids (Andreotti et al, 2006).
2.2.4 Thyroid Stimulating Hormones (TSH):

Thyroid-stimulating hormone (also known as TSH or thyrotropin) is a hormone that stimulates the thyroid gland to produce thyroxine (T4), and then triiodothyronine (T3) which stimulates the metabolism of almost every tissue in the body. It is a glycoprotein hormone synthesized and secreted by thyrotrpoe cells in the anterior pituitary gland, which regulates the endocrine function of the thyroid gland (Stephen Nussey et al, 2001).
Chapter Three

3. Materials and Methods

3.1 Study approach: Quantitative approach.

3.2 Study design: Analytical, case-control and hospital-based study.

3.3 Study area and period:

This study was done in Khartoum state in different hospitals (Khartoum, Omdurman and Khartoum North hospitals), during the period from November 2012 to January 2014.

3.4 Target population and sample size:

The study was conducted on Sudanese patients with type 1 diabetes mellitus as a test group (n = 500, 192 males and 308 females) and apparently healthy non-diabetic volunteers as a control group (n = 250, 94 males and 156 females).

3.5 Inclusion criteria:

a) – Test group: Sudanese patients with type 1 diabetes mellitus.

b) - Control group: healthy non-diabetics volunteers matched for age and sex.

3.6 Exclusion criteria:

Those with other types of diabetes (type 2, gestational and secondary diabetes) had been excluded from this study.

3.7 Ethical consideration:

- Permission of this study was obtained from the local authorities in the area of the study and the medical director of the above mentioned hospitals.
- The aim and benefits of the study had been explained to the participant with assurance and confidentiality.
- An informed consent was obtained from all participants.
- Health education had been provided to all participants.
3.8 Data collection and analysis:

1. Interview and questionnaire:
Interview with the patients were done to obtain clinical data and to provide health education. A questionnaire was specifically designed to obtain information which helps in either including or excluding certain individuals in or from the study respectively.

3.9 Clinical examination and diagnosis:
Clinical history & diagnosis of the test group and the control group were checked by a physician.

3.10 Study variables and methods of measurement:
- Serum levels of thyroid hormones T3, T4 and TSH were measured by Elisa technique using full automated Human chemistry analyzer.
- EDTA Blood Haemoglobin A1c % was measured spectrophotometric using NycoCard semi-automated analyzer.

3.11 Measurement of serum T3:

I. Principle:
Competition principle. Total duration of assay: 18 minutes.
- 1st incubation: 30 μL of sample and a T3-specific antibody labeled with a ruthenium complex; bound T3 is released from the binding proteins in the sample by ANS.
- 2nd incubation: After addition of streptavidin-coated microparticles and biotinylated T3, the still-free binding sites of the labeled antibody become occupied, with formation of an antibody-hapten complex. The entire complex becomes bound to the solid phase via interaction of biotin and streptavidin.
- The reaction mixture is aspirated into the measuring cell where the microparticles are magnetically captured onto the surface of the electrode. Unbound substances
are then removed with ProCell. Application of a voltage to the electrode then induces chemiluminescent emission which is measured by a photomultiplier.

- Results are determined via a calibration curve which is instrument-specifically generated by 2-point calibration and a master curve provided via the reagent barcode (Wheeler, 1994).

II. Assay Procedure:

For optimum performance of the assay, the directions given in this document for the analyzer concerned were followed. The appropriate operator’s manual for analyzer-specific assay instructions used. Resuspension of the microparticles takes place automatically before use. Read in the test-specific parameters via the reagent barcode. 15-digit sequence of numbers was entered. Reagents were brought to approx. 20 °C and placed on the reagent disk (20 °C) of the analyzer. Foam formation avoided. The system automatically regulated the temperature of the reagents and the opening/closing of the bottles (Wheeler, 1994).

3.12 Measurement of serum T4:

I. Principle:

Competition principle. Total duration of assay: 18 minutes.

- 1st incubation: 15 μL of sample and a T4-specific antibody labeled with a ruthenium complex; bound T4 is released from binding proteins in the sample by ANS.

- 2nd incubation: After addition of streptavidin-coated microparticles and biotinylated T4, the still-free binding sites of the labeled antibody become occupied, with formation of an antibody-hapten complex. The entire complex becomes bound to the solid phase via interaction of biotin and streptavidin.

- The reaction mixture is aspirated into the measuring cell where the microparticles are magnetically captured onto the surface of the electrode. Unbound substances
are then removed with ProCell. Application of a voltage to the electrode then induces chemiluminescent emission which is measured by a photomultiplier.

- Results are determined via a calibration curve which is instrument-specifically generated by 2-point calibration and a master curve provided via the reagent barcode (Wheeler, 1994).

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**3.13 Measurement of serum TSH:**

**I. Principle:**

Sandwich principle. Total duration of assay: 18 minutes.

- 1st incubation: 50 μL of sample, a biotinylated monoclonal TSH specific antibody and a monoclonal TSH-specific antibody labeled with a ruthenium complex react to form a sandwich complex.
- 2nd incubation: After addition of streptavidin-coated microparticles, the complex becomes bound to the solid phase via interaction of biotin and streptavidin.
- The reaction mixture is aspirated into the measuring cell where the microparticles are magnetically captured onto the surface of the electrode. Unbound substances are then removed with ProCell. Application of a voltage to the electrode then induces chemiluminescent emission which is measured by a photomultiplier.
• Results are determined via a calibration curve which is instrument-specifically generated by 2-point calibration and a master curve provided via the reagent barcode (Wheeler, 1994).

II. Assay Procedure:

For optimum performance of the assay the directions given in this document for the analyzer concerned were followed. The appropriate operator’s manual for analyzer-specific assay instructions used. Resuspension of the microparticles takes place automatically before use. Read in the test-specific parameters via the reagent barcode. 15-digit sequence of numbers was entered. Reagents were brought to approx. 20 °C and placed on the reagent disk (20 °C) of the analyzer. Foam formation avoided. The system automatically regulated the temperature of the reagents and the opening/closing of the bottles (Wheeler, 1994).

3.14 Measurement of Hemoglobin A1c :

• 5 ml of patient's blood sample was added to a test tube containing reagent after reached the room temperature then mixed and incubated for 2 – 3 minutes (Timer used).

• 25 µL of the reaction mixture was added to a test device and mixed again and the reaction mixture allowed to soak completely at the membrane in about 10 seconds.

• 25 µL of the washing solution was applied to a test device and mixed again and the washing solution allowed to soak completely at the membrane in about 10 seconds and pipette tip changed.

• After 5 minutes Nycocard reader II used for reading results.
3.15 **Quality Control:**

The precision and accuracy of all methods used in this study were checked each time a batch was analyzed by including commercially prepared control sera.

3.16 **Statistical analysis:**

The data collected in this study were analyzed using SPSS computer program. The means and standard deviations of the serum levels of T3, T4, TSH and HbA1c% were calculated and the independent t test was used for comparison (P value ≤ 0.05 was considered significant). Linear regression analysis was used to assess correlations of the serum levels of T3, T4 and TSH to HbA1c% and to the duration of diabetes.
Chapter Four

4. Results

This study was conducted in 500 patients with type 1 diabetes mellitus as test group and 250 healthy volunteers as control group. Frequency distribution and percentage according to gender among the test group were as follow: Male (n = 192) (38.40 %) and female (n = 308) (61.60 %) respectively and among the control group were as follow: Male (n = 94) (37.60 %) and female (n = 156) (62.40 %) respectively.

Serum T\textsubscript{3}:

Table (1): Shows a significant increase of the mean of the serum levels of T\textsubscript{3} among the test group compare to that of the control group. (Mean ± SD): (1.59±0.93) versus (1.37±0.30) ng/ml, (P= 0.041).

Fig (1) Shows the relationship between the age and the serum levels of T\textsubscript{3} among the diabetic group (r=0.01, P = 0.931)

Fig (4) Shows the relationship between the duration of diabetes and the serum levels of T\textsubscript{3} among the diabetic group (r=0.09, P = 0.731).

Fig (7) Shows the relationship between HbA\textsubscript{1c} % and the serum levels of T\textsubscript{3} among the diabetic group (r=0.00, P = 0.789).

Serum T\textsubscript{4}:

Table (1): Shows a significant increase of the mean of the serum levels of T\textsubscript{4} among the test group compare to that of the control group. (Mean ± SD): (10.05±3.32) versus (8.68±1.96) ng/ml, (P=0.028).

Fig (2) Shows the relationship between the age and the serum level of T\textsubscript{4} among the diabetic group (r=0.00, P = 0.917)
Fig (5) Shows a significant strong positive correlation between the duration of diabetes in years and the serum level of T₄ among the diabetic group (r=0.98, P = 0.000).

Fig (8) Shows no relation between HbA₁C% and the serum level of T₄ among the diabetic group (r=0.00, P = 0.811).

**Serum TSH:**
Table (1): Shows a significant reduction of the mean of the serum levels of TSH among the test group compare to that of the control group. (Mean ± SD): (1.44±0.96) versus (1.76±0.93) ng/ml, (P=0.037).

Fig (3) Shows no relation between the age and the serum levels of TSH among the diabetic group (r=0.00, P = 0.921)

Fig (6) Shows insignificant weak positive correlation between the duration of diabetes in years and the serum levels of TSH among the diabetic group (r=0.03, P = 0.852).

Fig (9) Shows no relation between HbA₁C% and the serum levels of TSH among the diabetic group (r=0.00, P = 0.141).

**Serum HbA₁C%:**
Table (1): Shows a significant increase of the mean of the blood HbA₁C% among the test group compare to that of the control group. (Mean ± SD): (7.45±2.21) versus (4.62±0.44) ng/ml respectively, (P=0.003).
Table (1): Comparison of the means of T₃, T₄, TSH and HbA₁C of the test group and the control group.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Test group (n=500)</th>
<th>Control group (n=250)</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Serum T₃ (ng/ml)</td>
<td>1.59±0.93 (0.32-6.91)</td>
<td>1.37±0.30 (0.61-1.99)</td>
<td>0.041</td>
</tr>
<tr>
<td>Serum T₄ (ng/ml)</td>
<td>10.05±3.32 (2.12-24.86)</td>
<td>8.68±1.96 (4.3-14.8)</td>
<td>0.028</td>
</tr>
<tr>
<td>Serum TSH (ng/ml)</td>
<td>1.44±0.96 (0.5-4.69)</td>
<td>1.76±0.93 (0.37-6.05)</td>
<td>0.037</td>
</tr>
<tr>
<td>Blood HbA₁C %</td>
<td>7.45±2.21 (3.40-16.1)</td>
<td>4.62±0.44 (4.00-5.90)</td>
<td>0.003</td>
</tr>
</tbody>
</table>

The table shows the mean±standard deviation, range between brackets and the level of significance (P-value).

t-test was used for comparison between the test group and control group.

P-value ≤0.05 is considered significant.
Table (2): Distribution of the test group and the control group according to gender.

<table>
<thead>
<tr>
<th>Sex</th>
<th>Test group</th>
<th>Control group</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Frequency</td>
<td>%</td>
</tr>
<tr>
<td>Male</td>
<td>192</td>
<td>38.40</td>
</tr>
<tr>
<td>Female</td>
<td>308</td>
<td>61.60</td>
</tr>
<tr>
<td>Total</td>
<td>500</td>
<td>100%</td>
</tr>
</tbody>
</table>
Fig. (1): The relationship between the age and the serum levels of T3 among the diabetic group ($r = 0.01$, $P = 0.931$)
Fig. (2): The relationship between the age and the serum levels of $T_4$ among the diabetic group ($r = 0.00$, $P = 0.917$).
Fig. (3): The relationship between the age and the serum levels of TSH among the diabetic group ($r = 0.00, P = 0.921$).
Fig. (4): The relationship between the duration of the diabetes and the serum levels of T3 among the diabetic group ($r = 0.09$, $P = 0.731$).
Fig. (5): The relationship between the duration of the diabetes and the serum levels of T₄ among the diabetic group (r = 0.98, P = 0.000).
Fig. (6): The relationship between the duration of the diabetes and the serum levels of TSH among the diabetic group ($r = 0.03$, $P = 0.852$).
Fig. (7): The relationship between HbA1c% and the serum levels of T3 among the diabetic group (r = 0.00, P = 0.789).
Fig. (8): The relationship between the HbA$_{1C}$% and the serum levels of T$_4$ among the diabetic group ($r = 0.00$, $P = 0.811$).
Fig. (9): The relationship between HbA$_{1c}$% and the serum levels of TSH among the diabetic group ($r = 0.00$, $P = 0.141$).
Chapter Five

5. Discussion

Type 1 and type 2 diabetes mellitus have in common high blood glucose levels (hyperglycemia) that can cause serious health complications, including: ketoacidosis, kidney failure, heart disease, stroke, and blindness. Patients are often diagnosed with diabetes when they see a physician for clinical signs such as excessive thirst, urination, and hunger. These symptoms result from the underlying hyperglycemia that is in turn caused by insufficient insulin. Type 1 diabetes (T1D) on the other hand usually starts in people younger than 30 and is therefore also termed juvenile-onset diabetes, even though it can occur at any age. T1D is a chronic autoimmune disorder that precipitates in genetically susceptible individuals by environmental factors (Atkinson, 2001).

In the current study a test group of 500 patients with type 1 diabetes mellitus were compared to 250 apparently healthy volunteers as control group, both groups were matched for age and sex.

In the test group of the current study there is a significant increase in the mean of the serum levels of T3 when compared with the control group (P = 0.041). This agrees with the results of a study done by (Notarbartolo, 1983) who found that the means of the serum levels of T3 in patients with type 1 diabetes mellitus were significantly higher than that in healthy subjects (P < 0.001). The above finding in the current study disagree with a study done by (Radetti, 1985) who reported T3 was appreciably diminished both in group A (P less than 0.05) and in group B (P less than 0.01) of type 1 diabetes mellitus and also disagrees with a study done by (Lin, 2003) who found the diabetic ketoacidosis group had significantly lower levels of T3.
In this study the test group has a significant increase of the mean of the serum levels of T4 when compared with that of the control group (P = 0.028). This disagrees with a study done by (Samaneh, 2011) who reported no significant differences between the diabetic patients with type 1 and non-diabetic ones regarding the mean serum of T4. Also disagrees with a study done by (Ditta, 2001) who reported that 30% of the diabetic patients with type 1 showed decreased T4 concentrations compared to controls.

In this study the test group has a significant reduction in the mean of the serum levels of TSH when compared with the control group (P = 0.037) and the reduction of TSH is an early and sensitive index of the occurrence of hyperthyroidism. This disagrees with a study done by (Sharifi, 2008) who reported that type 1 diabetic patients have higher TSH concentration (p=0.03). Also the above finding disagrees with a study done by (Ditta, 2001) who reported that the mean concentration of TSH was raised significantly (p<0.001) in type 1 diabetic patients as compared to the controls, This disagrees with a study done by (Samaneh, 2011) who reported there was no significant differences between diabetic patients with type 1 and non-diabetic ones regarding the mean serum TSH.

In the current study the test group has a significant increase in the mean of the serum levels of HA1C\% when compared with the control group (P = 0.003) and this may be due to presence of uncontrolled diabetes among the test group. This agrees with a study done by (Al-Hussaini et al, 2013) who reported that; there was a significant elevation in the mean of HbA1c.

In the current study there is no significant correlation between the age and both thyroid hormones (T3, T4) and thyroid stimulating hormones (TSH) among the
diabetic group. This disagree with study done by (Sharifi et al, 2008) who found there is a significant correlation between the age and T3, T4 and TSH.

In this study there is a significant strong positive correlation between the duration of diabetes and T4, and insignificant weak positive correlation with T3 among the diabetic group.

This agree with study done by (Sharifi et al, 2008) who reported the same findings and disagrees with a study done by (Radetti et al, 1985) who reported no significant correlation.
Chapter Six

Conclusion & Recommendations

6.1 Conclusion:

From this study, it is concluded that; in Sudanese patients with type1 diabetes:

1. Serum T3, T4, and HbA1c are significantly elevated in the diabetics compared to non diabetics.
2. Serum TSH is significantly decreased in the diabetics compared to non diabetics.
3. In the diabetic patients there is no correlation between the age and the thyroid hormones (T3, T4).
4. There is a strong positive correlation between the serum levels T4 and the duration of diabetes.
5. There is no significant correlation between the thyroid hormones and HbA1c%.
6.2 Recommendations:

1. Thyroid hormones and thyroid stimulating hormone should regularly be screened in diabetic patients.
2. HbA1c% should be assessed regularly in diabetic patients as a marker of glycemic control.
3. Diabetic patients should be assessed regularly to achieve good control in order to delay or minimized development of complications.
4. Further studies with greater sample size are needed to assess the magnitude of thyroid disorders in Sudanese with type 1 diabetes.
Reference


Holt, C. Cockram, A. Flyvbjerg and B. Goldstein (2010). Textbook of diabetes, Blackwell Publishing ; Chapter 3 ; Epidemiology of Type 1 Diabetes(Page 31 -44).


