KWAME NKRUMAH UNIVERSITY OF SCIENCE & TECHNOLOGY,

KUMASI

EVALUATION OF THYROID DYSFUNCTION AND THYROID AUTOIMMUNITY IN DIABETES MELLITUS SUBJECTS AT EFFIA NKWANTA REGIONAL HOSPITAL, SEKONDI, WESTERN REGION

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DECLARATION

I hereby declare that this submission is my own work towards the MPhil and that, to the best of my knowledge, it contains no material previously published by another person nor material which has been accepted for the award of any other degree of the university, except where due acknowledgment has been made in the text.

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ABSTRACT

Diabetes mellitus and thyroid disorders are the two most common endocrine disorders in clinical practice. Presence of thyroid dysfunction in diabetic patients makes management of the disease more difficult. The objective of this hospital based cross sectional study at the Diabetic Clinic of Effia Nkwanta Regional Hospital, Sekondi, was to investigate the prevalence of thyroid dysfunction and thyroid autoimmunity in clinically euthyroid type 1 and type 2 diabetes mellitus patients and also to correlate the glycated haemoglobin levels with thyroid function markers. One hundred and thirty-two (132) clinically euthyroid type 1 and type 2 diabetes mellitus patients and 132 age-matched and sex- matched non-diabetic control subjects were included in the study. Data on sociodemography of patients and controls such as age, sex, duration of diabetes, mode of glycaemic control were obtained using a pre-tested questionnaire. The serum levels of thyroid biomarkers (TSH, FT3, FT4, anti-TPO anti-TG) were evaluated using standard methods. The mean serum FT3 ($p=0.2044$) and TSH ($p=0.1869$) in diabetic subjects were not significantly different when compared to their control peers but the levels of FT4 was significantly higher in the diabetic mellitus subjects compared to the levels recorded among the control group ($p <0.0001$). The prevalence of thyroid dysfunction among the diabetic group was 11.36% (6.82% subclinical hypothyroidism and 4.54 % subclinical hyperthyroidism) with 117 (88.64 %) euthyroid. The prevalence of thyroid autoimmunity among the diabetic subjects was5.30% and 3.79 % respectively for anti-thyroid peroxidase autoantibody (anti-TPO) and anti- thyroglobulin autoantibody (anti-TG). Though not statistically different, the auto-antibody reactivity was observed to be higher among the diabetic group compared to the controls. Among the thyroid biomarkers assayed, increasing FT4 levels was significantly associated with increasing additive levels of fasting blood glucose and glycated haemoglobin after Bivariate Pearson correlations analysis.

In conclusion, this study has shown that, the prevalence of thyroid dysfunction among the diabetic population seen at Effia Nkwanta Regional Hospital in Sekondi, Ghana was 11.36 % and the prevalence of thyroid autoantibody reactivity (3.79-5.30 %) in the diabetes mellitus subjects was not significantly different from non-diabetic subjects.

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LIST OF ABBREVIATIONS

AAP-	Aminoantipyrine
ADA -	American Diabetes Association
AITD-	Auto-immune thyroid disease
Anti- TPO -	Anti -Thyroid peroxidase autoantibodies
Anti-TG -	Anti-Thyroglobulin autoantibodies
ATA -	American Thyroid Association
ATP-	Adenosine triphospate
cAMP-	cyclic Adenosine monophosphate
D1-	type 1iodothyronine deiodinase
D2 -	type2 iodothyronine deiodinase
DIT -	Diiodotyrosine
DM -	Diabetes mellitus
EDTA -	Ethylene Diamine Tetra Acetic Acid
ELFA -	Enzyme linked-fluorescent assay
ELISA -	Enzyme Linked Immunosorbent Assay
FBG-	Fasting Blood sugar
FFA -	Free fatty acids
FT3 -	Free Triiodothyronine
FT4 -	Free thyroxine
FTI -	Free thyroxine index

GAD -	Glutamic acid decarboxylase
GDM -	Gestational Diabetes Mellitus
GIGT -	Gestational impaired glucose tolerance
GLUT 2-	Glucose transporters 2
H_2O_2 –	Hydrogen peroxide
IgG -	Immunoglobulin G
IRD -	Inner ring deiodination
KATH -	Komfo Anokye Teaching Hospital
kDa-	Kilo Dalton
KNUST -	Kwame Nrumah University of Science and Technology
MIT -	Monoiodotyrosine
OGTT -	Oral glucose tolerance test
ORD -	Outer ring deiodination
PCV-	Packed cell volume
rT3 -	Reverse triiodothyronine
SPR -	Solid Phase Receptacle
T1DM -	Type 1 Diabetes Mellitus
T3 -	triiodothyronine
TBG -	Thyroxine binding globulin
TG -	Thyroglobulin
THBI -	Thyroid hormone binding inhibitor
TNF-α -	Tumour Necrosis Factor alpha
TPO -	Thyroid peroxidase
TRH –	Thyrotropin Releasing Hormone
TSH -	Thyroid stimulating hormone

TSHR - Thyroid stimulating hormone receptor

WHO - World Health Organization

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and constant patience have taught me so much about sacrifice, discipline and compromise.

DEDICATION

I dedicate this thesis to my parents, who sacrificed to educate me. To my lovely and caring wife Efua for her immense support, and to my children Kwadwo Sarpong, Kwame Asante and Afia Dufie who are the love of my life and hope of my future.

CHAPTER ONE INTRODUCTION

1.1 Background

Diabetes mellitus is a condition with multiple aetiologies arising from disturbances in glucose metabolism due to a defect either in insulin production and release, action or a combination of these leading to a high glucose level associated with other biochemical derangements (**World Health Organization**, 2002). Diabetes mellitus results from different metabolic disorders rather than a distinct pathological abnormality. Some known clinical manifestations are drowsiness associated with hyperglycaemia, excessive urination and thirst, weight loss, impaired vision and vulnerability to some infections. The severest form of increased glucose levels could lead to hyperosmolar syndrome and insulin deficiency as well as life-threatening ketoacidosis. Defects in carbohydrate metabolism coupled with the physiological system to undo the imbalance places a burden on other endocrine systems. Continuous breakdown in endocrine control worsens the metabolic imbalance and subsequently leads to hyperglycaemia (**Bailey**, 2000).

Worldwide, diabetes mellitus a well known endocrine metabolic abnormality is a main cause of mortality. It was projected that by the year 2000, 171 million people in the world would suffer from diabetes and at this rate the number is expected to increase to about 366 million in 2030 (**Wild** *et al.,* 2004). Presently, in every population in the world, there are people who live with diabetes mellitus and other subtle forms of glucose metabolic disorders. It is feared that without proper intervention and preventive control measures, the burden of the disease and the other lesser forms of glucose metabolic disorders are likely to increase globally (**Zimmet** *et al*., 2001; **Alberti.**, 2007). There is scanty information on the overall prevalence of diabetes among

Ghanaians, however; the rate has been estimated to be about 6.3% among some inhabitants in the capital, Accra (**Amoah** *et al*., 2002).

Thyroid diseases generally show as either over production of thyroid hormones or under production of the thyroid hormones. It can also result from swelling due to a neoplastic process or due to the pressure effects on the thyroid gland from close anatomical structures (**Jayakumar**, 2011). Thyroid disease is a pathological condition that interferes with the management and control of diabetes mellitus. According to **Der** *et al*., (2013), the prevalence of thyroid disorders varies according to some factors. For example dietary iodine intake of the population is known to affect the prevalence of thyroid disorders seen in that population. In clinical practice, thyroid diseases and diabetes mellitus are the two most common endocrine disorders seen. The state of glycaemic dysregulation and thyroid dysfunction have been observed to equally affect each other and the relationship between the two conditions have been established previously(**Feely and Isles**, 1979). Thyroid hormones contribute to the regulation of carbohydrate metabolism and pancreatic function, while diabetes mellitus affects thyroid function tests. **Rohdenburg** (1920) used the term 'thyroid diabetes' to explain the effect of thyroid hormone excess on the effective control of glucose metabolism. Thyroid hormones oppose the action of insulin and both insulin and thyroid hormones take part in metabolism of various cells. Functional abnormalities can occur as a result of overproduction or under production of either insulin or thyroid hormones (**Satish and Mohan**, 2003). The prevalence of thyroid disease in patients with diabetes has been shown to be significantly higher compared to the general population which suggests a possible relationship between thyroid status and insulin resistance (**Goswami** and **Mallika**, 2010). For example,

Papazafiropoulou (2010) showed that 12.3 % of Greek diabetic patients had thyroid dysfunction and **Akbar** *et al*., (2006) also showed that the thyroid dysfunction was prevalent in 16 % of type 2 diabetes mellitus subjects from Saudi Arabia.

1.2 Objectives of the study

1. To compare thyroid hormone levels and prevalence rates of thyroid dysfunction in diabetes mellitus subjects with age-matched and sex- matched nondiabetic control group.
2. To compare the prevalence of thyroid autoimmunity by estimating anti- thyroid peroxidase and anti-thyroglobulin levels in types 1 and 2 diabetes mellitus and their control subjects.
3. To determine the type of thyroid disorders associated with the diabetic subjects.
4. To determine the relationship between glycaemic control and thyroid dysfunction.

1.3 Justification of the objectives

The prevalence and pattern of various diseases including thyroid dysfunction vary from country to country and also shows temporal changes over centuries. This difference is mainly because of the fact that the causes of most of the diseases are multi factorial that is to say they depend on race, genetic makeup, dietary habits, lifestyle of individual as well as environmental factors which keep changing from place to place and time to time. Since the symptoms of hypothyroidism and hyperthyroidism are very similar to many other conditions and even in states of normal health (**Abalovich** *et al*., 2007), it is therefore important that thyroid function is tested biochemically alongside a careful clinical assessment of all diabetes mellitus patients. It is known that unidentified thyroid dysfunction could make management of diabetes and its complications more difficult.

Therefore, management of thyroid dysfunction in patients with diabetes may prove useful.

For example, American Diabetes Association (ADA) has proposed that people with diabetes to be checked for thyroid disorders (**Thakkar** and **Jain**, 2011). A study among type 2 diabetes mellitus subjects at Korle- Bu Teaching Hospital in Accra, Ghana showed that 10.1 % type 2 diabetes mellitus population studied had thyroid dysfunction. However, data is not available on thyroid dysfunction and thyroid autoimmunity among the diabetes mellitus patients seen at Effia Nkwanta Regional Hospital, Sekondi, Ghana. This study aims to determine the frequency and types of thyroid dysfunction and thyroid autoimmunity among diabetic subjects at Effia Nkwanta Regional Hospital.

1.4 Expected benefits of the study

Determination of frequency and types of thyroid dysfunction and thyroid autoimmunity among diabetic patients in Ghana will help inform clinicians whether it will be worthwhile screening all diabetic patients for thyroid dysfunction. Epidemiological data would also be available on thyroid dysfunction and thyroid autoimmunity among diabetes mellitus patients seen at Effia Nkwanta Regional Hospital in Sekondi, which will serve as foundation for further studies.

CHAPTER TWO

LITERATURE REVIEW

2.1 Anatomy and physiology of the thyroid gland.

2.1.1 Anatomy of the thyroid gland

In humans, the thyroid gland is important because of its capacity to synthesise and secrete the triiodothyronine (T3) and tetraiodothyronine (T4) hormones. These two

hormones are necessary for the required energy levels and active lifestyle. It has been shown that thyroid hormones are vital in the maintenance of the initial level of phospholipids in cell membranes and fatty acids composition of the lipids (Prasad and Kumar, 2005). In humans, the thyroid gland is known to be one of the biggest endocrine glands and can be found just below the larynx. It comprises of two lobes joined to either side of and anterior to the trachea. In a healthy adult, the thyroid gland has an average weight of about 20 grams. The thyroid gland is made of sphere-shaped follicles filled with a gel-like substance known as colloid and enclosed by follicular cells. The normal thyroid is attached loosely to neighbouring structures, and the fascial planes are distinct (Figure 2.1). In the posterior region of each pole of the thyroid are located four parathyroid glands. The follicular cells are positioned with their bases close to the capillary blood supply and the apices abutting the colloid. The mature thyroid gland contains numerous follicles made up of thyroid follicular cells that surround secreted colloid. The colloid is a protein-rich fluid that harbours large quantities of thyroglobulin; a requirement for thyroid hormones synthesis (**Fox**, 2003).

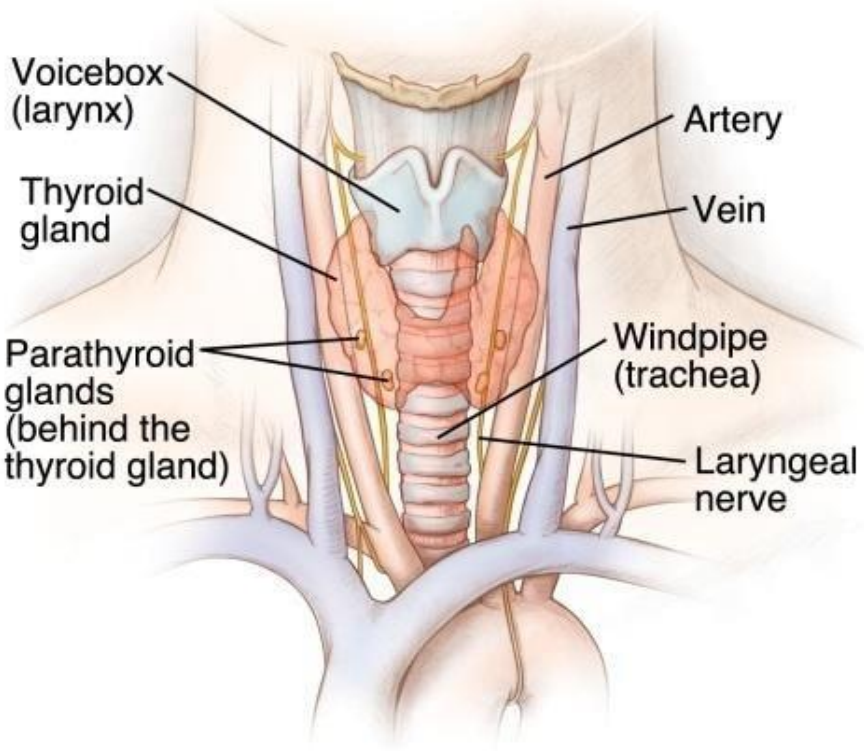


Figure 2.1: The location of thyroid gland (Adapted from **Fox**, 2003)

2.1.2.1 Physiology of thyroid gland

Synthesis, storage and release of the thyroid hormones, thyroxine (T4) and triiodothyronine (T3) are the physiological functions of the thyroid gland. The metabolic rate is controlled and regulated by thyroid hormones. Thyroid hormones also have important function in the metabolic pathways of carbohydrates, lipids and proteins. Thyroid hormones play critical function in the growth and maintenance of both mental and physical functions of the body (**Klein** and **Danzi**, 2007). Thyroid hormones dysfunction results in a wide range of clinical effects, since thyroid hormones are involved in almost every single organ function of the body. Evident among these is their role in the growth of the foetus and childhood development. According to **Fernandez-Real** *et al*., (2006), lack of thyroid hormone may result in delayed growth

in childhood, however; in adults, the influence of thyroid hormones is seen by changes in metabolism.

2.1.2.2 Iodine

The production of thyroid hormones largely depends on the availability of iodine. Iodine is therefore required for the optimal function of the thyroid gland (**Zimmermann**, 2010). The body receives iodine mainly from intake of iodine containing meals and iodized table salt. About 2 billion people from over 130 countries are at risk of developing brain damage due to iodine deficiency; a situation World Health Organization considers could have been avoided. Endemic cretinism, a syndrome of permanent mental retardation along with either a predominant neurological disorder or predominant hypothyroidism, is considered to be the most serious effect of iodine deficiency (**Chen** and **Hetze**, 2010). Low grade iodine deficiency is linked with fewer numbers of Hashimoto's disease, while too much iodine intake is known to be linked with a higher prevalence of Graves's disease (**Walsh** *et al*., 2006).

2.1.2.3 Thyroid hormones synthesis

TSH stimulation of thyroid gland triggers the production and release of thyroid hormones. TSH must first bind to TSH receptors on the cells of the follicles before thyroid hormone synthesis and release can take place. The first process in the synthesis of thyroid hormones is the absorption of accessible iodide into follicular cells. Iodide is changed into iodine (physiologically active form), in the follicular cells. The iodine then binds to tyrosine residues in the thyroglobulin molecules through what is called organification. Pairing of one iodine atom with a tyrosine molecule results in the formation of monoiodotyrosine (MIT). Further iodization of MIT results in one molecule of diiodotyrosine (DIT). Thyroid hormones are produced from pairing of DIT

and MIT. When one molecule of MIT molecule and one molecule of DIT pairs, one molecule of triiodothyronine (T3) is produced while one molecule of thyroxine (T4) is formed from pairing of two molecules of DITs. Four iodine atoms are contained in thyroxine while triiodothyronine contains three iodine atoms (Figure 2.2). The synthesized thyroid hormones remain in thyroglobulin and are only released on demand. TSH stimulates thyroid glandular cells to initiate the synthesis and secretion of thyroid hormones. In order for this to happen, follicular cells surround fragments of the colloid, internalize the colloid droplets into the cells, disintegrate them by the help of enzymes, and release the hormones from the thyroglobulin, and discharge them into the peripheral blood (**Guyton** and **Hall**, 2006).

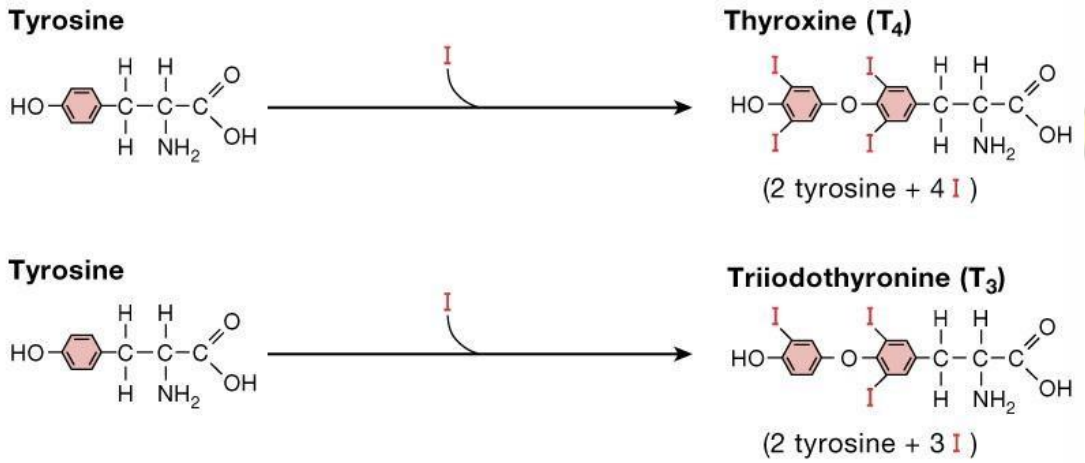


Figure 2 2: Thyroid hormones and their precursor compounds formulae (adapted from **Miot** et al., 2012).

2.1.2.4 Thyroid hormone transport

Thyroid hormones must be bound to proteins to be transported to their target cells when they are released from the thyroid gland. The binding to proteins is important because thyroid hormones are not water-soluble. Thyroid hormones are carried in the plasma to their target cells by the following proteins; thyroxine binding globulin (TBG), albumin and pre-albumin. Since bound thyroid hormones are not physiologically active, a small

proportion that remains unattached and free is physiologically active. The bound component of thyroid hormones serves as only a backup for the circulating thyroid hormones (**Refetoff,** 1989).

2.1.2.5 Thyroid hormone cellular transport and receptors

Thyroid hormones are carried into their target cells by particular carrier-mediated uptake process. Most of their actions are triggered by attachment of T3 to its nuclear receptors in target cells (**Yen**, 2001). The biological function of thyroid hormone depends mainly on the intracellular T3 concentration. The T3 intracellular concentration also depends on the circulating concentration of T3 and its precursor T4, activities of deiodinases that catalyze the production or degradation of T3 and functions of transporters which mediate the cellular uptake of T3 and T4 (**Pizzagalli** *et al*., 2002). Thyroid hormone receptors are of two isoforms, specifically TR α and TRβ determined by the TRα and TRβ genes, and each isoform presents as two or three subclasses (**Oetting** and **Yen**, 2007). The entry of free thyroid hormones into their target cells is by an energy-dependent, ATP necessitating stereospecific, and saturable transport process which is influenced by the monocarboxylate transporter 8 (MCT8) and MCT 1 (**Dumitrescu** *et al*. 2006), and other transporter proteins including OATP1c1, a member of the Na CO-independent organic anion transporter protein (OATP) family (**Williams** and **Bassett**, 2011). For example, transport via MCT8, increases uptake of T4 and T3 tenfold (**Friesema** *et al*. 2003).

2.1.2.6 Thyroid stimulating hormone (TSH)

Thyroid stimulating hormone is a 31-kDa hormone made up of two subunits called α and β. The α subunit is identical to the other glycoprotein hormones, but the TSH β subunit is unique to TSH. The β subunit, made up of 112 amino acids, determines the unique biological and immunological features of the hormone (**Wondisford** *et al*.,

1996). Secretion of TSH into the blood stream is according to circadian rhythm, peaking between at 1 and 2 am (**Scanlon** and **Toft**, 1996). Some drugs are known to alter TSH production, secretion, transport and also affect thyroid hormone metabolism (**Haugen**, 2009). It is also known that secretion of TSH can also be affected by other hormones and cytokines (**Kundra** and **Burman**, 2012). TSH assays with high levels of accuracy and efficient sensitivity have been classified as third generation assays. They have the advantage of distinguishing between the levels of TSH observed in thyrotoxicosis, compared with the low, but detectable, concentrations that happen in subclinical hyperthyroidism. The advantage of TSH measurement over the other diagnostic measurement is attributable to the physiologic inverse linear relationship between circulating TSH and FT4 levels (**Joshi**, 2011). The determination of TSH in a basal serum by a third generation assay remains the best diagnostic tool for thyroid function status in both overt and subclinical primary thyroid disorders. It must be noted however that; it is not adequate for evaluation of thyroid hormone status in inpatients or for investigation of suspected central hypothyroidism (**Garber** *et al*., 2012).

2.1.2.7 Regulation of thyroid hormone synthesis and control

Iodine and TSH are the most significant factors for thyroid hormone synthesis and control. Excess iodide affects thyroid hormone synthesis, through what is termed as *Wolff-Chaikoff* effect (**Zimmermann** *et al*., 2008), probably by reducing H_2O_2 production, and therefore, blocking thyroglobulin iodination. The thyroid hormone production and release is primarily under the influence of the hypothalamus and pituitary glands. The anterior pituitary gland serves as a detector for circulating thyroid hormone level through a negative feed-back system. Stimulation of the thyroid gland by TSH keeps on till sufficient levels of circulating thyroid hormone are sensed by the

anterior pituitary gland (**Kansagra** *et al.*, 2010). Figure 2.3 illustrates the hypothalamic-pituitary thyroid axis negative feedback process.

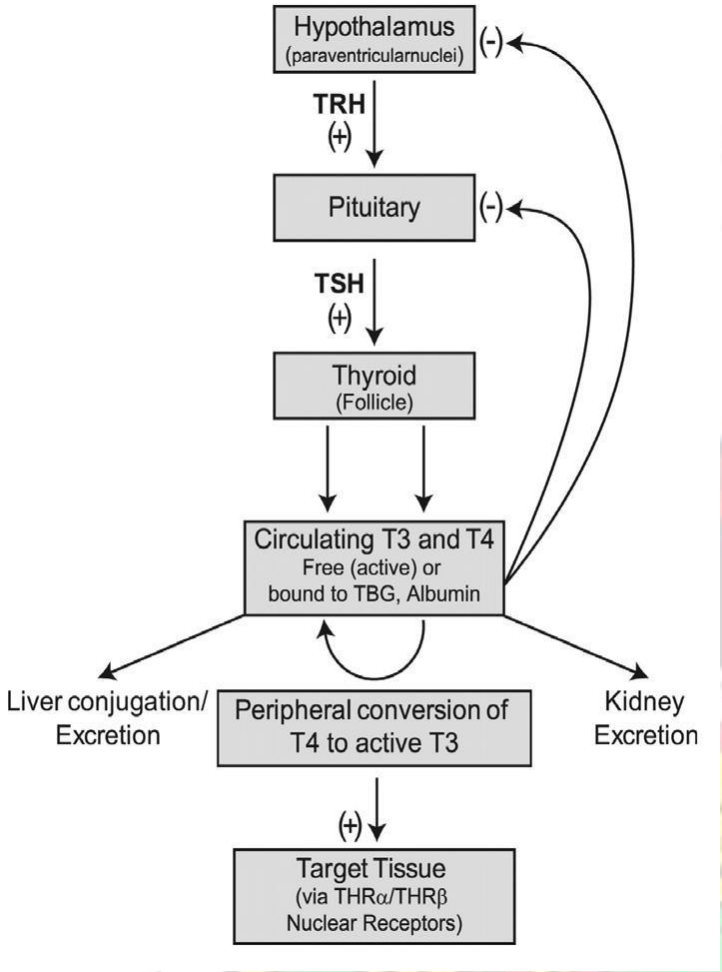


Figure 2.3: The mechanism of negative feedback loop of circulating thyroid hormone concentrations (**Kansagra** et al., 2010).

2.1.2.8 Thyroid hormone metabolism in humans

T3 is mainly made in the peripheral tissues through enzymatic outer deiodination (ORD) of T4. Inner ring deiodination (IRD) of T4 produces a metabolic product called reverse T3 (rT3). The amount of reverse T3 released by the by the thyroid gland is insignificant. Approximately, one-third of T4 is metabolized into T3 while about one-third is converted to rT3. Reverse T3 (rT3) is produced when T4 is deiodinated at the 5th position. Reverse T3 has no function biologically and mainly serves as an

escape route for T4. In a fasting state or profound physical exertion, the level of reverse T3 increases while T3 decreases. The rest of T4 is processed through dissimilar pathways such as glucuronidation and sulfation. The amount of T3 secretion from the thyroid gland rises when iodine is deficient and in conditions when the thyroid gland is stimulated by antibodies to TSH or TSH receptors. This is accompanied by an enhanced T3 production and thyroidal expression of both type 1 iodothyronine deiodinase (D1) and type2 iodothyronine deoidinse (D2). The enhanced expression of D1and D2 of the thyroid gland leads to intra-thyroidal conversion of T4 to T3. Both rT3 and T3 levels decrease in hypothyroidism. T3 and rT3 further loses iodide, mainly to produce common metabolite 3, 3′-diiodothyronine (3, 3′T2), produced by IRD of T3 and ORD of rT3 (**Bianco** *et al*., 2002).

2.1.2.9 Total T4 (TT4) and Free T4 (FT4)

Thyroxine (T4) was first isolated in 1914 by **Kendall** and was first synthetically produced in 1925 by **Harrington**. Thyroxine (T4) is used globally to treat millions of patients with thyroid dysfunction. Thyroxine or tetraiodothyronine (T4) produced by the thyroid gland, is mainly (> 99.9%) bound to carrier proteins (thyroxine binding globulin,thyroxine binding prealbumin, albumin). Unlike T3, T4 is only produced in the thyroid gland and is the main hormone produced from the thyroid gland (**Gereben** *et al*., 2008). For T4 to become physiologically active, T4 has to be converted to T3 by outer-ring deiodination. The fraction that remains free is considered as the physiologically active component of the hormone (**Sapin** and **D'Herbomez**, 2001).

2.1.2.10 Total (TT3) and Free (FT3) Triiodothyronine

Triiodothyronine (T3) is a hormone produced by thyroidal secretion and from the peripheral deiodination process which converts T4 to T3. FT3 circulates as a free

hormone or attached to carrier proteins such TBG, albumin and pre albumin. The free form of the hormone is the bioactive active fraction. Serum FT3 measurement has little contribution for diagnoses of hypothyroidism, because of increased rate of conversion of T4 to T3 maintains FT3 levels remain within the reference range until hypothyroidism develop into very severe state. However, measurement of FT3 levels plays a significant role in the diagnoses of hyperthyroidism, monitoring of patients on thyroxine and antithyroid agents and low-T3 syndrome (**Pearce and Byfield**, 1986). Serum FT3 concentration is increased in almost all hyperthyroidism cases and normally rises before the FT4 levels. FT3 assays also play extremely significant roles in differential diagnosis of T3 thyrotoxicosis, a type of hyperthyroidism that shows as abnormally increased T3 and reduced TSH levels, with normal T4 levels (**Gardner** and **Shoback**, 2007).

2.2. Thyroid function

Euthyroid is used to refer to individuals with normal thyroid function. Euthyroid is defined in individuals if the following criteria are met; no previous or current diagnosis of thyroid disorders or dysfunction, are not on thyroid hormone replacement therapy or medications for thyroid diseases, do not have positive thyroid autoantibodies in the serum, and have TSH, FT4 and FT3 levels within the reference ranges (**Bastemir** *et al*.,2007).

2.3 Thyroid dysfunction

Thyroid diseases can be broadly classified as functional or structural or a combination of both. Functional disorders produce alterations in hormonal levels. It can lead to either deficiency or excess of thyroid hormones, both having significant influence on health and well-being of the affected individual.

Structural disorders usually involve enlargement of the thyroid. However, this current study will focus on functional aspects of thyroid disease. Thyroid dysfunction is as a result of multiple aetiological factors such as a deficiency or excess of iodine; medication and autoimmunity (**Hadj-Kacem** *et al*., 2009; **Camargo** *et al*., 2008).

Serum TSH measurement is the first test to perform in evaluating thyroid function. TSH is primarily responsible for the negative-feedback system mechanism of the thyroid function system, slight variations in serum thyroid function cause dramatic response in TSH secretion. In most cases a normal TSH level is sufficient to rule out any thyroid dysfunction, however, when hypothalamic pituitary disease is suspected, FT4 level measurement is recommended (**Benhadi** *et al*., 2010).

2.3.1. Hypothyroidism

Hypothyroidism is a progressive disorder with a notable morbidity and the barely noticeable clinical manifestations which may be easily ascribed to other ailments, especially in women who have just been delivered and in the elderly. Hypothyroidism is firstly marked by an increased TSH levels accompanied by normal serum FT4 and FT3 levels. This phase progresses to a reduction in serum FT4 levels. Hypothyroidism is marked by inadequate amounts of thyroid hormones, can occur due to defects in the thyroid gland itself, pituitary or hypothalamus. The recommended screening test for hypothyroidism is a sensitive TSH test; which can be followed by a FT4 test (**Hoogendoorn** *et al*., 2006). The classical biochemical pattern for primary hypothyroidism is elevated TSH and low free T4 levels. However, FT3 levels could remain normal or low. The full-scale expression of hypothyroidism is called as myxoedema, a condition that escaped serious attention until it was described by **Gull** in 1874. Myxoedema is a very unusual, life-threatening medical condition in patients

with long-standing severe unmanaged hypothyroidism in whom the body mechanisms are unable to maintain normal homeostasis (**Wartofsky**, 2006).

2.3.1.1 Aetiology of hypothyroidism

In an environment with sufficient iodine consumption, hypothyroidism may be caused by antibodies raised against the thyroid gland or the damaging effect of treatment for hyperthyroidism. Hypothyroidism may also happen secondary to radiotherapy or surgery for hyperthyroidism and thyroid tumours. Central hypothyroidism results from inadequate synthesis and secretion of TSH because of the presence of pituitary or hypothalamic tumours (**Yamada and Mori**, 2008). Another cause of hypothyroidism, though, rare occurs in patients with haemangiomata and other tumours in which type 3 iodothyronine deiodinase is present (which causes increased rate of T4 and T3 degradation) and this is termed as consumptive hypothyroidism (**Mouat** *et al*., 2008).

2.3.2 Hyperthyroidism

Hyperthyroidism is a term given to a collection of disorders that entail increased production and release of thyroid hormones by the thyroid gland leading to an increased metabolism called thyrotoxicosis (**Braverman** and **Utiger,** 2005).

Thyrotoxicosis can result in a very serious and rare condition known as thyroid storm. This condition involves multiple organ dysfunction and can be life-threatening. It normally happens in patients who have unrecognized or inadequately treated thyrotoxicosis. It may be provoked by the stress of surgery, anesthesia, or thyroid manipulation (**Sarlis** and **Gourgiotis**, 2003).Symptoms that may suggest hyperthyroidism include anxiety, profuse sweating, heat intolerance, tremors and loss of weight (**Hoogendoorn** *et al*., 2004). The physical signs usually seen in hyperthyroid patients include tachycardia; goitre; skin changes and lid lag (**Frost** *et al*., 2004).

2.3.2.1 Aetiology of thyrotoxicosis

In iodine-deficient environment, the most important reasons of thyrotoxicosis are autoimmune Graves' disease and toxic multinodular goitre, while the minor causes consists of disproportionate thyroxine replacement, an autonomously functioning thyroid adenoma and thyroiditis (**Chu** *et al*., 2009).

2.3.3 Subclinical thyroid dysfunction

Subclinical thyroid dysfunction is a condition where TSH level falls outside the population reference range but FT4 level lies in the reference range (**Cooper**, 2001). Differential diagnosis between overt thyroid dysfunction and subclinical thyroid dysfunction is based on biochemical evaluation TSH and FT4.

2.3.3.1 Subclinical hypothyroidism

Subclinical hypothyroidism refers to the biochemical status of increaseed serum TSH concentration with normal levels of FT4 and FT3. However, the following conditions must have to be fulfilled; thyroid function has been stable for some weeks, the hypothalamic-pituitary thyroid axis is normal, and there is no existence of current severe illness (**Garber** *et al.*, 2012). A number of subjects with this condition may exhibit symptoms indicating hypothyroidism except that the symptoms are ambiguous and can be seen in subjects with normal thyroid function (**Canaris** *et al.*, 2000)

2.3.3.2 Subclinical hyperthyroidism

Subclinical hyperthyroidism is defined as the clinical status of decreased serum TSH levels with normal levels of FT4 and FT3. The designation is only applicable if the thyroid function has been steady for some days, the hypothalamic-pituitary thyroid axis is normal, and there is no indication of recent or current ill health (**Coope**r and **Biondi**, 2012**).**

2.3.4 Autoimmune thyroid disorders

Autoimmune thyroid disorders are the commonest form of organ specific autoimmune disorder especially in women. It can cause destruction of the gland leading to hypothyroidism. Many factors such as genetic factors, environmental pollutants, and iodine status can trigger autoimmune activation. The disease is made up of Graves' hyperthyroidism and Hashimoto hypothyroidism (**Stathatos and Daniels**, 2012). Autoimmune thyroid disease is the most commonly found autoimmune disease in type 1 diabetes mellitus subjects (**Montovani** *et al*., 2007).

2.3.4.1 Grave's disease

It is thyroid autoimmune disease which leads to over production and release of thyroid hormones. It affects about every 5 in 1000 of the population and is responsible for a considerable number of cases of hyperthyroidism (***Bürgi***, 2010). Graves's disease happens as a result thyroid-stimulating immunoglobulins (proteins that imitate the roles of TSH). These immunoglobulins attach to the receptors on thyroid cells and provoke the synthesis of thyroid hormones. In Grave's disease, levels of TSH and TRH are usually below the lower limits of their reference ranges and almost become undectable. A specific type of goitre known as diffuse toxic goitre is a feature of

Graves's disease which happens as a result of extended overstimulation and enlargement thyroid gland (**Weetman,** 2004).

2.3.4.2 Hashimoto's thyroiditis

Hashimoto's thyroiditis is considered among the list of autoimmune thyroid diseases in which thyroid cells are destroyed by cell and antibody-mediated immune processes. Hashimoto's thyroiditis is more observed in areas with a high dietary iodine intake.

Smoking is also known to increase the chances of developing Hashimoto's thyroiditis. Thyroid atrophy is more common than goitre in Hashimoto's thyroiditis. **Tomer** *et al*., (1999) have suggested a genetic role in developing Hashimoto's thyroiditis.

2.3.5 Thyroid autoantibodies

Autoimmune thyroid disease leads to cellular destruction and alteration in function of the thyroid gland through humoral and cell-mediated mechanisms. Cellular destruction happens once either sensitised T-lymphocytes or autoantibodies attach to thyroid cell membranes leading to break down of cells and inflammatory reactions. Modifications in thyroid gland function happen because of the action of provoking or blocking autoantibodies on cell membrane receptors. The three most important types of thyroid autoantigens involved in autoimmune thyroid diseases are thyroid peroxidase, thyroglobulin and the TSH receptor (**David** *et al*., 2001). Additional autoantigens, for example, Sodium Iodide Symporter (NIS) have also been identified although their roles in the development of AITD have not yet been explained (**Tonacchera** *et al*., 2001). Autoantibodies to pendrin, an iodide transporter located at the apical pole of thyroid follicular cells, have also been identified in most patients with Hashimoto's thyroiditis and Graves' disease (**Yoshida** *et al.*, 2009).

2.3.5.1 Anti-thyroglobulin autoantibodies (anti-TG).

Thyroglobulin (TG) is a 660-kDa glycoprotein made up of two similar subunits of 330 kDa each. Thyroglobulin is only synthesised in the thyroid gland and is the main component of colloid in the lumen of thyroid follicle. The major role is to give the support for production and storage of thyroid hormones (**Dunn** and **Dunn**, 2000). Anti-TG antibodies have all the four subclasses but IgG subclass is the dominant subclass (**Swain** *et al.*, 2005). Anti-TG antibodies are detected in many patients with autoimmune thyroid disorders. Anti-TG antibodies are detected in fewer than 60% of

patients with lymphocytic thyroiditis and 30% of Graves' disease patients. Anti-TG autoantibodies are less common than anti-TPO in thyroid auto immune diseases. It is known that anti-TPO antibodies can be detected in about 99% of cases when anti-TG antibodies are detected, however; only about35% of anti-TPO positive cases also shows anti-TG antibodies (**Ai** *et al*., 2003).

2.3.5.2 Thyroid peroxidase autoantibodies (anti-TPO)

Thyroid peroxidase autoantibodies (anti-TPO) are the main characteristics of autoimmune thyroid disease in humans. They are of IgG subclass and are linked with thyroid destruction and hypothyroidism. They are detectable in serum of subjects with thyroid disorders and patients with different types of immunologically involved thyroid conditions and small fraction of individuals who appear to be in good health. The development of anti-TPO antibodies normally happens before the development of thyroid disorders and it is even more common in patients with various autoimmune disorders (**Joshi**, 2011).

2.3.5.3 Thyroid stimulating hormone receptor autoantibodies (TRAb)

The TSH receptor (TSHR) is one of the main thyroid auto antigens (**Boelaert** and **Franklyn**, 2005). TSH binds to TSHR on thyroid gland cells plasma membranes and activates the cAMP and phospholipase C signaling pathways leading to the stimulation of the thyroid gland (**Davies** *et al*., 2002). The two categories are all linked with autoimmune thyroid disorders. They are thyroid stimulating autoantibodies that imitate the functions of TSH and can result in Graves' hyperthyroidism and blocking antibodies that block TSH binding to its receptor and may lead to hypothyroidism (**Ando** *et al*., 2005).

2.3.6 Thyroiditis

Inflammation of the thyroid results in a group of conditions known as thyroiditis. The most common cause of thyroiditis is Hashimoto's chronic lymphocytic thyroiditis. Inflammation of thyroid gland cells renders the cells incapable to synthesise and secrete thyroid hormones. Again, the inflammation causes interruption in the structural reliability and renders the follicles infective in keeping the already produced hormones. This leads to discharge of already formed hormones from disrupted follicles into peripheral blood (**Van den Berhe**, 2000).

2.3.6.1 Sub acute thyroiditis

Sub acute thyroiditis involves the three phases of medical path of hyperthyroidism, hypothyroidism and back to euthyroidism. In most cases, no treatment is required as the condition is self-limiting. The consequence of damage of thyroid follicle and subsequent discharge of already synthesised thyroid hormone into the peripheral blood leads to hyperthyroidism (**Volpe**, 1993).

2.3.6.2 Riedel's thyroiditis

Riedel's thyroiditis is an uncommon form of thyroid disease marked by extensive fibrosis of the thyroid gland. It is related with both Hashimoto's thyroiditis and Graves' disease (**Li** *et al*., 2004).

2.3.7 Drugs affecting thyroid function

Certain categories of drugs can interfere with thyroid hormone homeostasis. The interference can occur at four different levels. These drugs can impair the production and release of thyroid hormones, can alter the serum concentrations of thyroid hormones (through competing with thyroid hormone for binding sites), may interfere with intracellular transport and metabolism of thyroid hormone or may interfere with

thyroid hormone activities at the target tissue (**Barbesino**, 2010). Classical example is amiodarone which can induce both hypothyroidism and hyperthyroidism. It is iodinerich medicine mostly used for the control of atrial and ventricular arrhythmias. It is a benzofuranic derivative which has similar structure as that of T3 and T4 (**Ahmed** *et al*., 2011).

2.3.8 Euthyroid sick syndrome

Patients who are extremely ill may present with abnormal thyroid test results, even though they do not have thyroid dysfunction. The most significant changes are low serum total T3 and elevated rT3, leading to what is known as low T3 syndrome (**Warner** and **Beckett**, 2010). These alterations in circulating thyroid hormones levels in fasting and non thyroidal illness syndrome have been attributed to variations in deiodinases expression (**Kwakkel** *et al*., 2009).

2.3.9 Thyroid hormone resistance

Thyroid hormone resistance is a very rare condition caused by failure of target cells to respond effectively to thyroid hormones. It is an autosomal dominant inherited syndrome and **Refetoff** *et al.*, (2014) have named it as impaired sensitivity to thyroid hormone. Patients with this condition demonstrate increased serum FT4 and FT3 levels and TSH level can either be normal or slightly elevated (**Bonomi** *et al.*, 2009).

2.3.10 Thyroid Cancer

There are generally four forms of thyroid cancer: papillary, follicular, medullary, and anaplastic. Apart from anaplastic cancer, the rest of thyroid tumours have good prognosis. They are not normally malignant and have very low fatality (**Castro** and **Gharib,** 2000).

2.4 Classification, complications and diagnostic tests for diabetes mellitus

2.4.1 Classification of diabetes mellitus

The first widely adopted categorization of diabetes mellitus was published by **WHO** in 1980 (Expert Committee on Diabetes Mellitus) and was revised in 1985 (**World Health Organization**, 1985). According to American Diabetes Association (2015), diabetes mellitus can be broadly classified into the following four main types:

1. Type 1 diabetes mellitus

2. Type 2 diabetes mellitus

3. Gestational diabetes mellitus (GDM)

4. Specific types of diabetes due to other causes (secondary Diabetes)

2.4.1.1 Type 1 diabetes mellitus

Type 1 diabetes mellitus is the end result of lymphocytic penetration and damage of beta cells of islets of *Langerhans* in the pancreas. With decline in the number of beta (***β***) cells, insulin production diminishes to the point when insulin is no more sufficient to maintain blood glucose at physiologically acceptable level. In most cases, the onset happens in childhood, however; in few cases it can occur in adulthood. Type 1 diabetes mellitus is responsible for approximately one-tenth of all diabetes mellitus cases and happens because of a cellular-mediated autoimmune damage of the ß-cells of the pancreas. It has been suggested that the probability to develop these abnormal autoantibodies in type 1 diabetes mellitus is hereditary, although the mechanisms are not completely understood (**William** *et al*., 2002). It has also been mentioned that some viral infections and some pollutants may activate abnormal autoantibody reactions that cause destruction to the pancreas (**Hyoty**, 2002). The most normally detected islet cells antibodies are the ones targeted against glutamic acid decarboxylase (GAD), an enzyme

found within pancreatic beta cells. **Pilia** *et al.* (2011) found increased frequency of islet cell antibodies (IA2) and anti-GAD antibodies in patients with autoimmune thyroiditis indicating possible relationship between autoimmune thyroiditis and type 1 diabetes mellitus. Also, there is evidence to indicate that patients with other autoimmune diseases tend to have high incidence of type 1 diabetes mellitus.

Idiopathic type 1 diabetes mellitus is a class of type1 diabetes mellitus with unknown cause. Patients with this type of diabetes mellitus have permanent insulinopenia and are susceptible to develop ketoacidosis. It is predominantly found in Americans with African origin and Asians. This form of diabetes is strongly suspected to be inherited but does not show evidence of autoantibodies directed at parts of islet **ß** -cells (**Tan** *et al*., 2000).

Type 1 diabetic patients normally show acute symptoms of diabetes and severe hyperglycaemia, and in some cases ketoacidosis at the time of initial diagnosis. Biochemically, subjects with this type of diabetes are diagnosed with elevated blood glucose level, ketones in urine, and decreased serum levels of both insulin and Cpeptide and autoantibodies directed at parts of islet **ß**-cells (**Reinauer** *et al*., 2002)

2.4.1.2 Type 2 diabetes mellitus

Type 2 diabetes mellitus comprises what in the past was classified as non-insulindependent diabetes, or adult-onset diabetes. Subjects with this type of diabetes mellitus have relative insulin deficiency (**Ahrén and Corrigan**, 1984). Insulin resistance is the failure of insulin to have its biological effects at physiological level.

Type 2 diabetes mellitus happens as result of defect in insulin production and impairment of insulin activity in liver and peripheral tissues (**Corpeleijn** *et al.*, 2008). The specific causes are not well established, however; it has stronger genetic evidence

than type 1 diabetes mellitus (**DeFronzo**, 2004). Excess glucagon level is thought to play a role in the development of type 2 diabetes mellitus. In fact, type 2 diabetes mellitus is an islet paracrinopathy with inverse relationship between the glucagonsecreting alpha cell and the insulin-secreting beta cell altered causing high level of blood glucagon which ultimately leads to hyperglycaemia (**Unger** and **Orci**, 2010). **Wang** *et al.* (2011) have also established that amino acid metabolism may be vital in the development of type 2 diabetes. Unlike type 1 diabetes, most subjects with type 2 diabetes have no symptoms at the time of diagnosis. Major biochemical findings in type 2 diabetes mellitus are hyperglycaemia, hyperlipidaemia, and high serum levels of both insulin and C-peptide (**Reinauer** *et al*., 2002).

2.4.1.3 Gestational diabetes mellitus

It is defined as glucose intolerance which appears or is recognized during pregnancy (**Franz** *et al*., 2005). During pregnancy, the body's requirement for insulin is increased as result of the presence of biological substances for example, human placental lactogen and cortisol, which oppose the actions of insulin.

2.4.1.4 Secondary diabetes

Secondary diabetes occurs due to other illnesses or medications. Diseases of the pancreas that leads to destruction of the beta cells thereby affecting insulin release and action as well as some medications are known to be the principal causes. These forms of diabetes mellitus can either present as type1 diabetes mellitus or type 2 diabetes mellitus depending on the underlying cause. Conditions of the pancreas that destroy the pancreatic beta cells, hormonal disorders that interfere with insulin release and action and drugs are some of the common causes of this type of diabetes. Acromegaly and Cushing's syndrome have also been known to be strongly associated with impaired glucose tolerance and overt diabetes (Resmini *et al.*, 2009).

2.4.1.5 Varying forms of glucose intolerance

Most patients with cirrhosis may have glucose intolerance owing to impaired liver glucose uptake and glycogen synthesis. Other causes of glucose intolerance are hepatic and peripheral resistance to insulin and hormonal abnormalities. Glucose intolerance can also happen in uraemia, because of enhanced peripheral insulin resistance. The gastrointestinal tract is also known to play an important function in glucose tolerance. Incretin hormones such as glucagon-like peptide-1 and glucosedependent insulinotropic polypeptide are produced and released by specialized gut cells after food intake (**Joy** *et al.*, 2005).

2.4.2 Complications of diabetes mellitus

The basic expression of diabetes mellitus is hyperglycaemia which is linked with the development of some complications. Hyperglycaemia causes tissue destruction through repeated acute changes in cellular metabolism. Majority of diabetes mellitus complications which are caused by chronic hyperglycaemia, are similar irrespective of the type of diabetes mellitus. The long term consequences of diabetes mellitus may include development of retinopathy, nephropathy and neuropathy. Individuals with diabetes also stand the risk of developing diseases of the heart, brain and the blood vessels (**Fox** *et al*., 2007). Cataract and diabetic foot are also some of the longstanding complications of diabetes mellitus (**Harjutsalo** *et al*., 2011; **Krššák** *et al*., 2011). Another complication of diabetes mellitus is diabetic ketoacidosis (an acute condition which can easily result in fatality) normally seen in type 1 diabetes mellitus but can also be seen in some type 2diabetics (**Bowden** *et al*., 2008).

2.4.3 Diagnostic tests for diabetes mellitus

According to American Diabetes Association, diabetes mellitus may be detected using HbA1c levels or plasma glucose concentrations. The plasma glucose can either be

fasting plasma glucose or the 2-hour blood glucose level after a 75g oral glucose tolerance test (**Saudek** *et al*., 2008).

2.4.3.1 Blood glucose measurement

Blood glucose determination is the best available biochemical diagnostic tool to evaluate glucose tolerance status. Measurement of blood glucose level is used to identify glucose metabolic abnormalities such as diabetes mellitus, idiopathic hypoglycaemia and pancreatic disease (**Sacks**, 2001). Plasma glucose is about 11% greater than glucose assayed in whole blood. But, the disparity in the levels depends on packed cell volume (PCV) of the sample under consideration. This difference increases to 15% at a PCV of 0.55 and decreases to 8% at a PCV of 0.30 (**FoghAndersen** *et al*., 1990). National Diabetes Data Group and the WHO have recommended that fasting plasma glucose is appropriate for diabetes mellitus diagnosis (**Saudek** *et al*., 2006).

2.4.3.2 Oral glucose tolerance test (OGGT)

According to World Health Organization (2002), OGTT is a stimulation diagnostic tool to evaluate the ability of the body to ultilise glucose effectively. OGTT provides evidence on hidden diabetes states. The OGTT is useful in differentiating between subjects with normal glucose metabolism and subjects with impaired glucose tolerance and diabetes mellitus. The test is essentially employed in diagnosing diabetes when blood glucose level of an individual is unclear, during pregnancy, or in epidemiological studies. However, WHO proposes that OGTT should be maintained as a diagnostic test because fasting glucose alone is unable to detect about 30% of cases of hitherto undiagnosed diabetes. OGTT is also the only available diagnostic tool to identify people with impaired glucose tolerance. Persons with FBG of 6.1– 6.9mmol/L are recommended to undergo OGTT to determine their glucose tolerance status (**World Health Organisation**, 2006).

2.4.3.3 Glycated Haemoglobin (HbA1c)

In blood, glucose binds to proteins to form glycoproteins. This reaction is non enzymatic reaction and happens gradually under physiological conditions. Amino acids with sites available for glycation and the levels of glycoproteins that can be estimated are indications of the fluctuation of blood glucose levels at a point in time. Only glycated proteins which exhibit prolonged half-life are of diagnostic importance, because they show the exposure of these proteins to glucose for longer periods (**Reinauer** *et al*., 2002). The synthesis of glycated haemoglobin is fundamentally permanent and its concentration largely depends on the concentration of glucose to which the red blood cells are exposed and on life-span of the red blood cells (**Nathan**, *et al*., 2007). HbA1c is the main form of glycated haemoglobin in blood. The rate of HbA1c does not depend on variations related to diet and exercise. HbA1c value gives the average glucose level within the last 2–3 months and this can be undertaken at any time of the day without any special preparation of the subject. Factors affecting HbA1c values include anaemia, haemoglobin abnormalities and pregnancy. It must be noted that HbA1c results may be falsely decreased in subjects with a high red blood cell turnover while it may be falsely elevated in people with severe anaemia (**Gallagher** *et al*., 2009).

2.4.3.4 Serum insulin

Beta cells of the islets of *Langerhans* in the pancreas produce a peptide hormone called insulin which is initially produced in the endoplasmic reticulum and Golgi apparatus as proinsulin. The proinsulin produced then breaks into insulin and Cpeptide. Insulin is an anabolic hormone that enhances glucose uptake, glycogenesis, lipogenesis, and protein synthesis of skeletal muscle and fat tissue through tyrosine kinase receptor pathway. Insulin is also the most significant indicator in the control of plasma glucose metabolism, as it opposes glucagon and other catabolic hormones. (**Orci** *et al*., 1988).

Insulin concentrations are markedly decreased in type 1 diabetes mellitus subjects and other conditions for example, hypopituitarism, chronic pancreatitis and post-pancreatectomy. However; its concentration is elevated in type 2 diabetes mellitus especially at the initial stages, obesity, steroid administration, acromegaly, Cushing's syndrome, insulin receptor mutation. Insulin is also known to increase serum concentration of FT4 but it decreases serum concentration of T3 by impairing hepatic alteration of T4 to T3 (**Mannheim**, 1984).

2.4.3.5 Serum C-peptide

C-peptide is a peptide made up of 31 amino acids and has a half-life of about 3-4 times longer than that of insulin. It is largely excreted by the kidney. C-peptide levels are used to differentiate between insulin-dependent hypoglycaemia and insulinindependent hypoglycaemia in nondiabetic hypoglycaemia. C-peptide levels are elevated in insulinoma, sulfonylurea intoxication, non insulinoma pancreatogenous hypoglycaemia syndrome, insulin resistance state and chronic kidney disease. Cpeptide concentrations are however, suppressed in subjects with type 1 diabetes mellitus (Wahren *et al*., 2000).

2.5 Thyroid dysfunction and diabetes mellitus

2.5.1 Common pathological mechanisms to thyroid disorders and diabetes

Thyroid hormones produce significant changes in the control of glucose metabolism. The changes include changes in peripheral insulin levels and counter-regulatory hormones, intestinal absorption, hepatic synthesis and peripheral glucose uptake at tissue levels. Thyroid hormones are known to antagonise the action of insulin and encourage hepatic gluconeogenesis and glycogenolysis (**Weinstein** *et al*., 1994), they up-regulate the expression of genes such as GLUT-4 and phosphoglycerate kinase, involved in glucose transport and glycolysis respectively, in this way working together

with insulin (**Viguerie** *et al*., 2002) in facilitating glucose disposal and usage in peripheral tissues.

2.5.2 Thyrotoxicosis and glucose homeostasis

Hyperthyroidism is known to cause hyperglycaemia (**Maxon** *et al*., 1957). Thyrotoxicosis is linked with an increase in endogenous production of glucose, insulin resistance in the liver and associated hyperglycaemia (**Franklyn**, 2000). In hyperthyroid state, the half-life of insulin is shortened probably resulting from an increased rate of degradation and an improved secretion of physiologically inert insulin precursors (**O'Meara** *et al.*, 1993). Again, an untreated hyperthyroidism is known to be linked with fundamental deficiency in proinsulin processing. Another reason for the relationship between hyperthyroidism and hyperglycaemia is the increases in glucose gut absorption facilitated by the presence of excessive thyroid hormone production. Endogenous synthesis of glucose is also known to be increased in hyperthyroid state through a number of path ways. Thyroid hormones yield an increase in the liver cell plasma membrane concentrations of GLUT-2, the most important liver glucose transporter. This increased level of GLUT- 2 adds to the increased hepatic glucose output and abnormal glucose metabolism (**Kemp** *et al.*, 1997). Also in hyperthyroidism, there in increased lypolysis producing an increase in free fatty acids (FFA) that triggers gluconeogenesis in the liver. The increased release of FFA is probably due to an improved catecholamine-stimulated lipolysis seen in hyperthyroid state. Glucose tolerance is known to be deteriorated in thyrotoxicosis because thyroid hormones are known to increase the rate of beta-cell death (**Ximenes** *et al*., 2007).

2.5.3 Hypothyroidism and glucose homeostasis

Clamp studies have established that there is reduction in glucose disposal in patients with hypothyroidism (**Handisurya** *et al*., 2008). Hypothyroidism, however; is known

to cause decreased liver glucose output therefore compensating for insulin resistance found in peripheral tissues. This is the reason for the reduced requirement for insulin to achieve normoglycaemia in diabetes mellitus subjects who are also hypothyroid.

Also, in hypothyroid subjects beta-cell produces normal or decreased plasma insulin. Hypothyroidism has also been linked with impaired insulin secretion and resistance (**Maratou** *et al*., 2009).

2.5.4 Leptin, ghrelin, and adiponectin affecting metabolism

Through their association with adipocytokines and gut hormones, thyroid hormones can affect carbohydrate metabolism. Adiponectin, leptin and ghrelin produced by adipocytes influence insulin sensitivity and glucose homeostasis and these hormones are affected in hypothyroidism (**Yamauchi** *et al*., 2001).

2.5.5 Consequences of diabetes mellitus on thyroid hormones

Diabetes mellitus appears to affect thyroid functions at two levels. The first is at the point of hypothalamic control of TSH release while the second is at the point where T4 is changed to T3 in the peripheral tissues. In diabetes mellitus, the peak of nocturnal TSH is altered and TSH response to TRH also is diminished (**Gursoy** and **Tuncel,** 1999). Marked hyperglycaemia is known to reduce activity and hepatic concentration of T4-5-deiodinase, low serum concentration of T3, elevated levels of reverse T3 and low, normal or high level of T4 (**Shah**, 2007). The low levels of serum T3 is probably due to the impairment in alteration of T4 to T3 in the peripheral tissues that improves with normalization of the glycaemic levels. Type 2 diabetes mellitus subjects with subclinical hypothyroidism are known to be at a higher risk of developing nephropathy (**Chen** *et al*., 2007). This has been attributed to decline in cardiac output and improve peripheral vascular resistance associated with hypothyroidism and the resultant reduction in glomerular filtration rate (**Singer,** 2001).

Again, **Yang** *et al*., (2010) demonstrated that subjects with diabetes mellitus and subclinical hypothyroidism are at higher risk of developing retinopathy than diabetes mellitus subjects with normal thyroid function.

CHAPTER THREE STUDY DESIGN, METHODS AND MATERIALS

3.1 Study design

This research study was a hospital- based cross-sectional design involving both type 1 and 2 diabetic subjects attending Diabetes Clinic at Effia Nkwanta Regional Hospital in Sekondi.

3.2 Study site

The study was carried out at the Diabetic Clinic and Laboratory Unit of Effia Nkwanta Regional Hospital in Sekondi-Takoradi, Ghana.

3.3 Ethical considerations

This research protocol was approved after it was reviewed by the Committee for Human Research, Publications and Ethics of Kwame Nkrumah University of Science and Technology and Komfo Anokye Teaching Hospital both in Kumasi. The aim and purpose of the study were well explained to the diabetes mellitus patients and control subjects, verbal and written consent were obtained from all the study subjects.

3.4 Study Population

A total of one hundred and thirty-two (132) patients diagnosed with diabetes mellitus attending the Diabetic Clinic of Effia Nkwanta Regional Hosipital, Sekondi were recruited into the study. One hundred and thirty (132) subjects without diabetes or impaired fasting glucose were recruited from the general adult population seeking

services at the hospital's out- patient department using fasting plasma glucose and glycated haemoglobin levels.

The World Health Organisation (WHO) definition for diabetes was used to recruit the study subjects. WHO defines diabetes as having fasting plasma glucose greater or equal to 7.0 mmol/L on two consecutive occasions or on medication for hyperglycaemia. The cut-off point for Impaired Fasting Glucose (IFG) was
6.1mmol/L and the ideal levels of glycated hemoglobin (HbA1c) is 6.5% or below. Classification of types 1 and 2 diabetes by the physician was based on the age at which the disease commenced (<35 years) and whether or not glycaemic control was attained through insulin use. Those who met the above criteria were classified as type 1 diabetes.

3.4.1 Inclusion criteria

The study included diabetes mellitus subjects who were on treatment for the control of their fasting blood glucose levels, diagnosed using the World Health Organization standards, and who unreservedly agreed to participate in the study. All subjects were known diabetics with registration from the Diabetic Clinic and regularly attended the Diabetes Clinic of Effia Nkwanta Regional Hospital in Sekondi for management of their conditions. The control subjects were apparently healthy people with fasting blood glucose below 6.1 mmol/L and HbA1c below 6.5% after 8-12 hours overnight fast.

3.4.2 Exclusion criteria

Subjects who did not agree to participate in the study were excluded from the study. The study also excluded critically ill diabetes mellitus subjects and those with pregnancy, complications and documented record of thyroid dysfunction. The study also excluded subjects who had undergone thyroidectomy, amputation and those who had a goitre. Diabetics and control subjects with renal or liver diseases or any other

serious illnesses were excluded from the study. Also subjects with haemoglobinopathies or on medication affecting thyroid function were left out of the study.

3.5 Data collection

Socio demographic data such as age, sex, duration of diabetes, mode of glycaemic control were obtained using a standardized questionnaire. Also collected from participants were menopausal states for the women, intake of tobacco and alcohol. Information on type of diabetes mellitus participants were suffering from was extracted from their folders.

3.6 Biochemical investigations

The following biochemical tests were performed on blood samples of participants: serum thyroid stimulating hormone (TSH), free thyroxine (FT4), free triiodothyronine (FT3), anti-thyroid peroxidase autoantibody (anti-TPO) and anti-thyroglobulin autoantibody (anti-TG). Others were glycated haemoglobin (HbA1c) and fasting blood glucose.

After fasting for about 8-12 hours, 5 mls of venous whole blood sample was obtained from the antecubital fossa of the study participants.

About 2 mls of each participant's blood was put into fluoride oxalate tubes for plasma glucose determination, 1ml into EDTA tube for glycated haemoglobin measurement and 2 mls into serum separator tube. The fluoride oxalate blood was centrifuged at 3000x g for 5 minutes and the plasma used for the fasting plasma glucose measurement. The plasma was stored at 4˚C and used to for glucose levels measurement within six hours. The samples in the serum separator vacutainer tubes were also centrifuged after standing for 30 minutes at 3000 x g for 5 minutes at room temperature. Serum was put

into plain sample tubes and frozen at -20 ºC for up 5 days. This frozen plasma was later used for TSH, FT3, FT4, anti-TPO and anti-TG measurements. The blood sample in EDTA K3 tubes were used for glycated

haemoglobin within two hours after collection.

3.7 Statistical analysis

The data obtained from biochemical assays and the questionnaire were analysed using statiscal Package for Social Sciences and Microsoft Excel programmes for T-test and correlation coefficient calculations respectively. Variation of grouped data was assessed by two-way analysis of variance (ANOVA=F) using SPSS programme. A two-tailed p-value of <0.05 was considered statistically significant. Descriptive data was presented as mean ± SD. Comparison of paired data from the three groups of subjects was done using T-test, and correlations between groups were analyzed using Pearson correlation coefficient formula.

3.8 Assay methods 3.8.1 Determination fasting blood glucose and glycated haemoglobin (HbA1c)

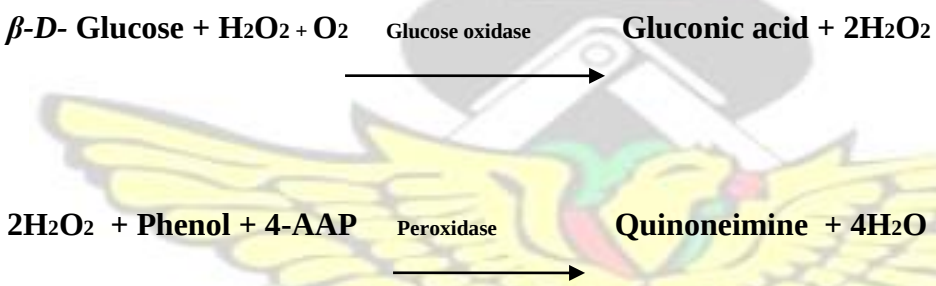
using Selectra Pro S Analyzer and Elitech reagents.

The Selectra *Pro S* is an automated table top chemistry analyzer, used in conjunction with reagents for in *vitro* investigative determination of various biological substances in samples of serum, blood, urine and other bodily fluids. The *Selectra Pro S* is designed with external computer and monitor. An embedded computer controls the analyzer unit, collects the raw data and provides the user interface. The analyzer is microprocessor controlled.

3.8.1.1 Principle of glucose determination using *Elitech* Glucose *Pap SL kit and Pro Selectra S*

The principle is based on Tinder's method of glucose determination of glucose.

Trinder's principle for glucose determination has almost all the features of a perfect automated glucose oxidase method (**Trinder**, 1969). The availability of the chemical reagents used in the colour reaction with peroxidase makes it useful. The solutions are stable and can be easily prepared, and the method is highly precise and fundamentally free of impediments. Glucose in the sample is oxidised to produce gluconic acid and hydrogen peroxide with the aid of glucose oxidase. The oxidative coupling of 4-aminoantipyrine (4-AAP) with phenol is catalysed by enzyme peroxidase to yield coloured quinonemine complex. The intensity of the coloured quinonemine complex produced measured at wavelength of 500nm is directly proportional to the concentration of glucose in the sample. The chemical reactions involved are shown below:



3.8.1.2 Procedure for blood glucose measurement

Blood glucose was measured using enzymatic method and the procedures of *Elitech* Clinical Systems (**Sees, France**, 2009) glucose *Pap SL* reagent on *Selecta Pro S.* Assays were carried out as recommended by the manufacturer for glucose determination (***Selectra Pro S* Analyzer Operator's Manual**, 2009). The reagent and the standard were ready-to -use. To ensure high quality results, reagents were calibrated and controlled according to the manufacturer's instruction.

3.8.2 Principle of glycated haemoglobin (HbA1c) determination using *Elitech*® HbA1c kit and *Pro Selectra S*.

HbA1c was measured using immuno-turbidimetry enhanced by latex particles method according to the following principle; first reaction: The sample is mixed with R1 which contains uncoated latex particles. As total haemoglobin and HbA1c have the same absorption affinity for these particles, the % HbA1c present in the sample is proportional to latex-bound HbA1c.

Second Reaction: Reagent R2 contains a mouse anti-human HbA1c mono-clonal antibody and a goat anti-mouse polyclonal- antibody. Agglutination complexes are formed from interaction between latex-bound HbA1c and the corresponding antibodies. Turbidity created by these aggregates is proportional to the % HbA1c in the sample.

3.8.2.1 Procedure for measurement of HbA1c using Selectra Pros S® and *Elitech HbA1c* reagent.

The HbA1c was measured with *Elitech HbA1c* on *Selectra Pro S*. The reagent was linear from 2.5-16 %. Assays were carried out as described by the manufacturer (**Selectra Pro S Analyzer Operator's Manual,** 2009). The samples and controls were prepared by dispensing 1 mL of Reagent 3 (haemolysis reagent) into clean pre labeled plastic tubes. Twenty (20) µL of well mixed blood samples and controls were added to the respective reagents tubes. The mixtures were allowed to stand for five minutes to achieve complete haemolysis. The haemosylates were put on board the analyzer for the measurement of HbA1c. To ensure that the results produced were of high standards, calibration and controls were run in line with manufacturer's instruction. The instrument without human intervention compared calibration factors against preprogrammed acceptance standards.

3.8.3 Principles for the determination of serum TSH, FT3, FT4, anti-TPO and anti- TG using Mini Vidas Analyzer and its reagents

TSH, FT3, FT4, anti- TPO and anti-TG were measured using *Mini Vidas* automated analyzer and its reagents **(BioMérieux S.A. Chemin de l'Orme 69280 Marcy l'Étoile / France**) (www.biomerieux.com/techlib). ***Mini Vidas*** is an automated quantitative analyzer that uses Enzyme linked-fluorescent assay (ELFA) method. This procedure was adopted because of its advantages over other methods such as Enzyme Linked Immunosorbent Assay (ELISA). The stages for performing the test are automatic, therefore the time needed for performing the assays is shorter, and operator and technical mistakes were omitted. The reproducibility of tests was high and personnel costs were low. The stability of calibration leads not only to the faster performance of the tests with higher precision, but also omission of the cost for multiple calibrations. Also, the higher limit of measurement enables application of undiluted serum. *Mini Vidas* ELFA method has the advantage of elimination of carry over due to application of specific Solid Phase Receptacle (SPR) for each strip (Iqbal and Khalid, 2007).

3.8.3.1 Principle of TSH determination using *Vidas*® TSH kit using *Mini Vidas* analyzer

The test theory combines a one-step enzyme immunoassay sandwich technique with final fluorescent detection (ELFA). The Solid Phase Receptacle (SPR) acts as the solid phase and also as a pipette for the testing procedures. Reagents for the test were already constituted and distributed in the sealed reagent strips. All the main steps of the assay were performed with minimal human intervention by the *Mini Vidas* instrument. The reaction medium was cycled in and out of the SPR repeatedly. The serum was dispensed into the well containing anti-TSH antibody tagged with alkaline phosphatase (conjugate). The TSH in mixture formed between the sample and conjugate attached

to antibodies plated on the SPR to the conjugate forming a "sandwich". The wash steps removed unattached conjugate. In the final detection steps, the substrate (4-Methyl-umbelliferyl phosphate) is cycled in and out of the SPR. The conjugate enzyme catalyzed the hydrolysis of this substrate into the fluorescent product (4-Methyl umbelliferone). The degree of fluorescence produced is measured at 450 nm by the ocular system in the analyzer and it was directly proportional to the TSH concentration in the serum. At the end of the process, results were evaluated without human intervention by the instrument in relation to the calibration curve stored in memory, and then printed.

3.8.3.2 Principle of free triiodothyrine (FT3) determination using *VIDAS*® FT3 and *Mini Vidas* analyzer

The serum was collected and dispensed into the well enclosing an alkaline phosphatase-tagged anti-T3 antibody (conjugate). The antigen present in the serum and the T3 antigen plated on the interior of the SPR compete for the available sites on the specific anti-T3 antibody conjugated to alkaline phosphatase. The reaction medium is cycled in and out of the SPR several times. During the ultimate detection step, the substrate (4-Methyl-umbelliferyl phosphate) is cycled in and out of the SPR. The conjugate enzyme catalyzed the hydrolysis of this substrate into a fluorescent product (4-Methyl-umbelliferone). The strength of the fluorescence produced was measured at 450 nm by optical system of the analyzer was inversely proportional to the concentration of free triiodothyronine in the sample. In the last part of the testing, results are automatically determined by the instrument in relation to the calibration curve programmed in the memory, and then printed.

3.8.3.3 Principle of free thyroxine (FT4) determination *VIDAS® FT4N* and *Mini Vidas* analyzer

The sample was collected and dispensed into the well containing an alkaline phosphatase-tagged anti-T4antibody (conjugate). The antigen present in the serum and the T4 antigen coated on the interior of the SPR compete for the available sites on the specific anti-T4antibody conjugated to alkaline phosphatase. During the concluding detection step, the substrate (4-Methyl-umbelliferyl phosphate) was cycled in and out of the SPR. The conjugate enzyme catalyzed the hydrolysis of this substrate into a fluorescent product (4-Methyl-umbelliferone). The strength of the fluorescence produced, measured at wavelength of 450 nm by the optical system of the analyzer, was inversely proportional to the concentration of free thyroxine present in the sample. At the end of the assay, results were automatically evaluated by the analyzer in relation to the calibration curve programmed in the memory, and then printed.

3.8.3.4 Principle of anti-thyroid peroxidase antibodies (IgG) using *VIDAS®* *AntiTPO* and *Mini Vidas* analyzer

The *VIDAS* Anti-TPO assay is an automated quantitative test for the detection of the IgG class of thyroid peroxidase autoantibodies (anti-TPO) in human serum. The reaction medium was cycled in and out of the SPR repeatedly. After first round of wash and sample dilution steps, the anti-TPO antibodies present in the sample bound to the recombinant protein covering the inner part of the SPR. The cycle of washings removed the unattached components. Anti-human IgG antibodies conjugated with alkaline phosphatase, attached to the immune complex coating the interior of the SPR. A final wash step removed the excess conjugate. In the last identification stage, the substrate (4-Methylumbelliferyl phosphate) was cycled in and out of the SPR. The conjugate enzyme catalyzed the hydrolysis of this substrate is into a fluorescent product (4-Methylumbelliferone). The strength of the fluorescence produced measured at 450 nm

by the optical system of the analyzer, was directly proportional to the anti-TPO (IgG) concentration autoantibodies in the serum. Finally the assay, results were calculated without human intervention by the analyzer using the standardization curve programmed in the memory, and then printed.

3.8.3.5 Principle of anti-thyroglobulin autoantibodies measurement using *VIDAS® anti-TG (ATG)* and *Mini Vidas* analyzer

The *VIDAS* Anti-TG assay is an automated quantitative test for the detection of the IgG subclass of thyroglobulin autoantibodies (anti-TG) in human serum. The reaction medium was cycled in and out of the SPR repeatedly. After preliminary wash and sample dilution stages, the anti-TG autoantibodies present in the sample bound to the protein coating the interior of the SPR. Repeated washings removed the unbound portions. Anti-human IgG antibodies conjugated with alkaline phosphatase, attached to the immune complex covering the interior of the SPR.

A concluding wash step eliminated the excess conjugate. In the last identification stage, the substrate (4-Methylumbelliferyl phosphate) was cycled in and out of the SPR. The conjugate enzyme catalyzed the hydrolysis of this substrate into a florescent product 4-methylumbelliferone. The intensity of the fluorescence produced was measured at 450 nm by the optical system of analyzer, was directly proportional to the concentration of anti-TG antibodies in the sample. Finally, results were automatically evaluated by the analyzer using the calibration curve programmed in the memory, and then printed.

3.8.3.6 Procedure for TSH, FT3, FT4, Anti-TPO and Anti-TG determination using Mini *Vidas*® analyzer and its reagents

1. All required reagents were stored at the refrigerator and kept at room temperature for at least 30 minutes before used.
2. One test strip and one SPR were used for each sample, control and calibrator.

3. The test was identified by test code on the instrument. The calibrator was identified by (S1) and tested in duplicate for TSH, FT3, Anti-TPO and AntiTG while FT4 calibrator was tested in triplicate. The calibrator, control and samples were gently mixed.
4. The SPRs and test strips were inserted into the instrument and checked to make sure the colour labels with the assay code on the SPRs and the reagents strips are match.
5. The assay was initiated as directed in the user's manual. All the assay steps are performed automatically by the instrument.
6. The vials were reclosed and returned to the required temperature after pipetting.
7. Assay was completed within approximately 40 minutes. After the assay was completed the SPRs and strips were removed from the instrument.
8. The used SPRs and strips were disposed into an appropriate recipient.

3.9 Definitions for thyroid function status

Classification of the thyroid status was based on the following reference ranges (Table 3.8) according to UK Guidelines for Thyroid Function Tests (**British Thyroid Association**, 2006) and algorithm for the diagnosis of thyroid dysfunction (**Joshi**, 2011) as shown in **figure 3.8**. Anti-TG and anti-TPO concentrations greater than 18.0 IU/mL and 8.0 IU/mL respectively, were classified as positive using the reference ranges provided by the manufacturer (**BioMérieux, France)**

Table 3.1 Reference ranges for thyroid hormone and TSH

MARKER	REFERNCE RANGE
TSH	0.4 – 4.5 μIU/ml
FT3	3.5 – 7.8 pmol/L
FT4	9.0 – 25.0 pmol/L

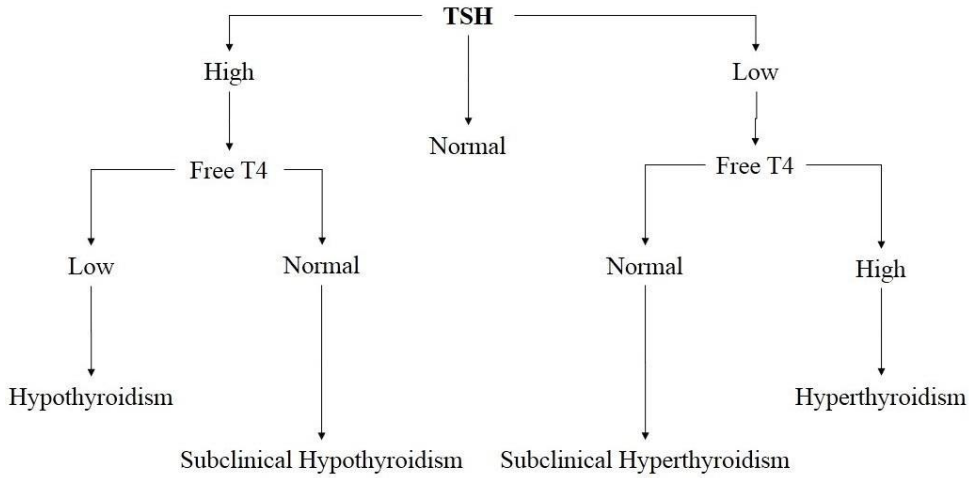


Figure 3.1 Algorithm for the diagnosis of thyroid dysfunction (Adapted from Joshi, 2011)

CHAPTER FOUR RESULTS 4.1 Demographic and clinical characteristics of the study subjects and control

subjects

The study participants numbered 264 with an equal ratio of 1:1 for diabetes mellitus patients (Case) and nondiabetic subjects (control). The average age of the study population were 57.88±0.60 years ranging from 31 years to 84 years.

The average age for type 1 diabetes mellitus subjects was found to be lower than that of type 2 diabetes mellitus subjects. The case and the control groups were agematched and sex-matched. The respondent gender distribution was skewed toward the female subjects 191(72.35%). As seen from table **4.1**, self-admitted cigarette smoking among both control group and case group was zero and only two diabetics representing 1.15% of the case group admitted to intake of alcohol. Majority of the case group 114 (86.36%) had type 2 diabetes mellitus.

All 132 diabetes patients who participated in this study were on either oral medication 101 (76.52%), insulin 18 (13.64%) or both 13 (9.85%).

Table 4. 1: Socio-demographic characteristics of study respondents stratified by diabetes status

Parameter	Total(n=264)	Cases(n=132)	Controls(n=132)
Gender Distribution			
Female	191(72.35)	96(72.73)	95(71.97)
Male	73(27.65)	36(27.27)	37(28.03)
Cigarette Smoking			
Smoker	0(0.00)	0(0.00)	0(0.00)
Non smoker	264(100)	132(100)	132(100)
Alcohol Consumption			
Non alcoholic	262(99.24)	130(98.48)	132(100)
Alcoholic	2(0.76)	2(1.52)	0(0.00)
Type of Diabetes Mellitus			
Type One	18(6.82)	18(13.64)	0(0.00)
Type Two	114(43.18)	114(86.36)	0(0.00)
Medication			
Insulin	18(6.82)	18(13.64)	0(0.00)
Oral	101(38.26)	101(76.52)	0(0.00)
Both	13(4.92)	13(9.85)	0(0.00)

Data is presented as figure with corresponding percentage in parenthesis

4.2: Demographic, glycaemic and thyroid function biomarkers stratified by diabetes status

The diabetes group presented with significantly higher levels of fasting blood glucose (9.15±0.37) and glycated haemoglobin (8.37±0.26) compared to their nondiabetic counterparts (4.84±0.04 and 3.88±0.06 respectively). The average levels of FT4 found among the case group was significantly higher compared to the levels recorded among the controls (p- <0.0001) as shown in table 4. 2.

Table 4.2: Demographic, glycaemic and thyroid function biomarkers stratified by diabetes status

Parameter	Total(n=264)	Case(n=132)	Control(n=132)	p-value
AGE (years)	57.88±0.60	59.51±0.94	58.25±0.72	0.0864
FBG(mmol/L)	7.00±0.23	9.15±0.37	4.84±0.04	<0.0001
HbA1c (%)	6.12±0.19	8.37±0.26	3.88±0.06	<0.0001
TSH(µIU/ml)	1.84±0.16	2.03±0.31	1.62±0.06	0.1869
FT3 (pmol/L)	4.59±0.03	4.63±0.05	4.55±0.04	0.2044
FT4 (pmo/L)	13.70±0.15	14.42±0.25	12.97±0.13	<0.0001

Data is presented as mean standard deviation of the mean. FBS-Fasting Blood Sugar, HbA1c-Glycated haemoglobin, TSH-Thyroid Stimulating Hormone FT3-Free Triiodothyronine, FT4-Free Tetraiodothyronine. p is significant at 0.05

4.3 Demographic, glycaemic and thyroid function biomarkers stratified by diabetes status and gender

No significant gender variations were observed both for the control group and the case group on all the variables presented in table 4.3, the exception however was seen with significant higher levels of TSH and FT3 among the diabetic males compared to the female in the same group. Significant higher levels of glycaemia (FBG and HbA1c) were observed for both genders in case group compared the control group (table **4.3**). Higher levels of FT4 were observed for both sexes when the case group was compared to the control group (p <0.0001, p=0.0100).

Table 4. 3: Demographic, glycaemic and thyroid function biomarkers stratified by diabetes status and gender

Parameter	Case			Control				
	Female	Male	P^1value	Female	Male	P^2value	P^3	P^4
AGE(years)	58.90±1.11	61.14±1.80	0.2909	56.42±0.80	55.81±1.55	0.7048	0.0723	0.0674
FBG(mmol/L)	9.57±0.45	8.02±0.64	0.0629	4.87±0.05	4.79±0.08	0.4211	<0.0001	<0.0001
HbA1c (%)	8.49±0.30	8.03±0.52	0.4213	3.88±0.07	3.86±0.09	0.8688	<0.0001	<0.0001
TSH(µIU/ml)	1.66±0.12	3.01±1.07	0.0488	1.57±0.07	1.72±0.10	0.2462	0.5420	0.2281
FT3(pmol/L)	4.57±0.06	4.80±0.09	0.0444	4.52±0.05	4.63±0.07	0.2187	0.5317	0.1244
FT4(pmol/L)	14.40±0.30	14.47±0.46	0.8964	12.93±0.15	13.08±0.27	0.6039	<0.0001	0.0100

Data is presented as mean ± standard deviation of the mean. FBG-Fasting Blood Glucose , HbA1c-Glycated Haemoglobin, TSH-Thyroid Stimulating Hormone), FT3-Free Triiodothyronine , FT4- Free Tetraiodothyronine. P^1-value-Female compared to Male in the case group, p2- Female compared to male in the control group p3- female compared to female between control and case groups, p4 –male compared to male between case and control groups, p is significant at 0.05.

4.4 Demographic, glycaemic and thyroid function biomarkers stratified by type of diabetes

There were significant variations in age, glycaemic levels and FT4 levels when the results were stratified by disease status. Regardless of type of diabetes the glycaemic levels were found to be higher among the case group in relation to the control group. Even though the fasting plasma glucose levels were comparable between the types 1 and 2 diabetes mellitus, glycaemic control was found to be better among the type 2 diabetes group (p=0.0023). FT4 levels ranged from the control group (12.97±0.13) through the type 1 diabetics (14.35±0.58) and peaking among the type 2 diabetes (14.43±0.28) as shown in table 4.4

Table 4. 4: Demographic, glycaemic and thyroid function biomarkers stratified by type of diabetes mellitus

Parameter	Control	Type One	Type Two	p	p1	p2	p3
AGE(years)	56.25±0.72	50.61±1.94	60.91±0.99	<0.0001	0.0501	0.0003	0.0001
FBG(mmol/L)	4.84±0.04	9.75±1.24	9.05±0.39	<0.0001	<0.0001	<0.0001	1.0000
HbA1c (%)	3.88±0.06	9.94±0.74	8.12±0.27	<0.0001	<0.0001	<0.0001	0.0023
TSH (µIU/ml)	1.62±0.06	1.81±0.38	2.06±0.35	0.3868	1.0000	0.5059	1.0000
FT3 (pmol/L)	4.55±0.04	4.54±0.15	4.65±0.06	0.3208	1.0000	0.4490	1.0000
FT4 (pmol/L)	12.97±0.13	14.35±0.58	14.43±0.28	<0.0001	0.0533	<0.0001	1.0000

Data is presented as mean ± standard deviation of the mean. FBG-Fasting Blood glucose, HbA1c- Glycated Haemoglobin, TSH- Thyroid Stimulating Hormone, FT3Free Triiodothyronine, FT4-Free Tetraiodothyronine p-ANOVA, p1- Control compared to Type One, p2- Control compared to Type Two, p3- Type One compared to Type Two. p is significant at 0.05

4.5. Demographic, glycaemic and thyroid function biomarkers stratified by type of medication

Stratification of diabetics into the type of medication used revealed no significant differences in the levels of thyroid function biomarkers assayed in this study. Though the results did not show any significant difference in fasting blood glucose levels among the various medication categories, glycaemic control was found to be better among the group taking oral medication compared to those placed on only insulin as shown in **Table 4.5**

Table 4.5: Demographic, glycaemic and thyroid function biomarkers stratified by type of medication

Parameter	Insulin	Oral	Both	p	p1	p2	p3
AGE(years)	50.61±1.94	61.04±1.05	59.92±3.05	0.0006	0.0004	0.0429	1.0000
FBG(mmol/L)	9.75±1.24	9.06±0.41	9.01±1.10	0.8147	1.0000	1.0000	1.0000
HbA1c (%)	9.94±0.74	8.07±0.30	8.48±0.55	0.0468	0.0410	0.5128	1.0000
TSH(µIU/ml)	1.81±0.38	2.12±0.39	1.65±0.26	0.8685	1.0000	1.0000	1.0000
FT3(pmol/L)	4.54±0.15	4.64±0.06	4.69±0.11	0.7421	1.0000	1.0000	1.0000
FT4(pmol/L)	14.35±0.58	14.44±0.29	14.37±0.86	0.9920	1.0000	1.0000	1.0000

Data is presented as mean ± standard deviation of the mean. FBG-Fasting Blood Glucose, HbA1c-Glycated Haemoglobin, TSH-Thyroid Stimulating Hormone, FT3Free Triiodothyronine, FT4-Free Tetraiodothyronine. p-ANOVA, p1- Insulin compared to Oral medication, p2- Insulin compared to both, p3- Oral medication compared to both. *p* is significant at 0.05.

4.6 Prevalence of thyroid dysfunction

The results from our study indicated that 15(11.36%) out of the 132 diabetic subjects studied had thyroid dysfunction (6.82%) subclinical hypothyroidism and 4.54% subclinical hyperthyroidism) and 117(88.64 %) were euthyroid. As seen from **table**

4.6, five out of nine with subclinical hypothyroidism were females while 4 were males. Out of the total number of six (6) with subclinical hyperthyroidism, five (5) were females while only one was a male. This study did not record any overt thyroid dysfunction and all the132 nondiabetic control subjects studied were euthyroid.

Table 4.6: Prevalence of thyroid dysfunction

Type of thyroid dysfunction	Case(n=132)			Control(n=132)		
	Male(n=36)	Female(n=96)	Total(n=132)	Male(n=35)	Female(n=97)	Total(132)
Subclinical Hypothyroidism	4(11.11)	5(5.21)	9(6.82)	0(0)	0(0)	0(0)
Subclinical Hyperthyroidism	1(2.78)	5(5.21)	6(4.54)	0(0)	0(0)	0(0)
Euthyroid	31(86.11)	86(89.58)	117(88.6)	35(100)	97(100)	132(100)

Data is presented as figure with corresponding percentage in parenthesis

4.7. Prevalence of anti-peroxidase and anti-thyroglobulin autoantibodies among the study population

The overall prevalence of anti-thyroid peroxidase autoantibodies (anti-TPO) was 3.41% among the study population (both control and case groups). Seven (7) persons out of the nine (9) who reacted positive to anti-TPO were among the diabetes group with six (6) coming from those suffering from type 2 diabetes mellitus and one from the type 1 diabetes mellitus group. Majority of the diabetic respondents (5) presenting with anti-TPO reactivity were on oral medication therapy.

The prevalence of anti-thyroglobulin autoantibodies (anti-TG) among the overall study participants (both control and case groups) was estimated at 3.41%. The results showed that 3.79 % of the diabetic group and 3.03% of the control were positive to anti-TG. As seen from table 4. 7, five (5) out of the nine (9) persons presenting with anti-TG reactivity had diabetes mellitus. Type 2 diabetes accounted for 4 (3.51%) and type 1 accounted for 1(5.56%).

Out of the 18 autoantibody reactivity (anti- TPO and anti-TG) recorded in this study; ten (10) representing 3.79% were single autoantibody reactions. The prevalence of both anti-TPO autoantibodies and anti-TG antibodies (double autoantibody) reactivity in a single subject was 1.52%. As shown in figure 4.1, single autoantibody reactivity was 4.5% among the controls with no double reactivity, Three percent (3.0%) of both single and double autoantibody reactivity was observed among the diabetics. Two (2) out of the four (4) positive single autoantibody reactions in the case group were found among type one diabetes and all double autoantibody reactivity occurred among participants presenting with type 2 diabetes mellitus.

Table 4.7: Prevalence of Anti-thyroid peroxidase autoantibodies and AntiThyroglobulin autoantibodies among the study population

	Parameter	Negative		Positive		p-Value
		Frequency	*Percentage*	*Frequency*	*Percentage*	
Anti-thyroid peroxidase autoantibodies (Anti -TPO)	Total Respondent	255	96.56	9	3.41	
	Case	125	94.70	7	5.30	0.0861
	Control	130	98.48	2	1.52	
	Female	185	96.86	6	3.14	0.4744
	Male	70	95.89	3	4.11	
	Insulin Only	17	94.44	1	5.56	0.9162
	Oral Tab Only	96	95.05	5	4.95	
	Both	12	92.31	1	7.69	
	Type One	17	94.44	1	5.56	1.000
	Type Two	108	94.74	6	5.26	

Anti-Thyroglobulin autoantibodies (Anti-TG)	Total Respondent	255	96.56	9	3.41	
	Case	127	96.21	5	3.79	0.5000
	Control	128	96.98	4	3.03	
	Female	186	97.38	5	2.62	0.2154
	Male	69	94.52	4	5.48	
	Insulin Only	17	94.44	1	5.56	0.6430
	Oral Tab	98	97.03	3	2.97	
	Both	12	92.31	1	7.69	
	Type One	17	94.44	1	5.56	0.5254
	Type Two	110	96.49	4	3.51	

Data is presented as figure and percentages .p is significant at 0.05.

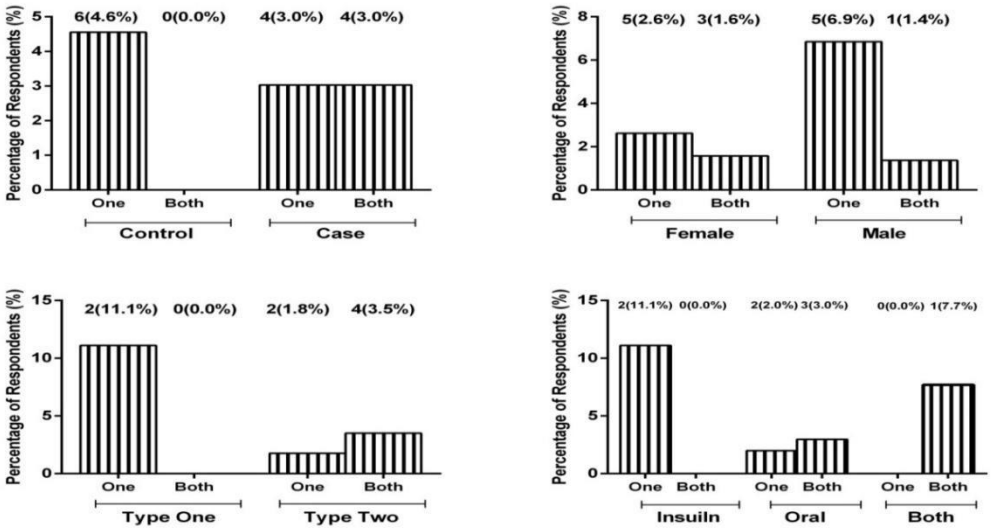


Figure 4. 1: Thyroid autoantibody reactivity characteristics: One: Positive reaction to either anti-thyroglobulin autoantibodies (TG) or anti-thyroid peroxidase autoantibodies (TPO), Two: Positive reaction to both as anti-thyroglobulin autoantibodies (TG) and anti-thyroid peroxidase autoantibodies (TPO).

4.8 Demographic, glycaemic and thyroid function biomarkers stratified by the presence of anti-thyroid peroxidase autoantibodies (TPO) and anti-thyroglobulin autoantibodies (TG)

Though not statistically significant, the levels of all the glycaemic as well as the thyroid function biomarkers assayed were found to be higher among participants presenting with autoantibody reactivity. However HbA1c level was significantly better among the anti-peroxidase antibody negative subjects and the mean TSH level was significantly higher among the autoantibody reactive subjects (**Table 4. 8)**

Table 4. 8 Demographic, glycaemic and thyroid function biomarkers stratified by the presence of anti-Peroxidase autoantibodies (TPO) and anti-thyroglobulin autoantibodies (TG)

	Parameter	Negative	Positive	p-value
Anti-Thyroid Peroxidase auto ntibodies Anti-TPO) (	AGE(years)	57.84±0.62	59.11±2.14	0.7005
	FBG(mmol/L)	6.91±0.23	9.33±1.51	0.0553
	HbA1c (%)	6.03±0.19	8.62±0.94	0.0139
	TSH(µIU/ml)	1.71±0.06	5.03±1.32	0.0001
	FT3(pmol/L)	4.59±0.03	4.74±0.11	0.3854
	FT4(pmol/L)	13.65±0.15	15.00±1.41	0.0987

Anti-Thyroglobulin	**Autoantibodies(Anti-TG)**	AGE(years)	58.11±0.60	51.44±3.94	0.0438
		FBG(mmol/L)	6.99±0.24	7.27±0.78	0.8245
		HbA1c (%)	6.08±0.19	7.19±1.16	0.2960
		TSH(µIU/ml)	1.69±0.06	5.52±4.26	<0.0001
		FT3(pmol/L)	4.59±0.03	4.65±0.12	0.7581
		FT4(pmol/L)	13.67±0.14	14.55±1.51	0.2775

Data is presented as mean±standard deviation of the mean. FBS-Fasting Blood Sugar, HbA1- Glycated Haemoglobin, TSH-Thyroid Stimulating Hormone (FT3-Free Triiodothyronine , FT4 -Free Tetraiodothyronine. *p* is significant at 0.05.

4.9 Pearson and partial correlation of demographic, glycaemic and thyroid function biomarkers

Among the general study population, increasing FT4 levels was significantly associated with increasing additive levels of fasting blood glucoseand glycated haemoglobin after Bivariate Pearson correlations analysis. Age and gender were found not to influence the association between FT4 and the glycaemic biomarkers assayed in this study.

Table 4.9: Pearson and partial correlation of demographic, glycaemic and thyroid function biomarkers

Parameter	FBG	HbA1c	TSH	FT3	FT4
Bivariate Correlations Unadjusted					
AGE(years)	0.089	0.126*	-0.007	0.026	0.022
FBG(mmol/L)		0.735**	0.018	0.096	0.260**
HbA1c (%)			0.005	0.042	0.217**
TSH(µIU/ml)				0.059	-0.067
FT3(pmol/L)					0.220**

Partial Correlations Adjusted for Age				
FBG (mmol/L)	0.732**	0.018	0.094	0.259**
		0.006	0.039	0.216**
HbA1c(%)				
TSH(µIU/ml)			0.059	-0.067
FT3(pmol/L)				0.219**
Partial Correlations Adjusted for Age and sex				
FBG(mmol/L)	0.732**			
		0.033	0.111	0.262**
HbA1c(%)		0.012	0.047	0.217**
TSH(µIU/ml)			0.041	-0.07
FT3((pmol/L)				0.219**

FBS-Fasting Blood Sugar, HbA1c-Glycated haemoglobin, TSH-Thyroid Stimulating Hormone, FT3-Free Triiodothyronine, FT4-Free Tetraiodothyronine. *p is significant at 0.05, **p is significant at 0.01.

4.10 Trend analysis of thyroid autoantibody reactivity

Among the general study population, HbA1c levels indicating glycaemic control was found to exhibit a significant linear incremental trend from autoantibody none reactive through single autoantibody reactivity to double autoantibody reactivity. TSH levels as well as tetraiodothyronine (FT4) significantly peaked among the double autoantibody reactive subjects (Figure 4. 2)

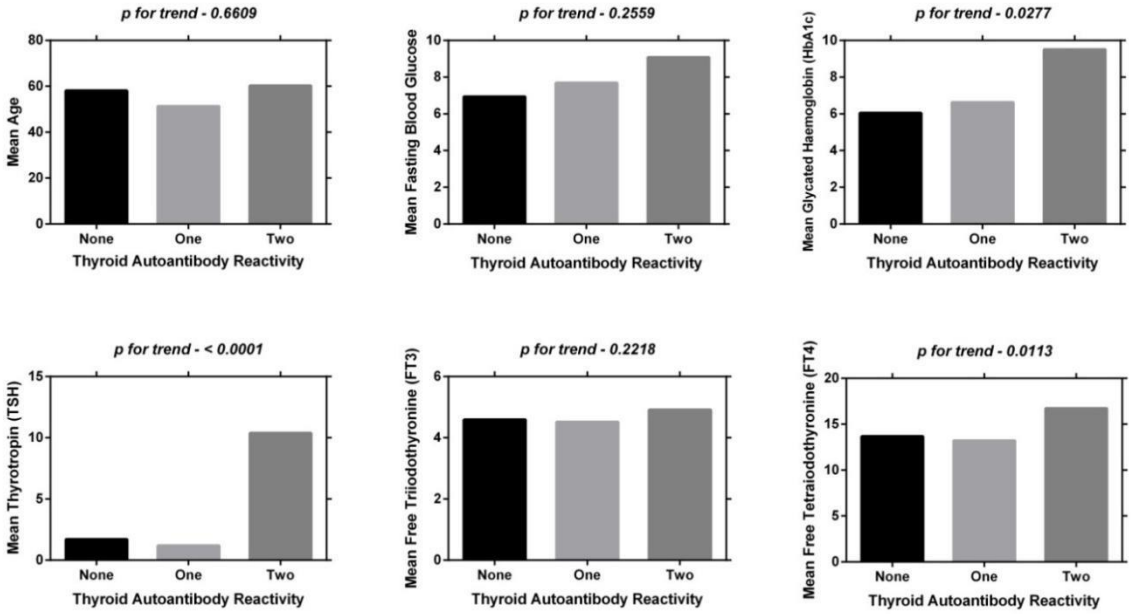


Figure 4.2: Trend analysis of thyroid autoantibody reactivity. None: No autoantibody reaction, One: Positive reaction to either anti- thyroglobulin autoantibodies (TG) or anti-thyroid peroxidase autoantibodies (TPO), Two: Positive reaction to both antithyroglobulin autoantibodies (TG) and anti-thyroid peroxidase autoantibodies (TPO).

CHAPTER FIVE DISCUSSION

The present study set out to look at the prevalence of thyroid dysfunction and thyroid autoimmunity and also to find possible relationship between thyroid dysfunction and glycaemic control among clinically euthyroid diabetes mellitus patients seen at Effia Nkwanta Regional Hospital, Sekondi. Literature is extensive when issues about thyroid dysfunction and diabetes mellitus are concerned; however, it is scarce when looking at the Ghanaian population. These objectives were successfully completed by means of a cross-sectional study design that evaluated thyroid biomarkers and glycaemic levels among 132 clinically euthyroid diabetes mellitus subjects and 132 age-matched and sex- matched control participants of this study.

5.1 Thyroid hormones and Thyroid stimulating hormone (TSH) levels of the study subjects

In this study, the average serum FT3 and TSH between diabetes mellitus subjects compared to their apparently normal counterparts were not significantly different. However, the mean level of FT4 found among the case group was significantly higher compared to the levels recorded among the controls. Though, the relationship between thyroid dysfunction and diabetes mellitus has been documented previously, various studies have reported inconsistent results. Some studies have shown reduced TSH levels, in combination with elevated FT3, FT4 or lowered FT3, FT4 levels in thyroid dysfunction among type 2 diabetes mellitus compared to non-diabetics (**Smithson,** 1998; **Roos** *et al*., 2007; **Vikram** *et al*., 2013; **Singh** *et al*., 2014). However, the results of our study corroborates findings of a study by **Udiong** *et al*., (2007) which evaluated thyroid hormone levels in both type 1 and type 2 diabetics in Nigeria. In that study, the levels of FT4 in diabetic subjects were significantly higher than those observed in the control ($p = 0.015$), but the levels of TSH did not differ significantly between the diabetic individuals and their control peers (**p** = 0.51). High levels of FT4 associated with diabetes mellitus has been attributed firstly to the existence of thyroid hormone binding inhibitor (alters the extra-thyroidal change of T4 to T3) and secondly to the dysfunction of the hypothalamo-pituitary thyroid axis; a phenomenon observed to worsen in poorly managed diabetes mellitus subjects (**Suzuki** *et al*., 1994).

In view of the fact that type 1 and type 2 diabetes mellitus have different aetiologies, the thyroid hormones and TSH in both groups were compared to look for any differences. It was observed that the levels of FT3, FT4 and TSH between type 1 and 2 diabetic subjects were similar (**Table 4.4**). However, in this study only 13.64 % of the diabetic subjects were classified as type 1 diabetics. The skewed nature of the

population towards subjects with type 2 diabetes mellitus may have obscured any differences in terms of the thyroid biomarkers assayed.

It is believed that abnormal thyroid hormones concentrations in diabetes mellitus subjects could result from difference in drug therapy (**Celani** *et al.*, 1994). In the current study, diabetes mellitus subjects were stratified into the type of medication used to control their glycaemic levels. The results did not show any significant difference in the levels of thyroid function biomarkers assayed in this study. This is in line with those observed by **Ghazali** and **Abbiyesuku** (2010) in a study to determine prevalence of thyroid dysfunction among type 2 diabetics attending the University College Hospital, Ibadan, Nigeria. However, our results contrasted with the findings of a study by **Jain** *et al*., (2013) among type 2 diabetes, which recorded statistically significantly higher ($p< 0.001$) thyroid dysfunction in patients who were on both oral hypoglycaemic agents and insulin than oral hypoglycaemia agents alone. In an attempt to explain why there was no significant association between medications used and thyroid function status in their study, **Ghazali** and **Abbiyesuku** (2010) postulated that some confounding variables, particularly treatment compliance could influence glycaemic control but this could not be monitored. The limitation in their view was ascribed to the cross-sectional nature of their study design. These assertions appear to be true in the current study.

Analyses of thyroid hormone levels according to sex in the diabetic group showed significantly higher levels of FT3 (4.80±0.09 vs4.57±0.06; $p=0.044$) and TSH (3.01±1.07 vs 1.66±0.12; $p=0.0488$) in the male diabetics compared with the female diabetics. However, FT4 levels did not differ statistically between the two sexes. Our results agree partly with the results from a study by **Begum** (2014) who evaluated thyroid function among diabetes mellitus subjects. In that study, FT3 level was

significantly higher in diabetic males compared with their female counterparts (4.79 ± 1.18 vs 3.95 ±1.57; p=00028). However, TSH level in diabetic males was significantly lower than their female peers (1.60 ±0.87 vs 2.23 ± 1.08; p=0.0017); FT4 did not show any significant difference with respect to gender consistent with our study.

5.2 Prevalence of thyroid dysfunction

In the present study, 15(11.36%) out of the 132 diabetic subjects studied had thyroid dysfunction and 117 (88.64 %) were euthyroid. The study did not record any overt thyroid dysfunction and all the control subjects were biochemically euthyroid. A study done earlier by **Tagoe** *et al*., (2014) which recorded 10.1 % prevalence of thyroid dysfunction among Ghanaian diabetes mellitus population attending the Korle-Bu Teaching Hospital in Accra was comparable to the results of the current study. However, in Nigeria and India, **Udiong** *et al*., (2007) and **Singh** *et al*., (2014) reported 46.5% and 29.0 % respectively among diabetic subjects with thyroid dysfunctions; rates much higher than what was recorded in the current study. The difference in the rate observed in this study and the previous studies cited above could be attributed to difference in population characteristics such as race, sex, and age as well as methodological differences. These factors have been shown to be determinants of a wide range of prevalence reports of thyroid dysfunction among persons with diabetes mellitus **(Moayeri** and **Rabbani**, 2004).

In the current study, thyroid dysfunction was found to be more prevalent in females than in males. Similar observations were made not only among individuals with diabetes mellitus but also persons in the general population (Udiong *et al*, 2007; Wu 2000; Johnson, 2006; **Sukkar** *et al*., 2000). Ghazali and Abbiyesuku, (2010) suggested age as a variable could be responsible for the drift in prevalence rate towards female gender since age increases with both type 2 diabetes and thyroid dysfunction.

5.3 Prevalence of anti-TPO and anti-TG antibodies among the study population

The overall prevalence of thyroid autoimmunity was 5.30% and 1.52% for anti-TPO positivity among diabetic and control group respectively with no significant difference between the two groups (p=0.0861). The anti-TG antibody positivity was 3.79% and 3.03% respectively among the diabetic and control group, also with no significant difference between the two groups (p=0.5000) as shown in **table 4.7** (page 53). The prevalence of thyroid autoimmunity recorded among the diabetic and control groups in this study is similar to those reported by **Mohammed** *et al*., (2009). It has been suggested that the sensitivity and specificity of a method influences the levels of anti-TPO and anti-TG (**La'ulu**, 2007). Again, factors such as ethnicity and geographical boundaries as well as dietary iodine intake have been shown to affect anti-TPO prevalence in population studies (**Meisinge**r *et al*., 2012). For example, antiTPO prevalence has been found to be significantly higher (estimated at 11%) in countries like the United States where there is adequate dietary iodine as compared with areas in Europe (estimated at 6 %) where there is inadequate dietary iodine intake (**d'Herbomez** *et al*.,2005;**Hollowell** *et al*., 2002;**Kasagi** *et al*., 2009). Again, **Radaideh** *et al*., (2004) recorded prevalence of 10.3 % of anti-TPO among nondiabetics in Jordan but few studies among Africans recorded much lower prevalence (0-2.7%) in apparent healthy subjects (**Olusi** *et al*., 1991;**Omar** *et al*., 1986). The lower prevalence of thyroid autoimmunity in Black populations is consistent with some studies in the United States of America, in which thyroid antibodies among Africans was lower than in caucasian Americans (**Hollowell** *et al*., 2002).

The mean concentration of TSH in the total population was associated with positive anti-TPO ($p<0.0001$) and anti-TG ($p<0.0001$) concentrations (**Table 4.8**) which is in agreement with studies reported previously (**Surks** and **Hallowell**, 2007;

Premawardhana *et al.*, 2003). The significantly higher levels of TSH in positive anti-TPO and anti-TG subjects could be an indication of the existence of chronic lymphocytic thyroiditis, which may slightly compromise thyroid hormone synthesis and release resulting in a small change of TSH levels towards higher values.

5.4 Correlation of serum FT3, FT4 and TSH, with and HbA1c in the study subjects

In our study, FT4 levels had a weak positive significant correlation with FBG(r=0.260 p=0.01) and HbA1c (r=0.217 p=0.01) contrary to what was recorded by **Begum** *et al*., (2014) in a study to evaluate thyroid dysfunction among type 2 diabetics. They showed that FT4 concentrations did not show any significant correlation with FBG levels (r= -0.066, p= 0.517) and HbA1c levels (r =0.001, p=0.99). **Saha** *et al.*, (2012) in a comparative study of thyroid hormones and lipid status in diabetic and nondiabetic adults in Bangladesh also showed that correlation between FT4 and HbA1c was insignificant(r=0.040, p=0.76).

In our study, serum levels of FT3 and TSH did not show any significant correlation with the glycaemic markers. In constrast**, Bazrafshan** *et al*., (2000) found a significant positive correlation between glycated haemoglobin levels and TSH levels among type 2 diabetes mellitus subjects.

5.5 Limitations of the study

The cross-sectional design of our study limits the inferences that can be made from the results. A causal path cannot be established that would lead to a conclusion that in fact, diabetes mellitus subjects who have positive thyroid autoantibodies will progress to develop thyroid dysfunction. Again , this study did not biochemically distinguish between type 1 and type 2 diabetes mellitus using serum C-peptide, serum insulin levels and any of antibodies against the β cells

CHAPTER SIX

CONCLUSION AND RECOMMENDATIONS

6.1 Conclusion

In conclusion, our study is important, because it has provided the prevalence rates of thyroid dysfunction and thyroid autoimmunity among diabetes mellitus subjects attending out patient's clinic at Effia Nkwanta Regional Hospital in Sekondi, Western Region of Ghana. The prevalence of thyroid dysfunction among the diabetic population seen at the hospital was 11.36%. The study did not record any overt thyroid dysfunction among the study population. This study also showed that 5.30 % of the diabetes mellitus population and 1.52% of the nondiabetic control group were thyroid anti- thyroid peroxidase autoantibody positive, while thyroid anti- thyroglobulin autoantibody positivity was prevalent in 3.79% of the diabetic mellitus subjects and in 3.03% of the nondiabetic. In our study, we did not record any significant difference in the anti-TPO positivity and anti-TG positivity between the diabetes mellitus subjects and nondiabetic control population.

6.2 Recommendations

Diabetes mellitus is a multifactorial disorder; hence future works should direct attention to the determination of other risk factors associated with the disease for deeper understanding of the pathogenesis. Future studies should also expand the sample size of the study population so that findings can generalised.

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