THYROID DYSFUNCTION AMONG PATIENTS WITH TYPE 2 DIABETES MELLITUS AT MOI TEACHING AND REFFERAL HOSPITAL, ELDORET, KENYA.

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A RESEARCH THESIS SUBMITTED IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE AWARD OF THE DEGREE OF MASTER OF MEDICINE IN INTERNAL MEDICINE, MOI UNIVERSITY.

DECLARATION

Candidate's Declaration

This research thesis is my original work and has not been presented for a degree at any other University. No part of this work may be reproduced without the permission of the author and /or Moi University.

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DEDICATION

I dedicate this work to my parents; John and Lucy Mwaura and my siblings Peter, Caroline and Christine for their prayers, support, encouragement and inspiration.

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I am grateful to my family, friends and colleagues for their constant encouragement and motivation. Finally, I wish to thank God for bringing me this far in good health and all that I am.

LIST OF ABBREVIATIONS

AACE	American Association of Clinical Endocrinologists
ATA	American Thyroid Association
BMI	Body Mass Index
BTA	British Thyroid Association
CHD	Chronic Heart Disease
DM	Diabetes Mellitus
DOPC	Diabetic Outpatient Clinic
FT3	Free Triiodothyronine.
FT4	Free Thyroxine.
GLUT-2	Glucose Transporter type 2
HbA1c	Glycosylated Hemoglobin
HTN	Hypertension.
LDL	Low Density Lipoprotein cholesterol
MTRH	Moi Teaching and Referral Hospital.
MUSOM	Moi University School of Medicine
PI	Principal Investigator.
T1DM	Type 1 Diabetes Mellitus
T2DM	Type 2 Diabetes Mellitus
TSH	Thyroid Stimulating Hormone.

DEFINITION OF TERMS

Case definition for Type 2 Diabetes Mellitus

This was defined as patients who had a previous diagnosis of Type 2 DM and were above the age of 35years at the time of diagnosis(Ladenson et al., 2000;Garber et al., 2012).

Glycemic Control

ADA Guidelines recommend that less stringent HbA1c goals (8%) may be appropriate for patients with history of severe hypoglycemia, limited life expectancy and advanced microvascular or macrovascular complications. Most of our patients fit the above description hence use of the below cut-offs(American Diabetes Association, 2017)

HBA1c $\leq$ 8% - Good control

HBA1c >8% - Poor control

Diagnosis of Thyroid Dysfunction

Diagnosis of thyroid dysfunction is usually based on local laboratory practices guidelines and assay specific manufacturer reference ranges (Kahapola- Arachchige, Hadlow, Wardrop, Lim, & Walsh, 2012). In this study, the diagnosis was based on the reference ranges provided by the AMPATH reference laboratory and the Elecsys assay manufacturer as shown in the table below.

REFERENCE RANGES FOR TSH AND FREE T4

Thyroid Status	TSH Level (mIU/l)	Free T4 Level (ng/dl)
Euthyroid	0.27- 4.2	0.932-1.71
Subclinical hypothyroidism	>4.2	0.932-1.71
Hypothyroidism	>4.2	< 0.932
Hyperthyroidism	<0.27	> 1.71

Reference ranges for LDL Cholesterol

LDL-C levels were defined as normal if ≤2.59mmol/l, while >2.59 mmol/l was abnormal. (Eldor & Raz, 2009)

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ABSTRACT

Background: Thyroid dysfunction is more prevalent among patients with diabetes (10-24%) compared to the general population (2-7%). Coexistence of thyroid disorders among patients with diabetes results in impaired lipid metabolism, endothelial dysfunction and impaired glycemic control. These risk factors have been implicated in worsening of cardiovascular disease which is the leading cause of death in diabetes. Early recognition and treatment of thyroid dysfunction in diabetes is important for mitigation of associated cardiac complications. Whereas diabetes is common in our region, little is known about the prevalence of thyroid dysfunction among patients with diabetes.

Objectives: To determine the prevalence and clinical correlates for thyroid dysfunction among patients with Type 2 Diabetes Mellitus (DM) in Moi Teaching & Referral Hospital (MTRH).

Methods: This was a cross-sectional study conducted at the diabetes outpatient clinic in MTRH between February and April 2018. The study population included 2500 ambulatory patients with Type 2 DM, aged 35 years and above at the time of diagnosis, out of which 368 participants were enrolled by systematic random sampling. Structured interviewer administered questionnaires were utilized to collect socio-demographic, clinical and laboratory data. Third generation immunoassays were used for measurement of Thyroid Stimulating Hormone (TSH), glycated hemoglobin (HbA1c) and low-density lipoprotein-cholesterol (LDL-C). Descriptive statistics such as the median and interquartile ranges were used to summarize continuous variables. Frequencies and percentages were used to summarize categorical variables. Association between thyroid dysfunction and categorical independent variables was assessed using Pearson's Chi Square test. Fisher's exact test was reported whenever the Chi Square assumptions were violated. Multiple logistic regression was used to determine the effect of independent variables on thyroid function.

Results: The median age of the participants was 59.2years, with 230(62.5%) of these being females. Prevalence of thyroid dysfunction among patients with Type 2 DM was 9.2% (95%CI: 6.4, 12.7) representing 34 participants. Subclinical hypothyroidism was the most frequent dysfunction at 7% (22). Sociodemographic (age, gender) and clinical characteristics (duration of Type 2 DM, insulin use, hypertension, body mass index and LDL-C) studied indicated no association with thyroid dysfunction. There was no significant correlation between thyroid dysfunction (TSH, freeT4) and glycemic control (HbA1c).

Conclusion: The prevalence of thyroid dysfunction among patients with Type 2 DM is high compared to what has been reported in the general population. There was no significant correlation found between thyroid dysfunction and the studied clinical correlates.

Recommendations: Routine assessment of thyroid hormone levels should be part of evaluation for patients with Type 2 DM. A prospective study should be considered to further evaluate other clinical correlates that may be associated with thyroid dysfunction.

CHAPTER ONE: INTRODUCTION

1.1: Background

Thyroid disease is the second most common endocrine disorder in the general population after diabetes mellitus worldwide. Hypothyroidism is by far the most common thyroid disorder in the adult population and its prevalence increases with age (Biondi, Kahaly, & Robertson, 2019), (Bjoro et al., 2000).

The worldwide prevalence of diabetes has been steadily increasing. It has been projected that by the year 2030, 366 million people will be living with diabetes. The prevalence among all age groups is also expected to rise from 2.8% in 2000 to 4.4% in 2030 with a marked increase in prevalence of diabetes in people above 65 years (Wild, Roglic, Green, Sicree, & King, 2004).

It has been suggested that thyroid disease and diabetes mellitus have a common genetic background(Akbar, Ahmed, & Al-Mughales, 2006). Current genetic evidence is almost exclusively restricted to autoimmune causes with a strong association between Type 1 diabetes and autoimmune thyroid disease. Thyroid antibody positivity has been found in upto 50% of T1DM patients (Huber, Menconi, Corathers, Jacobson, & Tomer, 2008).

Despite an almost similar frequency of thyroid disease in T2DM, genetic links have not been well characterized with only a few studies suggesting a direct genetic basis for thyroid disease in T2DM. Indirect links between thyroid disease and diabetes mellitus are better described with emphasis on effects of the thyroid hormone on glucose and lipid metabolism (Dora, Machado, Rheinheimer, Crispim, & Maia, 2010).

The presence of thyroid dysfunction in patients with Diabetes Mellitus affects glycemic control. Not only does it worsen glycemic control but it also worsens co-morbidities that

promote cardiovascular disease for example dyslipidemia and hypertension (Billic-Komarica, Beciragic, &Junuzovic, 2012).

Given the deleterious effect of untreated thyroid dysfunction on diabetes control and cardiovascular disease outcomes, screening for thyroid disease among patients receiving diabetes care in the community has been recommended (Smithson, 1998). In addition, demonstrated evidence of increased risk of nephropathy and retinopathy observed in patients with diabetes and hypothyroidism favors screening of T2DM patients for thyroid dysfunction and treating when present (Hage, Zantout, & Azar, 2011).

1.2: Problem Statement

Thyroid dysfunction has been found to be more prevalent among patients with diabetes (12.3- 61%) than in the general population (2-5%) in parts of the world. Not only does thyroid dysfunction affect glycemic control but it also compounds the complications through its effects on the cardiovascular system and cardiovascular risks.

Diabetes control has remained a challenge within our setup with high rates of suboptimal control. A study conducted in 6 sub-Saharan African countries including Kenya established that only one third of patients had appropriate glycemic control (Sobngwi et al., 2012). Consequently, there are higher complication rates especially cardiovascular.

Symptoms of thyroid dysfunction are non-specific and cannot be relied on for diagnosis thus require screening which is not frequently done despite recommendations for thyroid screening in T2DM (Billic-Komarica et al., 2012). That said, there is no data on the magnitude of this problem in Western Kenya, nor any data on any factors that can be used as surrogates to stratify or define who is at higher risk of TD among patients with diabetes.

1.3: Study Justification

Thyroid dysfunction has been found to be prevalent among diabetes patients in parts of the world with a wide range of prevalence reported.

Thyroid dysfunction affects glycemic control and also compounds the complications through its effects on the cardiovascular system and cardiovascular risks. These risk factors have been implicated in worsening of cardiovascular disease which is the leading cause of death among patients with diabetes (Raghavan et al., 2019).

Recognition and treatment of thyroid dysfunction is important for good glycemic control and reversal of associated cardiac dysfunction (Klein &Danzi, 2007).

The American Thyroid Association (ATA,2012), American Association of Clinical Endocrinologists (AACE,2012), British Thyroid Association (BTA,2006) guidelines recommend doing TSH levels in T2DM patients at baseline and annually for patients above the age of 35yrs. In the most recent Clinical guidelines for management of Diabetes in Kenya 2018 screening for thyroid dysfunction is now recommended among T2DM. Despite this recommendation it hasn't been adopted at all in our setup. Defining the burden and factors associated with thyroid dysfunction among T2DM would generate data that may help with adoption of these guidelines.

There is paucity of data on thyroid dysfunction in T2DM patients in Kenya. No studies have been done in our diabetes outpatient clinic at Moi Teaching and Referral Hospital to assess the magnitude of thyroid dysfunction and its correlates among T2DM patients.

1.4: Research Question

What is the prevalence and clinical correlates of thyroid dysfunction in patients with Type 2DM in MTRH?

1.5: Objectives

1.5.1: Broad Objective

To describe the prevalence and clinical correlates of thyroid dysfunction among patients with Type 2DM at MTRH.

1.5.2: Specific Objectives

1. To determine the prevalence of thyroid dysfunction among patients with Type 2 DM in Moi Teaching and Referral Hospital.
2. To describe factors associated with thyroid dysfunction in patients with Type 2 DM in Moi Teaching and Referral Hospital.
3. To correlate thyroid function with glycemic control in patients with Type 2DM in Moi Teaching and Referral Hospital Diabetes Outpatient Clinic.

1.6: Conceptual Framework

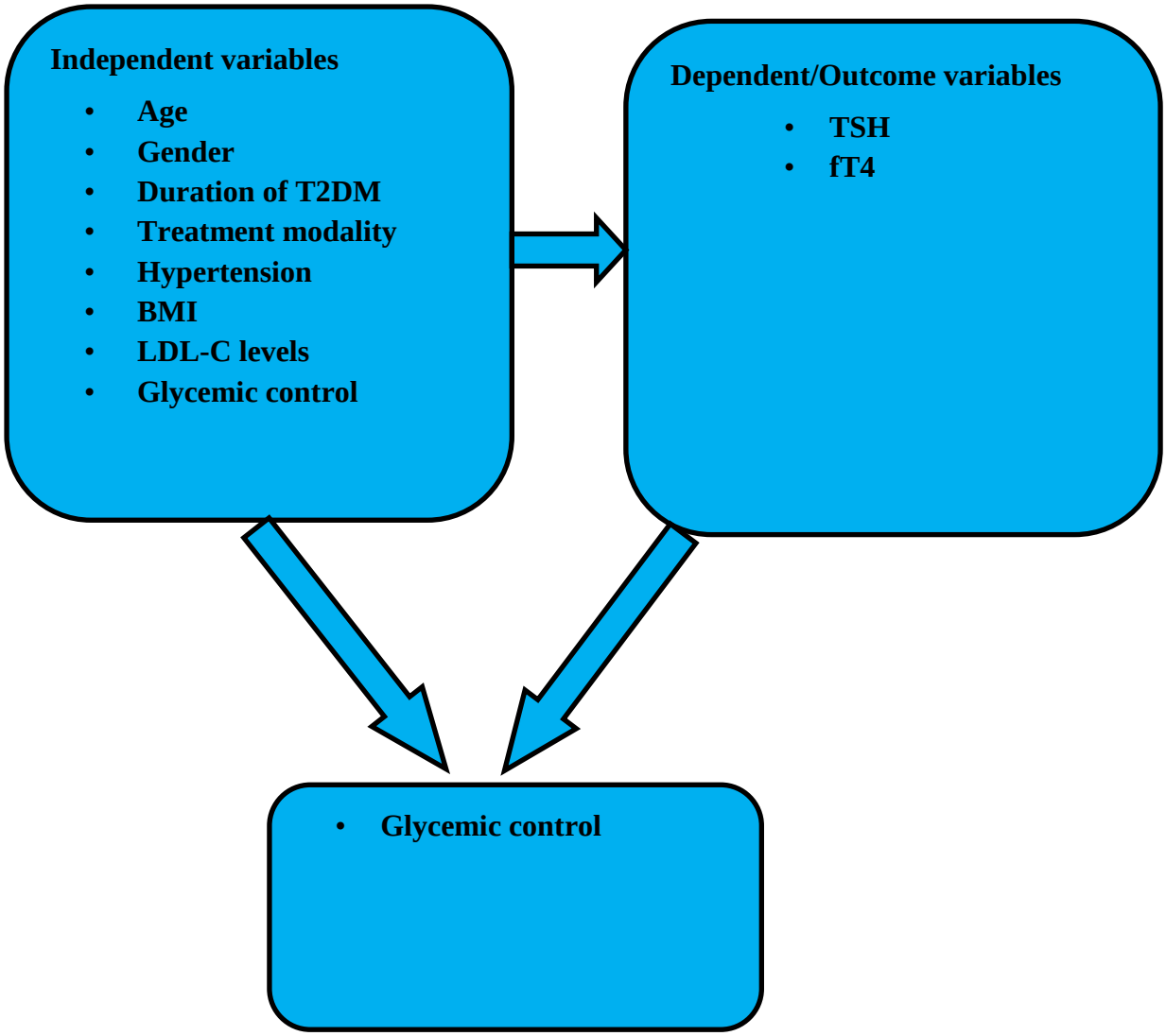


Figure 1: Conceptual Framework

CHAPTER TWO: LITERATURE REVIEW

2.1: Burden of Diabetes

The worldwide prevalence of diabetes has been steadily increasing. It has been projected that by the year 2030, 366 million people will be living with Diabetes. The prevalence among all age groups is also expected to rise from 2.8% in 2000 to 4.4% in 2030 with a marked increase in prevalence in people above 65 years of age (Wild et al., 2004).In 2011, 14.7 million adults in the African region of the World Health Organization (WHO) were estimated to be living with Diabetes Mellitus.

Of all the WHO regions, the African region is expected to have the largest proportional increase (90.5%) in the number of adult with diabetes by 2030 (Hilawe, Yatsuya, Kawaguchi, & Aoyama, 2013).A meta analysis of data on prevalence of Diabetes Mellitus in Sub Saharan Africa revealed between-country variation in the prevalence of Diabetes Mellitus among adults. However, an overall combined prevalence for all of the studies was high at 5.7% and is a reflection of the rapid transition from communicable to non-communicable diseases(Hilawe et al., 2013).

Kenya is not an exception in the rapid transition. Among 1459 participants of a cross-sectional study to assess the prevalence of glucose intolerance in rural and urban Kenyan population and in different ethnic groups; mean age 38.6 years (range 17–68 years), the overall age-standardized prevalence of diabetes and impaired glucose tolerance was 4.2% and 12.0%, respectively. On ethnicity, the Luo tribe had the highest prevalence of glucose intolerance among the rural ethnic groups(Christensen et al., 2009). A population based study done in Nairobi 4 years later revealed an age adjusted prevalence of diabetes was 5.3% (95% CI 4.2-6.4) and prevalence increased with age peaking at 10.5% (95% CI

6.8-14.3%) in the 45–54 year age category(Ayah *et al.*, 2013).

2.2: Prevalence of Thyroid Dysfunction

The prevalence of thyroid dysfunction continues to be debated upon with numerous studies indicating varying prevalence. However, most studies concluded that there was a higher prevalence of hypothyroidism in women which increased with advancement in age especially in those above 60 years. In the Colorado Study, 9.5% of the population was reported to have elevated TSH (Canaris, Manowitz, Mayor, & Ridgway, 2000). A study conducted in Whickham, England (M P J Vanderpump et al., 1995) found that 7.5% of women and 2.8% of men had elevated TSH level while the Framingham study found 13.6% of women had elevated TSH(Pearce, Yang, Benjamin, Aragam, & Vasan, 2010)(Mark P J Vanderpump, 2011).

In Africa, a systematic review done established that iodine deficiency is the leading cause of thyroid disorders with a wide range of reported prevalence of endemic goiter, ranging between 1% to 90% depending on the region of study. It was also noted that the prevalence of autoimmune thyroid disease was under diagnosed and underreported(Ogbera & Kuku, 2011).

In Kenya, studies done have looked at special populations like patients with chronic kidney disease (Kaggia SSN 2013) and T2DM (Ngugi et al, 2014) however there is no data relating to prevalence of thyroid dysfunction in the general population.

2.3: The Prevalence of Thyroid Dysfunction in Diabetes Mellitus

People with diabetes have a higher prevalence of thyroid dysfunction than the general population with a reported prevalence as shown in table 1 below.

Table 1: Summary of Prevalence Studies of Thyroid Dysfunction in Type 2 Diabetes

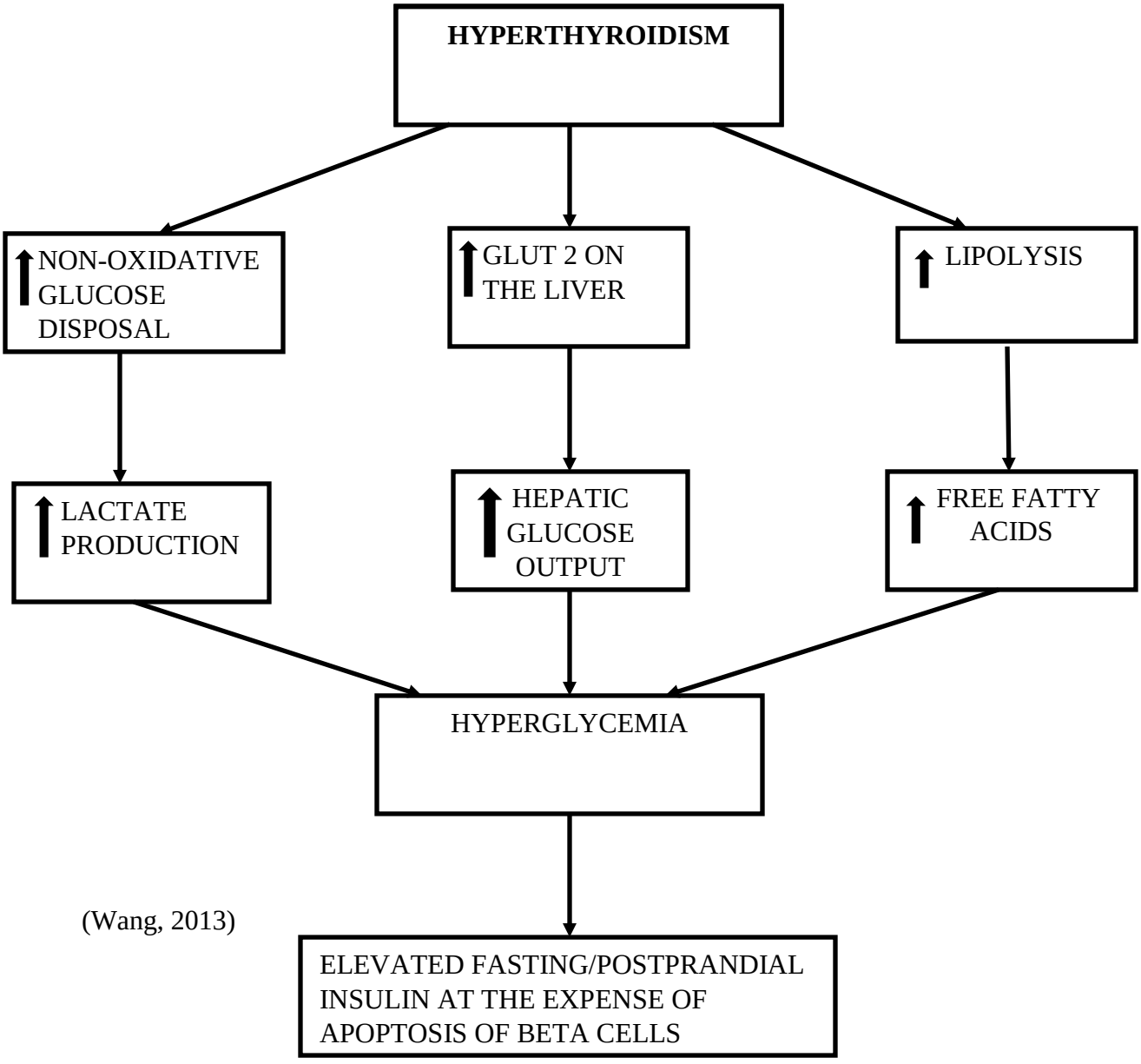
Study and location	Type of study	Sample size	Prevalence
Papazafiropoulou et al., 2010 –Greece	Cross-sectional	1,092	12.3%
Radaideh et al., 2004 – Jordan	Cross-sectional	908(304)	12.5%
Ghazali& Abbiyesuku, 2010- Nigeria	Cross-sectional	64(36)	29.7%
Diez, Sánchez & Iglesias, 2011-Spain	Cross-sectional	318	32.4%
Udiong, Udoh et al., 2007- Nigeria	Cross-sectional	161(105)	46.5%
Ngugi et al,.2014 Kenya	Cross-sectional	181	61%

2.4: The Effect of Diabetes on Thyroid Function

Patients who have poor glycemic control in both T1DM and T2DM, have blunted or abolished nocturnal TSH levels. In addition, there is impairment in conversion of T4 to T3 in the peripheral tissue resulting in a ‘low T3 state’. With normalization of the glycemic control, these effects are reversed(Hage et al., 2011).

2.5: The Effect of Hyperthyroidism on Glycemic Status

The stimulatory effects of excess thyroid hormone on appetite are well described. There is increased glucose absorption from the gastrointestinal tract eventually leading to hyperglycemia. In addition, excess thyroid hormone causes hyperproinsulinemia, hyperinsulinemia, increased free fatty acids, increased peripheral glucose transport and metabolism and increased hepatic glucose output. This cascade of events has been observed to precipitate subclinical diabetes and worsen glycemic control in already existing T2DM. A thyrotoxic state may further induce diabetic ketoacidosis as a result of the increase in growth hormone, glucagon and catecholamine levels associated with hyperthyroidism which contribute to increased insulin resistance (Hage et al., 2011). In addition, thyrotoxicity may aggravate diabetic heart conditions due to the arrythmogenic action of elevated thyroid hormone levels (Duntas, Orgiazzi, & Brabant, 2011)

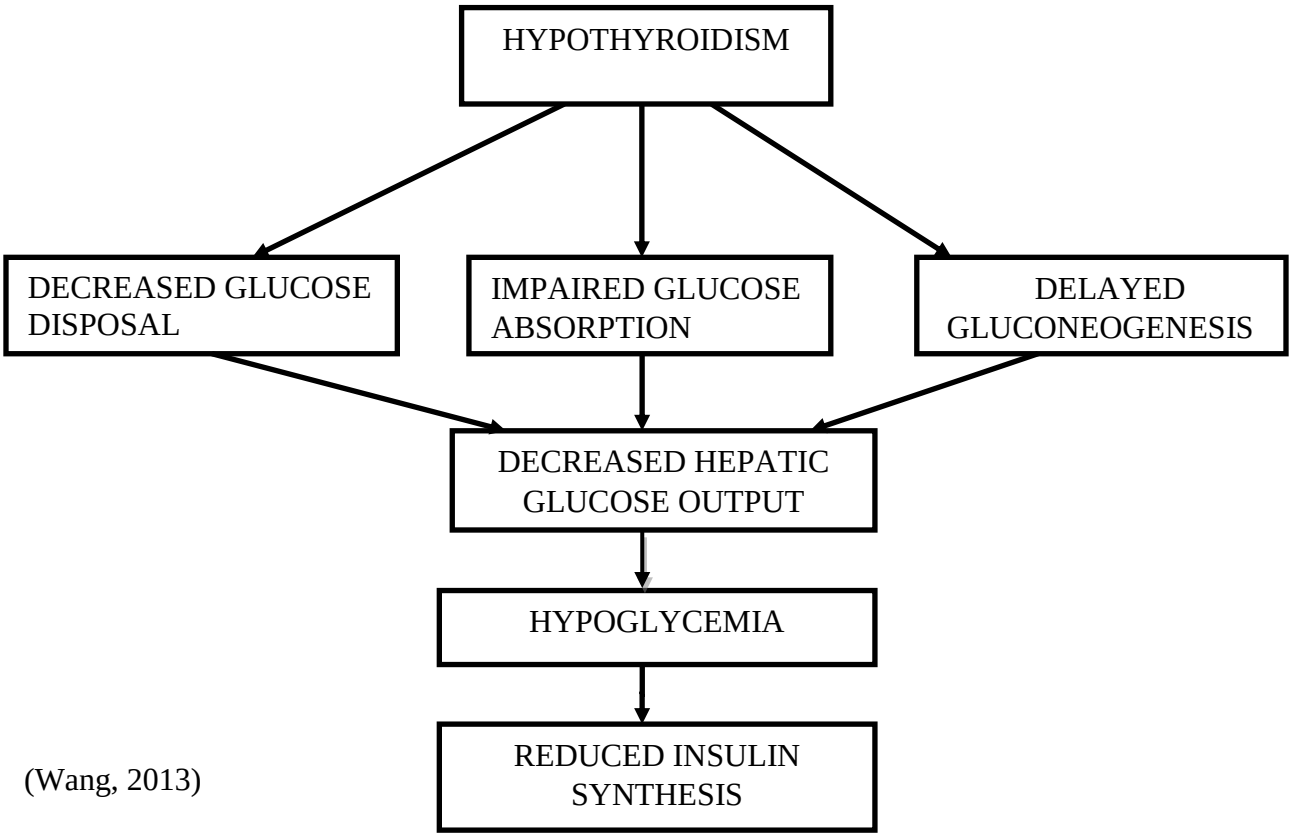


(Wang, 2013)

Figure 2: Hyperthyroidism Effect on Glucose Homeostasis

2.6: Effect of Hypothyroidism on Glycemic Control

Hypothyroidism may be characterized by reduced glucose absorption from the gut with a reduction in insulin production. Depending on what is predominant decreased glucose absorption or reduced insulin production this would lead to hypoglycemia or hyperglycemia. Strong evidence suggests that patients with hypothyroidism have reduced insulin clearance rate hence insulin requirements are decreased which could lead to hypoglycemia (Duntas et al., 2011)(Ogbonna & Ezeani, 2019). Both clinical and subclinical hypothyroidism are recognized as insulin resistant states due to impaired insulin stimulated glucose utilization in peripheral tissues which may cause hyperglycemia (Hage et al., 2011)(Dimitriadis et al., 2006). As a result, hypothyroidism my cause both hyperglycemia and hypoglycemia which results in reduced time in optimal glycemic range regardless of the HbA1c level. (Wang, 2013)



(Wang, 2013)

Figure 3a: Hypothyroidism Effect on Glucose Homeostasis

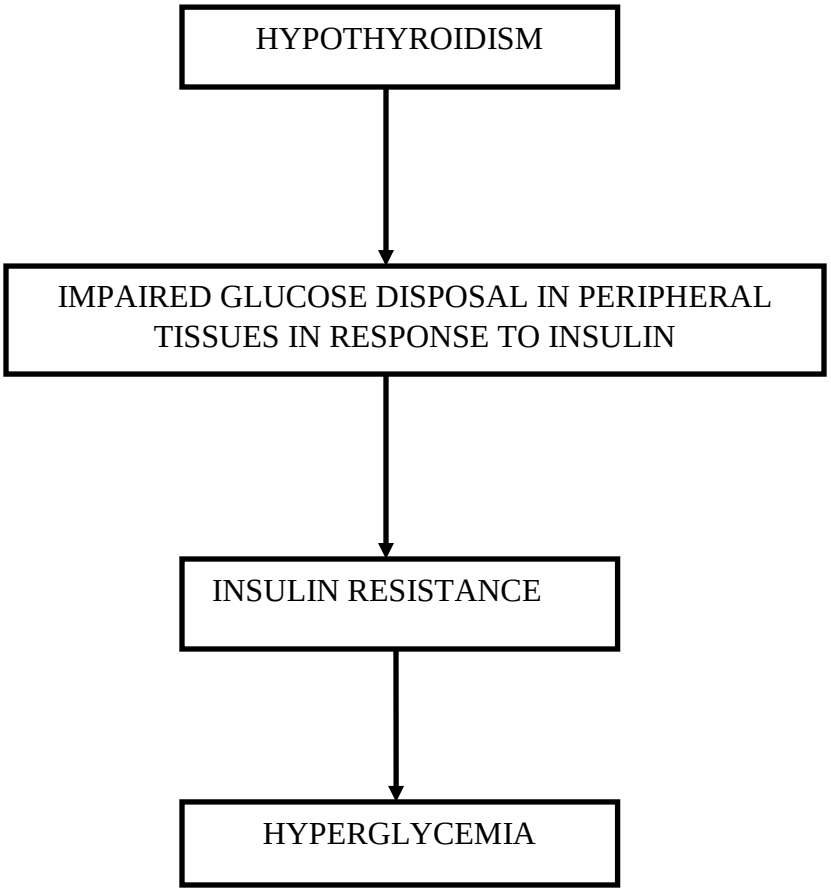


Figure 3b: Hypothyroidism Effect on Glucose Homeostasis

2.7: Effect of Thyroid Dysfunction on Other Organ Systems

The most clinically relevant findings in thyroid disease, both hyperthyroidism and hypothyroidism are cardiovascular signs and symptoms. By understanding the cellular mechanisms of the thyroid hormone actions on the cardiovascular system, then we are able to explain the changes that occur as a result of thyroid dysfunction such as changes in cardiac output, cardiac contractility, blood pressure, vascular resistance and rhythm disturbances. More importantly, hypothyroidism is associated with lipid abnormalities including elevated triglycerides and Low-Density Lipoprotein Cholesterol (LDL-C). This can exacerbate already existing dyslipidemia further increasing cardiovascular risk. Adequate thyroxin replacement has been shown to reverse the lipid abnormalities (Klein & Danzi, 2007).

Cardiac manifestations of hypothyroidism include bradycardia, mild diastolic hypertension, narrow pulse pressure and fatigue. Overt hypothyroidism is associated with increased systemic vascular resistance, decreased cardiac contractility, decreased cardiac output, accelerated atherosclerosis and coronary heart disease as a result of hypercholesterolemia and hypertension.

Subclinical hypothyroidism is characterized by high levels of TSH with normal T3& T4 levels. Patients with subclinical hypothyroidism are often asymptomatic but have elevated serum lipid levels and cholesterol levels which increase their risks for atherosclerosis coronary heart disease and myocardial infarction(Walsh et al., 2005). Cardiovascular disease is the most common cause of death among patients with diabetes hence coexistence of thyroid dysfunction multiplies their risk (Raghavan et al., 2019).

Among 55,287 adults from 11 cohorts (United States, Australia, Japan and Brazil)

followed up between 1972 and 2007, 3450(6.2%) had subclinical hypothyroidism. During follow-up, 9664 participants died (2168 of CHD), and 4470 participants had CHD events (among 7 studies). The risk of CHD events and CHD mortality increased with higher TSH concentrations particularly in those with TSH Levels greater than 10mlU/L(Rodondi et al., 2010).

Increased incidences of nephropathy have been reported in patients with Type 2 DM with subclinical hypothyroidism. This is partly due to the decreased renal blood flow and glomerular filtration rate occasioned by a decrease in cardiac output and peripheral vascular resistance. In addition, it has been observed that people with diabetes and subclinical hypothyroidism have more severe retinopathy than those who are euthyroid (Hage et al., 2011).

2.8: Effect of Thyroid Dysfunction on Lipid Metabolism

Dyslipedemia is almost always present in thyroid disease due to the effects of the thyroid hormone on the composition and the transport of lipoproteins (Peppa, Betsi, & Dimitriadis, 2011). Overt hypothyroidism is characterized by hypercholesterolemia and a marked increase in low-density lipoproteins (LDL) and apolipoprotein B (Apo B) because of a decreased fractional clearance of LDL caused by a reduced number of LDL receptors in the liver. The high-density lipoprotein (HDL) levels are normal or even elevated in severe hypothyroidism because of decreased activity of cholesteryl-ester transfer protein (CETP) and hepatic lipase (HL), which are enzymes regulated by thyroid hormones(Duntas, 2002). Elevation of LDL significantly increases cardiovascular risk which might explain the increased occurrence of atherosclerotic coronary artery disease especially in overt hypothyroidism (Rizos, Elisaf, & Liberopoulos, 2011).Thyroxine

therapy usually leads to considerable improvement of the lipid profile thereby reducing the cardiovascular risk(Duntas, 2002).

2.9: Effect of Drugs on Thyroid Dysfunction

Metformin is a widely used drug in the treatment of T2DM that has mostly been regarded as a safe drug due to its minimal interactions. In recent studies, it has been reported to lower TSH levels while T4 remains low hence may complicate interpretation of screening TSH(Dimic, Velojic Golubovic, Radenkovic, Radojkovic, & Pesic, 2016).Furthermore, it has been shown to have anti-proliferative activity on the thyroid gland lowering the risk of formation of thyroid nodules (Ogbonna & Ezeani, 2019).

2.10: Outcome of Treatment of Thyroid Dysfunction

Treatment of subclinical hypothyroidism has been observed to significantly decrease fasting and postprandial blood sugars as well as total cholesterol and triglycerides(Velija-Asimi & Karamehic, 2007). Potential benefits of early detection and treatment of subclinical thyroid dysfunction significantly outweigh potential side effects that may result from diagnosis and therapy(Gharib et al., 2005).

Glycemic control has been achieved in patients with diabetes and hyperthyroidism who undergo treatment with antithyroid agents (Fica, Lazar, Albu, Barbu, & Grigorescu, 2006).

CHAPTER THREE: METHODOLOGY

3.1: Study site

The study was conducted at Moi Teaching and Referral Hospital (MTRH) Diabetes Outpatient Clinics (DOPC). The hospital is located in Eldoret town, Uasin Gishu County, which is approximately 300 kilometers northwest of Nairobi. MTRH is the second largest referral hospital in Kenya after Kenyatta National Hospital. It is the teaching hospital for Moi University, School of Medicine and other middle level health colleges as well as the main referral hospital for the western part of Kenya. It has a bed capacity of about 991 patients and serves the greater western Kenya region representing about 40% (approximately 16.2 million people) of the country's population, Eastern Uganda and parts of Southern Sudan. It admits patients who require specialized treatment that cannot be offered in the county referral hospitals including, cancer treatment, renal replacement therapy, neurosurgical treatment among others. It also runs several specialist clinics including Diabetes clinic which runs on Monday, Thursday and Friday. On average, there are about 50 patients attending the DOPC on a weekly basis.

3.2: Study design

A cross-sectional analytical study was performed.

3.3: Study population

Ambulatory adult patients with Type 2 Diabetes Mellitus seeking care from DOPC at Moi Teaching and Referral Hospital.

3.4: Eligibility Criteria

3.4.1: Inclusion criteria

Patients above the age of 35 years at the time of diagnosis with Type 2 DM

3.4.2: Exclusion criteria

Patients with documented or reported history of conditions known to interfere with HbA1c assay such as anemia (Hb<10g/dl), recent blood transfusion (3months), hemodialysis, erythropoietin therapy and hemoglobinopathies (sickle cell disease) were excluded. Also exclude were patients who were acutely ill requiring admission since acute illness is known to interfere with thyroid function tests.

3.5: Sample Size Determination

Sample size determination was done for each objective.

Objective 1: Prevalence of thyroid dysfunction

The sample size was calculated using the Fisher's formula (Heinisch, 1963)

$$n = \frac{Z_{\alpha 2}.pq}{d^2}$$

n=minimum sample size required

z=confidence interval at 95% (standard value of 1.96)

p=estimated prevalence of thyroid dysfunction in patients with T2DM (Ngugi2014) - 61%

d=margin of error (0.05)

$$\frac{1.96*1.96*0.61*0.39}{(0.05)^2} \qquad n = 366 \text{patients}$$

Correction for missing or incomplete data = 366÷0.9 = **406 participants**

Objective 2: Factors associated with thyroid dysfunction

A formula for logistic regression was used. The minimum sample size (N) based on logistic regression model was obtained using the formula suggested by Peduzzi et al, **N = 10k/p**, where k is the number of independent variables and p is the number or events or prevalence of the condition of interest as determined from previous studies (Peduzzi *et al.*, 1996). The number of independent variables in this study were 8; age, gender, duration of T2DM, treatment modality, HTN, BMI, HbA1c, LDL-C. p was derived from a study by Uppal et al.,2013 where prevalence was 24.5%. The calculated sample size was. N= 10*8/0.245=**326 patients**

Objective 3: Correlation between TSH/FT4 and HbA1c

A formula for correlation by Hulley SB Cumming (2013) was used.

$N = [(Z_\alpha + Z_\beta)/C]^2 + 3.$

The correlation coefficient (r) was derived from a study by Uppal et al., 2013 where r-0.42

The calculated sample size was $N = [(Z_\alpha + Z_\beta)/C]^2 + 3 =$ **55 patients**

Overall, the sample size calculated from Fishers formula and adjusted to account for missing data was enough to answer all the objectives. The total sample size used therefore was **406 patients.**

3.6: Sampling Method

Systematic random sampling was done. On average, there were about 50 patients attending the DOPC on a weekly basis. The study period was three months, giving a total of approximately 800 patients on follow up for diabetes during the proposed study period. This number divided by the sample size of 406 gave a value of 2 for k. Every second patient was thus enrolled into the study over the study period after obtaining an informed consent.

$k=N\div n$ N=800, n=406 k=2

3.7: Study Procedure

3.7.1: Patient Recruitment

Patients were recruited from the DOPC clinics at MTRH on Monday, Thursday and Friday between February and April 2018. Systematic random sampling was done, whereby every second patient was approached and those who met the criteria were requested to participate in the study. Figure 3 below shows a summary of study flow algorithm.

3.7.2: Consenting

The consenting process was carried out by either a Good Clinical Practice (GCP) trained research assistant or the principal investigator. Literate participants were provided with written consent form in English (appendix 2A) or Kiswahili (appendix 2B) as per their preference. Participants who could not read had the consent read to them in a language of their choice and a translator was sought when necessary. Data collection was done after an informed written consent was obtained.

3.7.3: Clinical and Laboratory Procedures

The PI and research assistants administered the structured pre-tested questionnaires which were utilized to collect socio-demographic, clinical and laboratory data for each consenting patient (Appendix 1). Blood pressure, height and weight (Appendices 3&4) were measured before participants were taken to the phlebotomy area where blood samples for HbA1c, TSH, fT4 and LDL-C were then taken (Appendix 5). All phlebotomy procedures were carried out under sterile conditions. The collected samples were delivered to the AMPATH Reference Laboratory and analyzed on the day of collection. An automated Cobas e 411electrochemilumiscence immunoassay (ECLIA) analyzer was used for analysis of TSH and Ft4 (Appendix 8&9). An automated Cobas Integra 400 Plus chemistry analyzer was used for analysis of glycated hemoglobin. The principle method was based on Turbidimetric inhibition immunoassay for hemolyzed whole blood (Appendix 7). Homogenous enzymatic colorimetric test was the principle used for analysis of low-density lipoprotein cholesterol (Appendix 10).

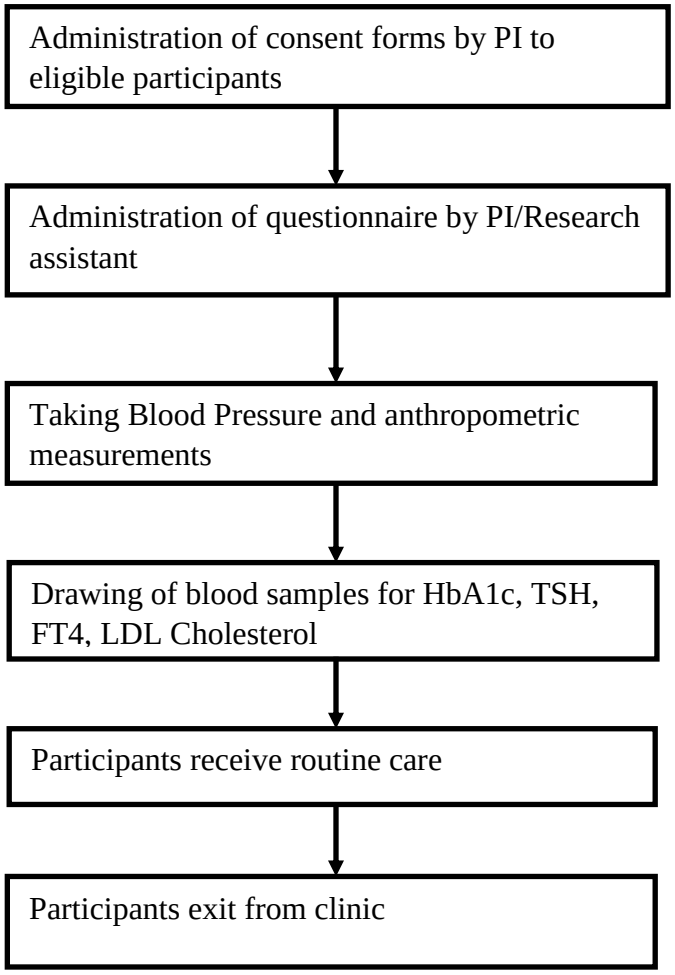


Figure 4: Study procedure

3.8: Study Variables

The dependent variables for this study were serum TSH and Serum fT4**.** These were used to determine thyroid dysfunction (Surks, Chopra, Mariash, Nicoloff, & Solomon, 1990).

The independent variables were good glycemic control and poor glycemic control. Covariates were sociodemographic variables (age, gender), Duration of T2DM, Treatment modality, Hypertension, BMI and LDL-C levels (Appendix 6)

3.9: Data Management

3.9.1: Materials and Data collection

A structured interviewer administered questionnaire was used to collect demographic and clinical information. The only unique identifier that the questionnaire contained was the subject's birth dates and the study number. Laboratory results were recorded on a data collection sheet. Once completed, questionnaires and data collection sheets were checked for errors and completeness then double entered into REDCap (a secure, web-based application designed to support data capture for research studies).

3.9.2: Data Cleaning

Data was cleaned during collection, data entry and analysis. Data collection sheets and questionnaires were checked for completeness and errors at the end of each week during data collection. Missing data was excluded from the analysis. Validation checks were incorporated into the database to ensure accuracy of data.

3.9.3: Data Protection and Security

Paper records were kept under lock and key only accessible to the PI. Computer was password protected, with up-to-date Kaspersky antivirus, internet firewall protected and with a backup. Data validation was done through the same application. Thereafter the data was converted into a database and exported to STATA version 15.1 for analysis.

3.9.4: Data Analysis

Descriptive statistics such as the median and corresponding interquartile range were used to summarize continuous variables such as age, BMI, serum TSH levels, serum T4 levels, HBA1c levels, and LDL-C levels.

Frequencies and the corresponding percentages were used to summarize categorical variables such as gender, level of education, and marital status.

Serum TSH levels, serum T4 levels, HBA1C levels, LDL-C levels, and BMI were categorized using clinically acceptable limits. Hyperthyroidism was defined as TSH < 0.27mIU/l, Euthyroid was defined as TSH in the range of 0.27–4.20 mIU/l, hypothyroidism was defined as having TSH >4.20mIU/l and subclinical hypothyroidism was defined as TSH >4.20mIU/l and fT4 0.932-1.71ng/dl.

Good control was defined as a HBA1C $\leq$ 8% while poor control was defined as HBA1C >8%. LDL-C levels were defined as normal if $\leq$2.59mmol/l, while >2.59 mmol/l was abnormal.

BMI (kg/m^2) was categorized as underweight <18.5, normal 18.5-24.9, overweight 25.0 – 29.9, obese$\geq$30.0- 39.9 and extreme obesity$\geq$ 40.

Association between thyroid dysfunction and categorical independent variables were assessed using Pearson's Chi Square Test, Fisher's Exact Test and Wilcoxon Rank Sum Test.

Chi- Square Test of Independence was used to compare distribution of thyroid dysfunction across comparison groups of categorical variables that showed homogeneity in their distribution, with a cell size of >5 in all 4 cells of the 2by2 table were subjected to this test. These were Glycemic control (good control HbA1c $\leq$ 8%, poor control >8%), Insulin (use or no use), Hypertension (present or absent), Sex (male, female), LDL Cholesterol level (Normal $\leq$ 2.59mmol/l, Abnormal >2.59 mmol/l).

Fisher's Exact Test, a non parametric alternative to Chi-Square Test was used to test association between categorical variables which violated Chi-Square Test assumptions and had small cell sizes of <5 in any of the 4 cells in the 2by2 tables. The variables subjected to this test were History of diagnosis of thyroid disease and BMI.

Wilcoxon Rank Sum Test, a non parametric comparing median between two populations was used to determine if the difference in median age between participants with thyroid dysfunction and those without dysfunction was significant.

Logistic regression model was used to assess the variables associated with thyroid dysfunction. Bivariate logistic regression model was used to calculate the unadjusted odds ratios (OR), and the multivariate logistic regression model was used to calculate the adjusted odds ratios. In the multivariate model, the variables that were significantly associated with the thyroid dysfunction in the bivariate analysis were included. These variables included age, gender, duration of diabetes, insulin use, hypertension, BMI, LDL-Cholesterol and HBA1C. None of the variables was dropped. We reported the OR and the corresponding 95% confidence intervals.

Relationship between continuous variables (Serum TSH & Serum T4) with HBA1C was assessed using scatter plots. Spearman rank correlation coefficient was calculated to determine the strength and direction of the relationship.

Results were presented using tables, bar graphs and scatter plots.

3.10: Ethical Considerations

Approval was sort from IREC and permission to conduct the study obtained from MRTH management. The purpose of the study was clearly explained to eligible participants in English or Kiswahili prior to inclusion into the study. Only patients who gave informed written consent were enrolled. Patients who declined to participate in this study were not discriminated at all but rather continued receiving their care as usual. The clinician directly managing the patient did not take part in recruitment or consenting to avoid coercion.

All patient information was kept confidential by storing data in key-locked cabinet and use of REDCap, a safe password protected database. Findings were communicated to the attending clinician for follow up and further care. There was no conflict of interest in this study.

CHAPTER FOUR: RESULTS

4.1: Recruitment

A total of 406 participants were enumerated for this study. Twenty-one participants did not meet the inclusion criteria and were excluded (7 participants were ill requiring admission, 9 were under 35 years of age at diagnosis of Diabetes and 5 had hemoglobin of <10g/dl). Six participants declined to participate. Eleven participants had incomplete results and were excluded in the final analysis which included results of 368 participants.

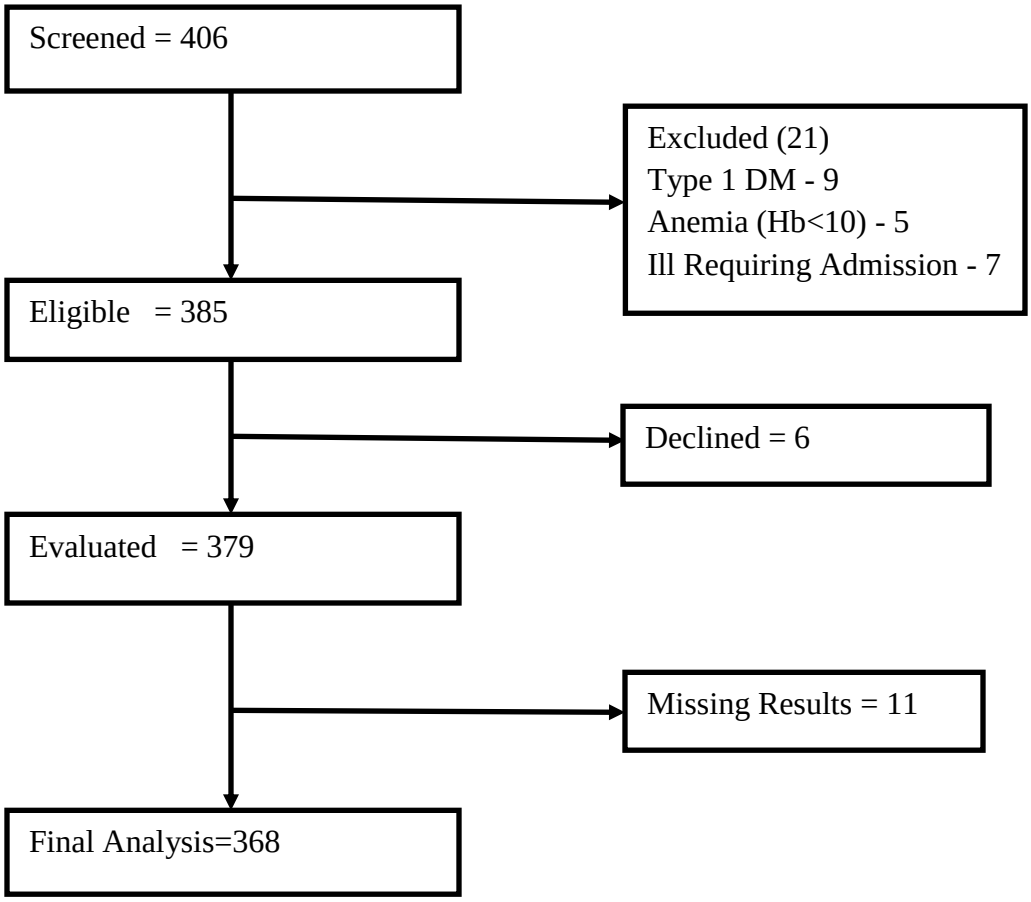


Figure 5: Recruitment Schema

4.2: Socio demographic Characteristics

A total of 368 participants were enrolled in the study, 230(62.5%) were female. The median age was 59.2 (IQR: 52, 67) years with the youngest being 35.0 and the oldest 95.0 years. Less than half of the participants (41.6%) had secondary or tertiary education, and most of them were married (89.7%).

Table 2: Participants socio demographic characteristics

Variable	Freq/Median	%/ IQR
Age in years	59.2	(52,67)
Gender		
Female	230	62.50%
Male	138	37.50%
Marital status		
Married	330	89.70%
Separated	4	1.10%
Single	15	4.10%
Widowed	19	5.20%
Level of education		
None	49	13.30%
Primary	166	45.10%
Secondary	118	32.10%
Tertiary	35	9.50%

4.3: Clinical Characteristics

4.3.1: Medical History

The median duration since diagnosis of diabetes was 60 (IQR: 24,132) months with a range of 1 and 420 months. Two hundred and two (54.5%) participants reported a diagnosis of hypertension. However few patients (≅4%) were aware of any family history of thyroid related disease. History of thyroid surgery was present in 9 (2.4%) participants. Six participants (1.6%) had a history of thyroid disease with half of them having a diagnosis of hypothyroidism and the other half hyperthyroidism. The table below summarizes these findings.

Table 3: Medical History

Variable	Median/Freq	IQR/ %
Duration of diabetes since diagnosis (months)	60	(24, 132)
Hypertension	202	54.9
Family History of thyroid disease	15	4.1
History of thyroid surgery	9	2.5
History of radioiodine ablation	1	0.3
History of diagnosis of thyroid disease requiring medical treatment	6	1.6
Hyperthyroidism	3	0.8
Hypothyroidism	3	0.8

4.3.2: Medication History

More than half of the patients, 196 (53.2%) were on insulin only or a combination of insulin and an oral anti-diabetes agent while 46.8% were on only oral anti-diabetes agents for glycemic control. Almost two thirds 220 (59.8%) were on a Metformin based therapy. Majority of the participants 150 (40.8%) were on an ARB/ACE while 86 (23.4%) & 83 (22.5%) were on a CCB and diuretics respectively.

Only 54 (14%) of the participants were on a lipid-lowering agent, Atorvastatin and of the 3 patients with a history of hypothyroidism 2 were on thyroxine replacement.

Table 4: Medication History

Variable	**Freq**	%
Insulin	196	**53.2**
Oral Anti-Diabetes Agents	172	46.7
ACE Inhibitors /ARB's	150	**40.8**
Calcium Channel Blockers	86	23.4
Beta Blockers	19	5.2
Diuretics	83	22.5
Amiodarone	0	0
Atorvastatin	54	**14.7**
Thyroxine	2	0.5

4.3.3: Physical Examination and Laboratory Findings

Majority of the participants were either overweight or obese accounting for 63% of the total number as shown in the bar graph below.

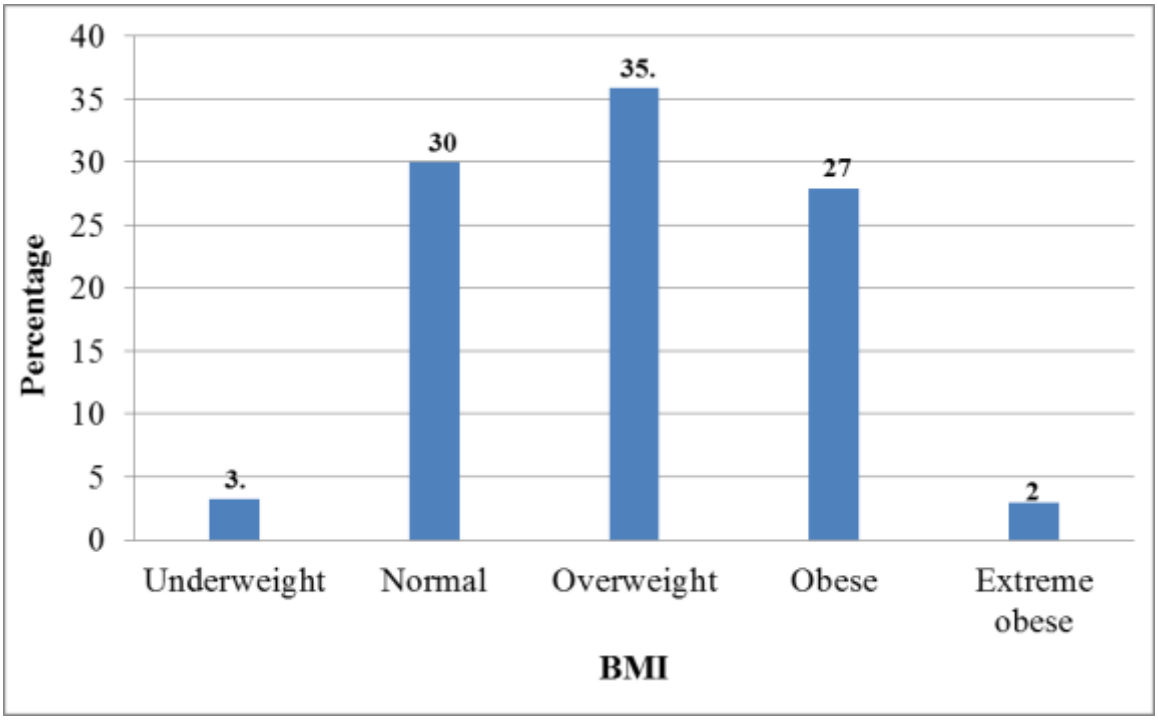


Figure 6: Distribution of Participants by BMI Categories

Few participants (1.6%) reported symptoms related to thyroid dysfunction and on physical examination, 6 participants (1.6%) were found to have signs of thyroid dysfunction mainly an enlarged thyroid gland. Hand tremors, facial puffiness and protruding eyes were the other signs elicited. Nearly half of the patients 162(42%) had an elevated blood pressure on examination.

The median serum TSH levels were 1.61(IQR 1.08, 2.5) mIU/l, median serum free T4 was 1.24(IQR 1.1, 1.35) ng/dl and the median HbA1c was high at 8.82 (IQR 7, 10.86) %.

The median LDL cholesterol levels were elevated at 2.89 (IQR 2.25, 3.62) mmol/l

Table 5: Physical Examination and Laboratory Findings

Variable	Median	%/ IQR
TD signs and symptoms	6	1.6
Blood Pressure >140/90	162	42.0
Serum TSH	1.61	(1.08,2.5)
Serum free t4	1.24	(1.1,1.35)
HbA1c	8.82	(7,10.86)
LDL-Cholesterol	2.89	(2.25,3.62)

4.4: Prevalence of thyroid dysfunction

The prevalence of thyroid dysfunction was 9.2% (95%CI: 6.4, 12.7). Of the 34 Type 2 DM patients who had thyroid dysfunction, 31 (91.2%) had hypothyroidism while the remaining 3 (8.8%) had hyperthyroidism.

Among those with hypothyroidism, 22(71%) had subclinical hypothyroidism.

4.5: Factors associated with thyroid dysfunction

On bivariate analysis as per table 7 it is notable that despite differences in age, glycemic control, insulin use, hypertension, sex, BMI and LDL Cholesterol in the comparison groups, none of these factors were statistically significantly associated with thyroid dysfunction (all P-Values >0.05).

However, history of diagnosis of thyroid disease was found to be associated with thyroid dysfunction with a significant P-Value of 0.000.

Table 6: Factors associated with thyroid dysfunction bivariate analysis

Variable	Thyroid dysfunction Freq (%)/ Median (IQR)		P-value
	No	**Yes**	
Age	59 (52, 66)	59.5 (55,69)	0.406[1]
HbA1c			
Control	127 (87.0)	19 (13.0)	**0.046[2]**
No control	205 (93.2)	15 (6.8)	
History of diagnosis thyroid disease			
No	332 (91.7)	30 (8.3)	**0.000[3]**
Yes	2 (33.3)	4 (66.7)	
Insulin use			
No	154 (91.1)	15 (8.9)	0.824[2]
Yes	180 (90.5)	19 (9.5)	
Hypertension			
No	149 (89.8)	17 (10.2)	0.547[2]
Yes	185 (91.6)	17 (8.4)	
Sex			
Male	125 (90.6)	13 (9.4)	0.926[2]
Female	209 (90.9)	21 (9.1)	
LDL			
Normal	135 (91.8)	12 (8.2)	0.561[2]
Abnormal	199 (90.0)	22 (10.0)	
BMI			
Underweight	11 (91.7)	1 (8.3)	
Normal	96 (90.6)	10 (9.4)	1.000[3]
Overweight	117 (90.7)	12 (9.3)	
Obese	93(90.3)	10 (9.7)	
Extreme obese	10 (90.9)	1 (9.1)	

[1]Wilcoxon rank sum test,[2]Chi square test,[3] Fishers' Exact test

Multiple logistic regression model results as seen in table 8 showed that participants who had good glycemic control as assessed by HbA1c had a 57% greater chance of having

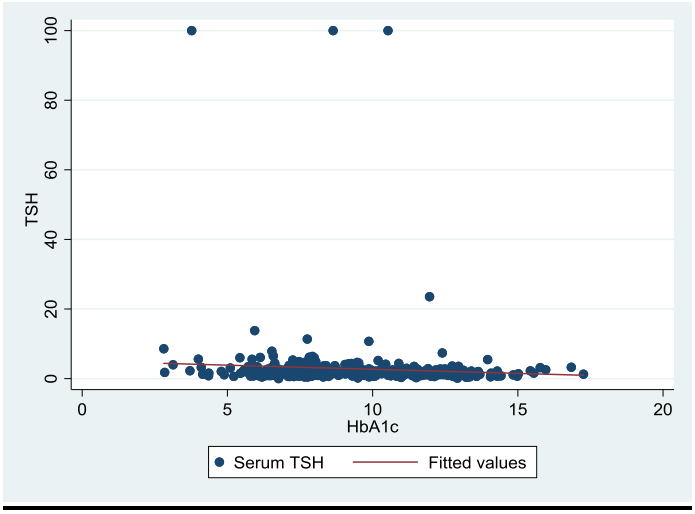
thyroid dysfunction compared to those with poor glycemic control, OR: 0.431 (95% CI: 0.201, 0.920). However, there was no association found between age, sex, duration of diabetes, insulin use, hypertension, BMI, LDL-Cholesterol and thyroid dysfunction.

Table 7: Multivariate analysis factors associated with thyroid dysfunction

Variable	Odds ratio	P-value	[95% Conf.	Interval]
Age	1.025	0.140	0.991	1.059
Sex				
Male	1			
Female	1.109	0.798	0.501	2.454
Duration of Diabetes (months)	1.001	0.791	0.996	1.005
Insulin use				
No	1			
Yes	1.307	0.487	0.617	2.774
Hypertension				
No	1			
Yes	0.566	0.156	0.257	1.242
BMI				
Normal	1			
Underweight	1.027	0.981	0.112	9.397
Overweight	0.924	0.865	0.372	2.296
Obese	1.014	0.977	0.389	2.644
Extreme obese	1.145	0.904	0.125	10.496
LDL				
Normal	1			
Abnormal	1.244	0.584	0.570	2.715
HbA1c				
Control	1			
No control	**0.431**	0.030	0.201	0.920

4.6: Correlation between thyroid function and glycemic control

There was an insignificant negative correlation between serum TSH and HbA1c however no correlation was found between free T4 and HbA1c.



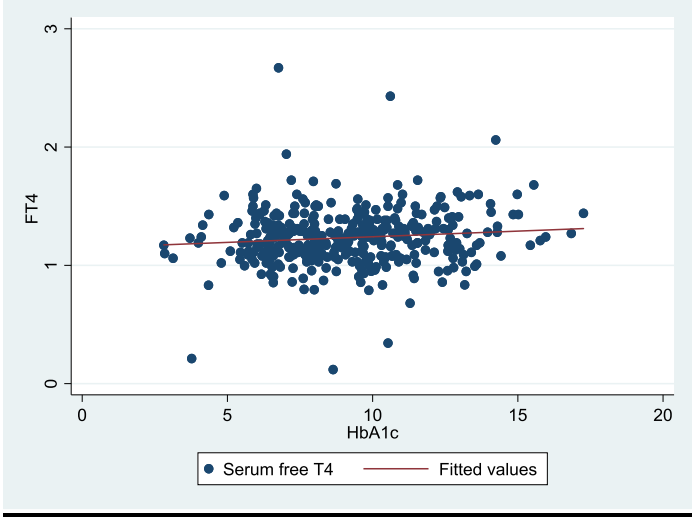
Serum TSH and HbA1c are negatively correlated though insignificant.

r = -0.069

95% confidence interval

-0.170, 0.034

Figure 7: Correlation between TSH and HbA1c



Serum free T4 and HbA1c show no correlation.

r= 0.106

95% confidence interval

0.004, 0.206

Figure 8: Correlation between FT4 and HbA1c

CHAPTER FIVE: DISCUSSION

The prevalence of thyroid dysfunction in patients with diabetes has not been studied at all in western Kenya yet; thyroid disease and diabetes are important co-morbidities that are bound to impact cardiovascular outcomes of both conditions. Our study set out to evaluate the prevalence of thyroid dysfunction, factors associated with thyroid dysfunction and correlate glycemic control and thyroid function among Type 2 DM patients in a Kenyan population in Eldoret.

5.1: Prevalence of thyroid dysfunction

This study found a prevalence of thyroid dysfunction of 9.2% (34) among the 368 patients with Type 2 Diabetes studied. This is slightly lower than the wide range of prevalence of thyroid dysfunction globally which varies between 9.5 - 46% (Aljabri, n.d. et. al., 2019).The wide range could be explained by the use of different criteria for the diagnosis of thyroid dysfunction; thyroid function test, presence of anti-thyroid peroxidase (anti-TPO), antithyroglobulin antibody (anti-TG), or both (Kalra, Aggarwal, & Khandelwal, 2019). In addition, factors such as iodine status in different regions, selenium deficiency and thiocyanate toxicity have been found to be responsible for variations of thyroid disease epidemiology (Ogbera & Kuku, 2011). However, further exploration may be needed to determine if these factors are responsible for the lower prevalence of thyroid dysfunction among our population in Eldoret.

The prevalence in this study was comparable to that of a Greek population of 1029 patients with Type 2 DM who were found to have a prevalence of 12.3% (Papazafiropoulou et al., 2010). The similarities in prevalence could be attributed to similar methodology in regards to the laboratory procedures for HbA1c and thyroid

function tests. However, the slight higher prevalence in the Greece study may be attributed to the much older mean age of participants (67yrs) compared to 59yrs in this study, given that the prevalence of thyroid dysfunction increases with age (Ogbonna & Ezeani, 2019).

The prevalence of 9.2% found in this study was much lower than that reported in a local study done at Kenyatta National Hospital (KNH) which found a prevalence of 61% among 181 patients with Type 2 DM (Ngugi et al 2014, unpublished master's dissertation in University of Nairobi). Some factors that may have been responsible for the high prevalence in the Kenyatta study include: differences in the population characteristics with a higher percentage of patients having a previous diagnosis of thyroid dysfunction (10.6%, compared to 1.6% in this study), 22% of patients reported a positive family history of thyroid disease (hence higher risk) compared to 4.1% in this study; Difference in definition of Type 2 DM with the KNH study including all patients above the age of 30years regardless of the age at diagnosis hence higher likelihood of inclusion of Type 1 DM patients who are at a higher risk of thyroid dysfunction(Palma et al., 2013); The use of different test methods in the two studies with our study using electrochemiluminescence immunoassay (ECLIA) technology of Elecsys 2010 which has shown advantages in system performance and superiority to other laboratory methods such as ELISA (KNH study) and Immunoradiometric assay (IRMA) for the measurement of serum TSH with higher specificity(Kazerouni & Amirrasouli, 2012). Subclinical hypothyroidism was the most frequent dysfunction found at 7% (22 participants) among our study population. Subclinical hypothyroidism is most common among females above

the age of 60 years, which comprised majority of our study population. This is consistent with a number of studies(Kalra et al., 2019),(Papazafiropoulou et al., 2010).

Other studies done in Nigeria(Ghazali & Abbiyesuku, 2010) and India (Uppal, Vij, Bedi, Vij, & Banerjee, 2013)also reported a higher prevalence of subclinical hypothyroidism at 26% and 24.5% respectively. This difference may be attributed to their use of ELISA for measurement of thyroid hormones compared to ELCIA in this study.

5.2: Sociodemographic and clinical factors associated with thyroid dysfunction

The social demographic characteristics of our study population are comparable to a number of previous studies. Our study found a female predominance at 61% .This is in keeping with the expected epidemiologic distribution of type 2 DM as reported in IDF 2018 .These findings are similar to (Ngugi et al in Nairobi, Kenya, and Ghazali et al in Nigeria at 62.6%).(Ngugi 2016),(Ghazali & Abbiyesuku, 2010). The median age of the study population was 59 years with a range between 35-95 years. Majority of the participants were above the age of 60. This is in keeping with the IDF 2018 fact sheet which reports the highest prevalence of Diabetes in Africa among adults aged 55-64years.

Few participants reported a family history of thyroid dysfunction or history of diagnosis of thyroid dysfunction. Thyroid dysfunction symptoms are often nonspecific and overlap with those of diabetes. Furthermore, subclinical thyroid disease may not have overt symptomatology (Billic-Komarica et al., 2012).The majority of our patients had subclinical hypothyroidism and hardly reported any symptoms despite inquiry. This was a shared observation among a number of studies with less than 3% of patients reporting symptoms (Ngugi et al, 2014),(Uppal et al., 2013).

This study established a largely poorly controlled diabetes population at 58% (HbA1c >10%) in whom you would have expected higher rates of insulin use given the recommendations of the 2019 ADA Guidelines of early introduction of insulin when HBA1c is >10%. More than two thirds of patients on oral anti-diabetes agents are poorly controlled (HbA1c >10%). Despite evidence that insulin secretory capacity deteriorates over time hence the need for insulin with longer duration of diabetes, some of the factors that may have contributed to lower use of insulin therapy among our study population include, physician inertia to initiate insulin therapy, patient's preference and views about injectable therapy (Home et al., 2014).

Regarding factors associated with thyroid dysfunction, patients with thyroid dysfunction had a higher chance of good glycemic control (HbA1c <8%). This is an uncommon finding and although there is a scientific explanation, it could also be a chance finding as demonstrated by the wide confidence interval for the odds ratio. Majority of the participants had hypothyroidism which increases susceptibility to recurrent hypoglycemia which may lower the overall HbA1c level and hence be interpreted as better glycemic control. In the absence of self blood glucose monitoring data most hypoglycemic episodes may have gone unnoticed. Hypothyroidism is often associated with a relative adrenal insufficiency which further results in blunting of the hypothalamo- pituitary – adrenal response to hypoglycemia. Hypothyroid patients also have a reduced cortisol response to insulin- induced hypoglycemia further worsening hypoglycemia in these patients (Duntas et al., 2011). Hypoglycemia has been identified as a risk factor for both major cardiovascular events and death(Chow et al., 2014). The knowledge that hypothyroidism has a significant role in hypoglycemia necessitates consideration of the

appropriate dosing of insulin and oral hypoglycemic agents and treatment of hypothyroidism to alleviate further risks of hypoglycemia. Similarly, Telwani et al found patients with thyroid dysfunction had a higher chance of good glycemic control. Despite Papazafiropoulou et al. finding no association between glycemic control and thyroid dysfuction, patients who had thyroid dysfunction had a lower mean HbA1c (7.38% vs 7.81%) compared to those without.

On the other hand, Uppal et al found that poor glycemic control was associated with thyroid dysfunction. However, this is not surprising given the complexity of the interaction between thyroid disorders and diabetes. Insulin resistance seems to be the possible link between T2DM and thyroid dysfunction. Insulin resistance and βcell function are inversely correlated with thyroid stimulating hormone which may be explained by the antagonistic effects of insulin on thyroid hormones along with an increase in TSH (Wang, 2013).Hence, patients with poor glycemic control are equally at risk of thyroid dysfunction.

In this study, age, treatment modality, duration of T2DM and HTN were not associated with thyroid dysfunction. Similarly, Papazafiropoulou et al found no association. Perhaps this could be explained by the fact that majority of our patients were on metformin which has counter regulatory effects on the thyroid hormone release and activity. Metformin decreases thyrotropin levels in patients with hypothyroidism and it has also been shown to have anti-proliferative effects on the thyroid gland. However, Uppal et al and Ogbonna et al found longer duration >12yrs and >6.5 yrs respectively of T2DM was associated with TD. Longer duration of DM increases risk of chronic hyperglycemia which impairs the peripheral deiodination of T4 to T3 leading to thyroid dysfunction (Telwani, Wani,

Ashraf, & Shah, 2017).Perhaps there was no association in this study since the median duration of T2DM was shorter (5yrs).

The other factors studied were gender, BMI, LDL-C, which were not associated with thyroid dysfunction. However, Telwani et al found an association between female gender and LDL-C and TD. This may be due to differences in methodology. While this was a cross sectional study, Telwani et al conducted a case control with age and sex matched controls.

5.3: Correlation between thyroid function and HbA1c

There was no significant correlation between TSH and fT4 and HbA1c in this study. However, the correlation tended towards the negative and this could have resulted from majority of the thyroid dysfunction being subclinical hypothyroidism. This may have increased susceptibility to hypoglycemic episodes as earlier alluded to.

However, Uppal et al and Imdad et al found a positive correlation which is thought to be as a result of the complex interaction between insulin resistance and thyroid hormones as earlier discussed.

5.4 Study Limitation

The definition of Type 2 DM is based on age cut-off at the time of diagnosis rather than objective test of insulin function and resistance. This may have underestimated the proportion of patients with T2DM given the recent trends where younger patients are being diagnosed with T2DM. However, a higher age cut-off of 35 years at diagnosis was used which reduced the chance of recruiting a participant who had Type 1 DM or MODY.

Glycemic control was assessed using HbA1c rather than time in range which may have been more appropriate given the complex association of thyroid dysfunction with glycemia. The low socio-economic status of the country though makes it impossible to do continuous blood glucose monitoring at the moment and using HbA1c was more in keeping with what is practiced and hence its results are more applicable.

CHAPTER SIX: CONCLUSION AND RECOMMENDATIONS

6.1 Conclusion

The prevalence of thyroid dysfunction among patients with Type 2 DM is high compared to what has been reported in the general population. There was no significant correlation found between thyroid dysfunction and the studied clinical correlates.

6.2 Recommendations

Routine assessment of thyroid hormone levels should be part of evaluation for patients with Type 2 DM. A prospective study to evaluate the effect of routine screening of thyroid dysfunction on glycemic control and its impact on cardiovascular risk in these patients should be done.

REFERENCES

Akbar, D. H., Ahmed, M. M., & Al-Mughales, J. (2006). Thyroid dysfunction and thyroid autoimmunity in Saudi type 2 diabetics. *Acta Diabetologica*, *43*(1), 14–18.

Aljabri, K. S. (n.d.). *The Prevalence of Thyroid Disorders in Patients with Type 2 Diabetes Mellitus in Saudi Community Based Hospital*.

American Diabetes Association. (2017). Standards of Medical care in diabetes - 2017. *The Journal of Clinical and Applied Research and Education*, *40*(January), 1–142.

Ayah, R., Joshi, M. D., Wanjiru, R., Njau, E. K., Otieno, C. F., Njeru, E. K., & Mutai, K. K. (2013). A population-based survey of prevalence of diabetes and correlates in an urban slum community in Nairobi, Kenya. *BioMedical Centre public health*, *13*(1), 1-11.

Billic-Komarica, E., Beciragic, A., & Junuzovic, D. (2012). The importance of HbA1c control in patients with subclinical hypothyroidism. *Materia Socio-Medica*, *24*(4), 212.

Biondi, B., Kahaly, G. J., & Robertson, R. P. (2019). Thyroid dysfunction and diabetes mellitus: two closely associated disorders. *Endocrine Reviews*, *40*(3), 789–824.

Bjoro, T., Holmen, J., Kruger, O., Midthjell, K., Hunstad, K., Schreiner, T., … Brochmann, H. (2000). Prevalence of thyroid disease, thyroid dysfunction and thyroid peroxidase antibodies in a large, unselected population. The Health Study of Nord-Trondelag (HUNT). *European Journal of Endocrinology*, *143*(5), 639–647.

Canaris, G. J., Manowitz, N. R., Mayor, G., & Ridgway, E. C. (2000). The Colorado thyroid disease prevalence study. *Archives of Internal Medicine*, *160*(4), 526–534.

Chow, E., Bernjak, A., Williams, S., Fawdry, R. A., Hibbert, S., Freeman, J., … Heller, S. R. (2014). Risk of cardiac arrhythmias during hypoglycemia in patients with type 2 diabetes and cardiovascular risk. *Diabetes*, *63*(5), 1738–1747.

Christensen, D. L., Friis, H., Mwaniki, D. L., Kilonzo, B., Tetens, I., Boit, M. K., … Borch-Johnsen, K. (2009). Prevalence of glucose intolerance and associated risk factors in rural and urban populations of different ethnic groups in Kenya. *Diabetes Research and Clinical Practice*, *84*(3), 303–310.

Dimic, D., Velojic Golubovic, M., Radenkovic, S., Radojkovic, D., & Pesic, M. (2016). The effect of metformin on TSH levels in euthyroid and hypothyroid newly diagnosed diabetes mellitus type 2 patients. *CLINICAL STUDY*, *433*, 435.

Dimitriadis, G., Mitrou, P., Lambadiari, V., Boutati, E., Maratou, E., Panagiotakos, D. B., … Raptis, S. A. (2006). Insulin action in adipose tissue and muscle in hypothyroidism. *The Journal of Clinical Endocrinology & Metabolism*, *91*(12), 4930–4937.

Dora, J. M., Machado, W. E., Rheinheimer, J., Crispim, D., & Maia, A. L. (2010). Association of the type 2 deiodinase Thr92Ala polymorphism with type 2 diabetes: case–control study and meta-analysis. *European Journal of Endocrinology*, *163*(3), 427–434.

Duntas, L. H. (2002). Thyroid disease and lipids. *Thyroid*, *12*(4), 287–293.

Duntas, L. H., Orgiazzi, J., & Brabant, G. (2011). The interface between thyroid and diabetes mellitus. *Clinical Endocrinology*, *75*(1), 1–9.

Eldor, R., & Raz, I. (2009). American Diabetes Association Indications for Statins in Diabetes. *Diabetes Care*, *32*(suppl 2), S384 LP-S391.

Fica, S., Lazar, A., Albu, A., Barbu, C., & Grigorescu, M. (2006). The impact of hyperthyroidism on glycemic control in patients with diabetes mellitus. *ACTA ENDOCRINOLOGICA-BUCHAREST-*, *2*(2), 203.

Garber, J. R., Cobin, R. H., Gharib, H., Hennessey, J. V, Klein, I., Mechanick, J. I., … Woeber for the American Association of Clinical Endocrinologists and American Thyroid Association Taskforce on Hypothyroidism in Adults, K. A. (2012). Clinical practice guidelines for hypothyroidism in adults: cosponsored by the American Association of Clinical Endocrinologists and the American Thyroid Association. *Thyroid*, *22*(12), 1200–1235.

Gharib, H., Tuttle, R. M., Baskin, H. J., Fish, L. H., Singer, P. A., & McDermott, M. T. (2005). Subclinical thyroid dysfunction: a joint statement on management from the American Association of Clinical Endocrinologists, the American Thyroid Association, and the Endocrine Society. *The Journal of Clinical Endocrinology & Metabolism*, *90*(1), 581–585.

Ghazali, S. M., & Abbiyesuku, F. M. (2010). Thyroid dysfunction in type 2 diabetics seen at the University College Hospital, Ibadan, Nigeria. *Nigerian Journal of Physiological Sciences*, *25*(2), 173–179.

Hage, M., Zantout, M. S., & Azar, S. T. (2011). Thyroid disorders and diabetes mellitus. *Journal of Thyroid Research*, *2011*.

Heinisch, O. (1963). *Cochran, w. G.: Sampling techniques*. John wiley and sons, new york, london.

Hilawe, E. H., Yatsuya, H., Kawaguchi, L., & Aoyama, A. (2013). Differences by sex in the prevalence of diabetes mellitus, impaired fasting glycaemia and impaired glucose tolerance in sub-Saharan Africa: a systematic review and meta-analysis. *Bulletin of the World Health Organization*, *91*(9), 671-682D.

Home, P., Riddle, M., Cefalu, W. T., Bailey, C. J., Bretzel, R. G., del Prato, S., … Raz, I. (2014). Insulin therapy in people with type 2 diabetes: opportunities and challenges? *Diabetes Care*, *37*(6), 1499–1508.

Huber, A., Menconi, F., Corathers, S., Jacobson, E. M., & Tomer, Y. (2008). Joint genetic susceptibility to type 1 diabetes and autoimmune thyroiditis: from epidemiology to mechanisms. *Endocrine Reviews*, *29*(6), 697–725.

Kahapola- Arachchige, K. M., Hadlow, N., Wardrop, R., Lim, E. M., & Walsh, J. P. (2012). Age- specific TSH reference ranges have minimal impact on the diagnosis of thyroid dysfunction. *Clinical Endocrinology*, *77*(5), 773–779.

Kalra, S., Aggarwal, S., & Khandelwal, D. (2019). Thyroid Dysfunction and Type 2 Diabetes Mellitus: Screening Strategies and Implications for Management. *Diabetes Therapy*, *10*(6), 2035–2044.

Kazerouni, F., & Amirrasouli, H. (2012). Performance characteristics of three automated immunoassays for thyroid hormones. *Caspian Journal of Internal Medicine*, *3*(2), 400.

Klein, I., & Danzi, S. (2007). Thyroid disease and the heart. *Circulation*, *116*(15), 1725–1735.

Ladenson, P. W., Singer, P. A., Ain, K. B., Bagchi, N., Bigos, S. T., Levy, E. G., … Daniels, G. H. (2000). American Thyroid Association Guidelines for Detection of Thyroid Dysfunction. *Archives of Internal Medicine*, *160*(11), 1573.

Ogbera, A. O., & Kuku, S. F. (2011). Epidemiology of thyroid diseases in Africa. *Indian Journal of Endocrinology and Metabolism*, *15*(6), 82.

OGBONNA, S., & EZEANI, I. (2019). Risk Factors of Thyroid Dysfunction in Patients With Type 2 Diabetes Mellitus. *Frontiers in Endocrinology*, *10*, 440.

Palma, C. C. S. S. V., Pavesi, M., Nogueira, V. G., Clemente, E. L. S., Pereira, M. de F. B. M., Pereira, L. C., … Soares, J. O. (2013). Prevalence of thyroid dysfunction in patients with diabetes mellitus. *Diabetology & Metabolic Syndrome*, *5*(1), 58.

Papazafiropoulou, A., Sotiropoulos, A., Kokolaki, A., Kardara, M., Stamataki, P., & Pappas, S. (2010). Prevalence of thyroid dysfunction among greek type 2 diabetic patients attending an outpatient clinic. *Journal of Clinical Medicine Research*, *2*(2), 75–78.

Pearce, E. N., Yang, Q., Benjamin, E. J., Aragam, J., & Vasan, R. S. (2010). Thyroid function and left ventricular structure and function in the Framingham Heart Study. *Thyroid*, *20*(4), 369–373.

Peppa, M., Betsi, G., & Dimitriadis, G. (2011). Lipid abnormalities and cardiometabolic risk in patients with overt and subclinical thyroid disease. *Journal of Lipids*, *2011*.

Raghavan, S., Vassy, J. L., Ho, Y., Song, R. J., Gagnon, D. R., Cho, K., … Phillips, L. S. (2019). Diabetes Mellitus–Related All- Cause and Cardiovascular Mortality in a National Cohort of Adults. *Journal of the American Heart Association*, *8*(4), e011295.

Rizos, C. V, Elisaf, M. S., & Liberopoulos, E. N. (2011). Effects of thyroid dysfunction on lipid profile. *The Open Cardiovascular Medicine Journal*, *5*, 76.

Rodondi, N., Den Elzen, W. P. J., Bauer, D. C., Cappola, A. R., Razvi, S., Walsh, J. P., … Collet, T.-H. (2010). Subclinical hypothyroidism and the risk of coronary heart disease and mortality. *Jama*, *304*(12), 1365–1374.

Smithson, M. J. (1998). Screening for thyroid dysfunction in a community population of diabetic patients. *Diabetic Medicine*, *15*(2), 148–150.

Surks, M. I., Chopra, I. J., Mariash, C. N., Nicoloff, J. T., & Solomon, D. H. (1990). American Thyroid Association guidelines for use of laboratory tests in thyroid disorders. *Jama*, *263*(11), 1529-1532.

Telwani, A. A., Wani, Z. H., Ashraf, Y., & Shah, A. A. (2017). Prevalence of thyroid dysfunction in type 2 diabetes mellitus: a case control study. *Int J Res Med Sci*, *5*(10), 4527–4531.

Uppal, V., Vij, C., Bedi, G. K., Vij, A., & Banerjee, B. D. (2013). Thyroid disorders in patients of type 2 diabetes mellitus. *Indian Journal of Clinical Biochemistry*, *28*(4), 336–341.

Vanderpump, M P J, Tunbrldge, W. M. G., French, Jm., Appleton, D., Bates, D., Clark, F., … Tunbridge, F. (1995). The incidence of thyroid disorders in the community: a twenty- year follow- up of the Whickham Survey. *Clinical Endocrinology*, *43*(1), 55–68.

Vanderpump, Mark P J. (2011). The epidemiology of thyroid disease. *British Medical Bulletin*, *99*(1).

Velija-Asimi, Z., & Karamehic, J. (2007). The effects of treatment of subclinical hypothyroidism on metabolic control and hyperinsulinemia. *Med Arh*, *61*(1), 20–21.

Walsh, J. P., Bremner, A. P., Bulsara, M. K., O'Leary, P., Leedman, P. J., Feddema, P., & Michelangeli, V. (2005). Subclinical thyroid dysfunction as a risk factor for cardiovascular disease. *Archives of Internal Medicine*, *165*(21), 2467–2472.

Wang, C. (2013). The relationship between type 2 diabetes mellitus and related thyroid diseases. *Journal of Diabetes Research*, *2013*.

Wild, S., Roglic, G., Green, A., Sicree, R., & King, H. (2004). Global prevalence of diabetes estimates for the year 2000 and projections for 2030. *Diabetes Care*, *27*(5), 1047–1053.

APPENDICES

Appendix1: Questionnaire

Study number ---------- Date**:** ----------

1. Age: ----------

2. Gender: Male ☐ Female ☐

3. Marital status (Tick one):

Single ☐ Married ☐ Divorced ☐ Separated ☐ Widowed ☐

4. Level of education (tick one):

Primary ☐ Secondary ☐ Tertiary ☐ None ☐

5. Duration of diabetes (months) ----------

6. Type of medication used for diabetes

---

7. Other medication other than Oral hypoglycemic agents or Insulin

---

---

8. Hypertension: Present ☐ Absent ☐

9. History of Atrial fibrillation: Yes ☐ No ☐

10. Family history of thyroid disease: Yes ☐ No ☐

11. History of Thyroid surgery: Yes ☐ No ☐

12. History of radioiodine ablation: Yes ☐ No ☐

13. History of Diagnosis of Thyroid disease Yes ☐ No ☐

If yes, which type of thyroid dysfunction ----------?

14. Is the patient on medication for thyroid dysfunction? Yes ☐ No ☐

If yes which medication----------

15. Do you have any of the symptoms below? (Tick where appropriate)

Symptoms of Hypothyroidism like:		Symptoms of Hyperthyroidism like:	
Sensitivity to cold	☐	Insomnia	☐
Puffy face	☐	Irritability	☐
Poor memory	☐	Unexplained weight loss	☐
Constipation	☐	Palpitations	☐
Depression	☐	Brittle hair	☐
Hoarse voice	☐	Warm, flushed skin	☐
Weight gain	☐	Protruding eyes	☐
Dry skin	☐	Hand tremors	☐

Physical Examination

Temp..... PR...../min RR...../min BP.....mm/Hg

Weight.....Kg Height.....m BMI.....Kg/m^2

Local exam: Evidence of Thyroid enlargement. Yes ☐ No ☐

Evidence of protruding eyes Yes ☐ No ☐

Evidence of puffy face Yes ☐ No ☐

Evidence of hand tremors Yes ☐ No ☐

Laboratory Investigations

TEST	RESULTS	DATE
Serum TSH		
Serum Free T4		
HBA1c		
LDL cholesterol		

Appendix 2A: Informed Consent (English)

English

My name is Dr Wandia Mwaura. I am a medical doctor currently pursuing a Masters degree in

Internal Medicine at Moi University. I would like to recruit you today into my research which is looking at thyroid dysfunction in T2DM patients that are receiving care at MTRH DOPC.

Purpose of the study:

The purpose of this study is to establish the burden of thyroid dysfunction among patients with T2DM in MTRH in order to institute appropriate measures to manage the condition and improve glycemic control.

What does the study involve?

In this study, you will be asked to provide your sociodemographic information which includes age, sex, marital status, occupation, level of education among others. Blood will also be drawn from you and taken to the laboratory for biochemical assessment of the levels of serum Thyroid Stimulating Hormone (TSH), Free T4, HBA1c and LDL Cholesterol

There are minimal risks involved in this study. These include but not limited to pain at the injection site and swelling at the site of the injection. Blood samples will be discarded after the study.

The result of the study will be stored in a database that is password protected and only accessible by those conducting the study. No one will be able to identify you or your results should data be published; no individual information will be disclosed. Your

participation in this study is voluntary. If you decide to participate you can change your mind later and quit the study before the end. If you decide not to participate or if you quit the study it will not affect the health care services you receive at the clinic. By signing this document you are voluntarily agreeing to participate. You are free to decline to answer any particular question you don't wish to answer for any reason

Consent form

I ……., having been explained to and well understood the nature and purpose of this study by……., do hereby voluntarily agree to participate in the study. I agree to willingly answer the questions honestly and undergo the laboratory investigations that have been explained to me.

Signature (Patient)…….

Date…….

If you need further clarifications, feel free to contact IREC using the address below.

The Administrator IREC

Moi Teaching and Referral Hospital,

PO Box 3,

Eldoret.

Tel: 33471/2/3

You can also contact me (Dr. Wandia Mwaura) on 0722960519

Appendix 2B:Informed Consent (Kiswahili)

Jina langu ni Daktari Wandia Mwaura.Nimehitimu udaktari na nimesajiliwa na bodi ya madaktari ya Kenya (Kenya Medical Practitioners and Dentists Board). Mimi ni msomi wa shahada ya juu (Masters) ya udaktari (Internal medicine) katika chuo kikuu cha Moi. Nimekuona leo kwa sababu ninafanya utafiti wa kuangalia matatizo ya tezi kati ya watu walio na ugonjwa wa sukari.

Lengo kuu la utafiti huu ni kutambua jinsi shida hii ilivyo kidhiri katiya wale wanao ugua ugonjwa wa sukari na wanaopata matibabu yao katika hospitali kuu ya MTRH. Hii itatuwezesha kuweka mikakati bora ya matibabu kabla ugonjwa huu haujaenea na kuleta madhara mwilini.

Katika utafiti huu, utahitajika kutueleza maswala kadha wa kadha kukuhusu. Kwa mfano, miaka, jinsia, kiwango ulichokifikia cha masomo na kadhalika. Utahitajika pia kutolewa damu itakayo pelekwa mahabara nakuangalia kiwango cha homoni za tezi, kiwango cha mafuta mwilini na kipimo cha sukari mwilini.

Utafiti huu hauna madhara kuu kwako mbali na uchungu utakaosikia unapotolewa damu ama uvimbe mdogo mahala utakapotolewa damu. Hata hivyo, uvimbe huu hautakawia kwa muda mrefu.

Una huru wa kukubali ama kukataa kuwa katika utafiti huu. Unaweza pia kujiondoa wakati wowote unapohisi. Kukataa kwako ama kujiondoa katika utafiti huu hauta dhuru jinsi utakavyo hudumia katika hospitali hii sasa wala siku za usoni.

Unajulishwa kwamba kushiriki kwako katika utafiti huu ni kwa hiari yako na sio kwa kulazimishwa naunaweza jiondoa katika utafiti huu wakati wowote. Habari zote tutakazopata kuhusiana nawe na afya yako italindwa na uaminifu wote.

Mimi……baada ya kufafanuliwa nakuelezwa na…… kiini cha utafiti huu na jinsi utakavyotekelezwa nimekubali kushiriki katika utafiti huu bila kushurutishwa.Nakubali kuyajibu maswali yote kwa uaminifu na pia kutolewa damu itakayopelekwa kwenye mahabara kwa uchunguzi.

Sahihi (mshiriki)…….

Tarehe …….

Iwapo utahitaji maelezo zaidi, usikose kuwasilianana IREC ukitumia anwani ifuatayo

Administrator IREC

Moi Teaching and Referral Hospital,

Sanduku la Posta 3,

Eldoret.

Simu: 33471/2/3

Unaweza pia wasilianana mi (Dr.Wandia Mwaura) ukitumia nambari ya simu ifuatayo: **0722960519**

Appendix 3: Blood Pressure Measurement

Measurement of blood pressure was then done using Omron M2 compact upper arm blood pressure monitor (Omron Healthcare, Inc., 1200 Lakeside Drive, Bannockburn, Illinois 60015). The arm cuff position was maintained at the heart level during rest in a seated position and readings taken after at least 15 minutes of being seated in the waiting area. The examiner would take the blood pressure with the arm on rested on the table. The cuff was wrapped on the arm with the bottom of the cuff one centimeter above the elbow, while allowing for finger breadth allowance on application of the cuff. The machine was then be switched on, the cuff inflated and the blood pressure reading recorded. Three blood pressure readings will be taken one minute apart with the average of the last two being recorded.

Appendix 4: Weight and Height Measurement

The patient's weight and height were measured. Weight was measured using a digital weighing scale rounded to the nearest one decimal point. Height was measured in centimeters using a Detecto-beam scale with height rod. Participants were requested to remove excessive clothing and shoes before measurements are taken. Excessive clothing was described as more than two layers of clothes. The participants would then proceed for routine clinic care.

Appendix 5: Phlebotomy Procedure

After explaining the procedure and obtaining verbal consent, the PI would apply a tourniquet to the arm distal to the elbow. The brachial vein in the ante cubital fossa was identified and the site cleaned with antiseptic solution. The vein was then be punctured gently by a gauge 21 needle at an angle of approximately 30 degrees and 8 mls of venous blood drawn. The needle would be withdrawn from the vein and an adhesive bandage applied at the punctured site to help stop bleeding. The samples were put in two labeled red top vacutainers and one purple top vacutainer and the needle and syringe disposed of safely in a sharps container.

Appendix 6: Description of Variables

Age- was the nearest number of years as from the reported or documented date of birth

Gender- was determined by the observed phenotypical sex and secondary sexual characteristics of male or female sex.

Duration of Type 2 DM- was determined as the period to the nearest whole month from the reported or documented date of disease onset. Date of onset was to the first date which the patient learnt of the diagnosis or first documented date of diagnosis

Treatment modality- was defined as the current drugs being used by the patient to achieve glycemic control. There were 3 categories of treatment modalities oral hypoglycemic agents, insulin, both insulin and oral hypoglycemic agents.

Comorbidities (Presence of thyroid disease and/or hypertension)

Appendix 7:SOP for HbA1c

AMPATH Reference Laboratory
SOP for HBA1C

SOP #: ARL429 Version 1.0

1. **Introduction**

Hemoglobin consists of four protein chains with four heme portions, and is the red pigmented protein located in the erythrocytes. The relative amount in the erythrocyte of the Hb A converted to HbA1c increases with the average concentration of glucose in the blood and is limited to the Rbc life span of approximately 100-120 days thus reflecting the average glucose levels in the preceding 2-3 months (HbA1c).

2. **Purpose**.

To describe the procedure for the quantitative determination of percent hemoglobin A1c [HbA1c (%)] in whole blood on Cobas Integra 400 plus Chemistry analyzer.

3. **Principle**

Is based onTurbidimetric inhibition immunoassay (TINIA) for hemolyzed whole blood.
Anticoagulated whole blood is hemolysed automatically on the Cobas Integra 400 plus with Hemolyzing Reagent .This method uses TTAB as the detergent in the hemolyzing reagent to eliminate interference from leukocytes (TTAB does not lyse leukocytes). Sample pretreatment to remove labile HbA1c is not necessary.all glycated Hb variants which are glycated at Beta chain N-terminus and which have antibody-recognizable regions identical to that of HbA1c are determined by this assay.
Glycohemoglobin in the sample reacts with anti-HbA1c antibody in R1 to form soluble antigen antibody complexes. Since the specific HbA1c antibody site is present only once on the HbA1c molecule, complex formation does not take place. Polyhaptens inR2 reacts with excess anti-HbA1c antibodies to form an insoluble antibody-polyhapten complex which is determine turbidimetrically.
Liberated hemoglobin in the hemolyzed sample is a derivative having a characteristic absorption spectrum which is measured bichromatically during preincubation phase of the above immunological reaction.

4. **Responsibility**

All laboratory technologists assigned to work at the AMPATH – Modular Routine Laboratory who have documented training and competency for this test.

5. **Procedure**

Accept sample in accordance to SOP ARL 828.00 for laboratory information system.

Proceed and run the samples as described in the SOP for *the COBAS INTEGRA 400 PLUS Chemistry Analyzer*

a. Precautions

General laboratory precautions are required for handling reagents, calibrators and bleach. Treat all samples and controls as infectious agents. Comply with Laboratory Safety Policy ARL201, SOP for Handling Spills and Tube Breakages ARL210 and dispose off laboratory waste in line with SOP ARL213

b. Reagents

i. R1 Antibody reagent

ii. R2 Polyhapten reagent

iii. Hemolyzing reagent

c. Reagent stability

i. Unopened kit components are stable up to expiration date at 2-8^0 C. On board, reagents are stable for 12 weeks.

d. Equipment/Materials Required

i. COBAS INTEGRA 400 PLUS Chemistry Analyzer

ii. Computerized Workstation

iii. Printer

iv. 1000 µl pipettors

v. Cobas cups

e. Specimen Collection And Preparation

i. Wholeblood is the specimen of choice and is collected from EDTA/li Heparin blood using standard sampling tubes as described in the sop for *Venipuncture ARL 702.00*. Whole Blood may be stored for 3 days at 15-25ºC, up to 7 days at 2-8 ºC and up to 6 months at (-15)-(-25)º C.

ii. Centrifuge samples containing precipitates before performing the assay.

f. Calibration

1. Calibrate the machine, when reagent lot is changed or when internal quality controls give repeatedly out-of-range values.
2. Each new lot number of calibrators needs to be registered on the analyzer, either by barcode reader or manually. Check that the values of the calibrators are adjusted in line with the new lot number.
3. Verify the calibration of a new calibrator lot by running calibrators as sample.

g. Quality Control

1. Control N and P and other suitable control is run for the internal quality control.
2. In order to validate new chemistry controls, the new lot of controls will be run in parallel with the old lot of controls until the mean and standard deviation is obtained for the new lot of controls. The mean and standard deviations for the new lot of controls will be reviewed and signed off by the laboratory supervisor or director before being put into use.
3. In case of QC failure, refer to ARL202. Quality Control Plan for further action.

h. Specimen Rejection

i. Whole blood collected in wrong specimen bottles or samples not meeting the specimen collection time frame. (See also under "Limitations").

a. Results

I. Machine automatically measures the analyte in each sample.

ii. SOP number ARL 828 is used to complete and authenticated the results from the Laboratory information system.

iii. Interpretation of results is based on comparison with the normal ranges provided.

iv. Critical values are telephoned to the clinician as soon as possible. . Log all phone calls reporting critical values.

b. Repeat Criteria

i. High values above AMR (analytical measurable range): Dilute sample and multiply result with the dilution factor

ii. Low values below AMR: If low on repeat, report result as "Below lowest detectable limit"

iii. Critical values: Re-run to confirm

iv. Flags for blockages, slope, or calibration: Confirm quality of sample and reagents and re-run the sample.

c. Limitation

i. N/A

d. Measuring Range

i. Refer to SOP 229

e. Reference Values

i. HbA1c 4.8-5.9%

6. Reference

a. Roche Cobas Integra 400 Operators Manual, Indianapolis 2004
b. Roche Cobas Integra 400 Test Manual, Indianapolis 2004
c. Roche Cobas Benchtop Guide

Appendix 8:SOP for

TSH

TSH

cobas®

REF	Σ	SYSTEM
		MODULAR ANALYTICS E170 **cobas e** 411 **cobas e** 601 **cobas e** 602
11731459 122	200	

English

System information

For **cobas e** 411 analyzer: test number 010
For MODULAR ANALYTICS E170, **cobas e** 601 and **cobas e** 602 analyzers: Application Code Number 001

Intended use

Immunoassay for the in vitro quantitative determination of thyrotropin in human serum and plasma.

The **e**lectrochemiluminescence **i**mmuno**a**ssay "ECLIA" is intended for use on Elecsys and **cobas e** immunoassay analyzers.

Summary

Thyroid-stimulating hormone (TSH, thyrotropin) is a glycoprotein having a molecular weight of approximately 30000 daltons and consisting of two subunits. The β-subunit carries the TSH-specific immunological and biological information, whereas the α-chain carries species-specific information and has an identical amino acid sequence to the α-chains of LH, FSH and hCG.

TSH is formed in specific basophil cells of the anterior pituitary and is subject to a circadian secretion sequence. The hypophyseal release of TSH (thyrotropic hormone) is the central regulating mechanism for the biological action of thyroid hormones. TSH has a stimulating action in all stages of thyroid hormone formation and secretion; it also has a proliferative effect.[1,2]

The determination of TSH serves as the initial test in thyroid diagnostics. Even very slight changes in the concentrations of the free thyroid hormones bring about much greater opposite changes in the TSH level. Accordingly, TSH is a very sensitive and specific parameter for assessing thyroid function and is particularly suitable for early detection or exclusion of disorders in the central regulating circuit between the hypothalamus, pituitary and thyroid.[3,4,5,6]

The Elecsys TSH assay employs monoclonal antibodies specifically directed against human TSH. The antibodies labeled with ruthenium complex[a)] consist of a chimeric construct from human and mouse-specific components. As a result, interfering effects due to HAMA (human anti-mouse antibodies) are largely eliminated.

a) Tris(2,2'-bipyridyl)ruthenium(II)-complex ($Ru(bpy)_3^{2+}$)

Test principle

Sandwich principle. Total duration of assay: 18 minutes.

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

Reagents - working solutions

The reagent rackpack is labeled as TSH.

M Streptavidin-coated microparticles (transparent cap), 1 bottle, 12 mL:
Streptavidin-coated microparticles 0.72 mg/mL, preservative.

R1 Anti-TSH-Ab~biotin (gray cap), 1 bottle, 14 mL:
Biotinylated monoclonal anti-TSH antibody (mouse) 2.0 mg/L; phosphate buffer 100 mmol/L, pH 7.2; preservative.

R2 Anti-TSH-Ab~$Ru(bpy)_3^{2+}$ (black cap), 1 bottle, 12 mL:
Monoclonal anti-TSH antibody (mouse/human) labeled with ruthenium complex 1.2 mg/L; phosphate buffer 100 mmol/L, pH 7.2; preservative.

Precautions and warnings

For in vitro diagnostic use.
Exercise the normal precautions required for handling all laboratory reagents.
Disposal of all waste material should be in accordance with local guidelines. Safety data sheet available for professional user on request.

Avoid foam formation in all reagents and sample types (specimens, calibrators and controls).

Reagent handling

The reagents in the kit have been assembled into a ready-for-use unit that cannot be separated.

All information required for correct operation is read in from the respective reagent barcodes.

Storage and stability

Store at 2-8 °C.

Do not freeze.

Store the Elecsys reagent kit **upright** in order to ensure complete availability of the microparticles during automatic mixing prior to use.

Stability:	
unopened at 2-8 °C	up to the stated expiration date
after opening at 2-8 °C	12 weeks
on MODULAR ANALYTICS E170, **cobas e** 601 and **cobas e** 602	6 weeks
on **cobas e** 411	8 weeks

Specimen collection and preparation

Only the specimens listed below were tested and found acceptable.

Serum collected using standard sampling tubes or tubes containing separating gel.

Li-, Na-, NH_4-heparin, K_2-EDTA, sodium citrate and sodium fluoride/potassium oxalate plasma.

Criterion: Recovery within 90-110 % of serum value or slope 0.9-1.1 + intercept within < ± 2x analytical sensitivity (LDL) + coefficient of correlation > 0.95.

Stable for 7 days at 2-8 °C, 1 month at -20 °C.[7] Freeze only once.

The sample types listed were tested with a selection of sample collection tubes that were commercially available at the time of testing, i.e. not all available tubes of all manufacturers were tested. Sample collection systems from various manufacturers may contain differing materials which could affect the test results in some cases. When processing samples in primary tubes (sample collection systems), follow the instructions of the tube manufacturer.

Centrifuge samples containing precipitates before performing the assay.

Do not use heat-inactivated samples.

Do not use samples and controls stabilized with azide.

Ensure the samples, calibrators and controls are at 20-25 °C prior to measurement.

Due to possible evaporation effects, samples, calibrators and controls on the analyzers should be analyzed/measured within 2 hours.

Materials provided

See "Reagents – working solutions" section for reagents.

Materials required (but not provided)

- REF 04738551190, TSH CalSet, 4 x 1.3 mL
- REF 11776479122, PreciControl TSH, 4 x 2.0 mL
- REF 11731416190, PreciControl Universal, for 4 x 3.0 mL
- REF 06445918190, PreciControl Thyro Sensitive, for 4 x 2.0 mL
- REF 03609987190, Diluent MultiAssay, 2 x 16 mL sample diluent
- General laboratory equipment
- MODULAR ANALYTICS E170 or **cobas e** analyzer

Accessories for **cobas e** 411 analyzer:

- REF 11662988122, ProCell, 6 x 380 mL system buffer
- REF 11662970122, CleanCell, 6 x 380 mL measuring cell cleaning solution
- REF 11930346122, Elecsys SysWash, 1 x 500 mL washwater additive
- REF 11933159001, Adapter for SysClean
- REF 11706802001, AssayCup, 60 x 60 reaction cups
- REF 11706799001, AssayTip, 30 x 120 pipette tips
- REF 11800507001, Clean-Liner

Accessories for MODULAR ANALYTICS E170, **cobas e** 601 and **cobas e** 602 analyzers:

- REF 04880340190, ProCell M, 2 x 2 L system buffer
- REF 04880293190, CleanCell M, 2 x 2 L measuring cell cleaning solution
- REF 03023141001, PC/CC-Cups, 12 cups to prewarm ProCell M and CleanCell M before use
- REF 03005712190, ProbeWash M, 12 x 70 mL cleaning solution for run finalization and rinsing during reagent change
- REF 12102137001, AssayTip/AssayCup, 48 magazines x 84 reaction cups or pipette tips, waste bags
- REF 03023150001, WasteLiner, waste bags
- REF 03027651001, SysClean Adapter M

Accessories for all analyzers:

- REF 11298500316, ISE Cleaning Solution/Elecsys SysClean, 5 x 100 mL system cleaning solution

Assay

For optimum performance of the assay follow the directions given in this document for the analyzer concerned. Refer to the appropriate operator's manual for analyzer-specific assay instructions.

Resuspension of the microparticles takes place automatically prior to use. Read in the test-specific parameters via the reagent barcode. If in exceptional cases the barcode cannot be read, enter the 15-digit sequence of numbers (except for the **cobas e** 602 analyzer).

Bring the cooled reagents to approximately 20 °C and place on the reagent disk (20 °C) of the analyzer. Avoid foam formation. The system automatically regulates the temperature of the reagents and the opening/closing of the bottles.

Calibration

Traceability: This method has been standardized against the 2nd IRP WHO Reference Standard 80/558.

Every Elecsys reagent set has a barcoded label containing specific information for calibration of the particular reagent lot. The predefined master curve is adapted to the analyzer using the relevant CalSet.

Calibration frequency: Calibration must be performed once per reagent lot using fresh reagent (i.e. not more than 24 hours since the reagent kit was registered on the analyzer).

Calibration interval may be extended based on acceptable verification of calibration by the laboratory.

Renewed calibration is recommended as follows:

- after 8 weeks when using the same reagent lot
- after 7 days when using the same reagent kit on the analyzer
- as required: e.g. quality control findings outside the defined limits

Quality control

For quality control, use PreciControl Universal, PreciControl TSH or PreciControl Thyro Sensitive.

In addition, other suitable control material can be used.

Controls for the various concentration ranges should be run individually at least once every 24 hours when the test is in use, once per reagent kit, and following each calibration.

The control intervals and limits should be adapted to each laboratory's individual requirements. Values obtained should fall within the defined limits. Each laboratory should establish corrective measures to be taken if values fall outside the defined limits.

If necessary, repeat the measurement of the samples concerned.

Follow the applicable government regulations and local guidelines for quality control.

Calculation

The analyzer automatically calculates the analyte concentration of each sample either in µIU/mL or mIU/L (selectable).

Limitations - interference

The assay is unaffected by icterus (bilirubin < 701 µmol/L or < 41 mg/dL), hemolysis (Hb < 0.621 mmol/L or < 1 g/dL), lipemia (Intralipid < 1500 mg/dL), biotin (< 102 nmol/L or < 25 ng/mL), IgG < 2 g/dL and IgM < 0.5 g/dL.

Criterion: Recovery within ± 10 % of initial value.

Samples should not be taken from patients receiving therapy with high biotin doses (i.e. > 5 mg/day) until at least 8 hours following the last biotin administration.

No interference was observed from rheumatoid factors up to a concentration of 3250 IU/mL and samples from dialysis patients.

There is no high-dose hook effect at TSH concentrations up to 1000 µIU/mL.

In vitro tests were performed on 26 commonly used pharmaceuticals. No interference with the assay was found.

The presence of autoantibodies may induce high molecular weight complexes (macro-TSH) which may cause unexpected high values of TSH.[8]

In rare cases, interference due to extremely high titers of antibodies to analyte-specific antibodies, streptavidin or ruthenium can occur. These effects are minimized by suitable test design.

For diagnostic purposes, the results should always be assessed in conjunction with the patient's medical history, clinical examination and other findings.

Limits and ranges

Measuring range

0.005-100 µIU/mL (defined by the lower detection limit and the maximum of the master curve). The functional sensitivity is 0.014 µIU/mL.[9] Values below the lower detection limit are reported as < 0.005 µIU/mL. Values above the measuring range are reported as > 100 µIU/mL (or up to 1000 µIU/mL for 10-fold diluted samples).

Lower limits of measurement

Lower detection limit of the test

Lower detection limit: 0.005 µIU/mL

The lower detection limit represents the lowest analyte level that can be distinguished from zero. It is calculated as the value lying two standard deviations above that of the lowest standard (master calibrator, standard 1 + 2 SD, repeatability study, n = 21).

Dilution

Samples with TSH concentrations above the measuring range can be diluted with Diluent MultiAssay. The recommended dilution is 1:10 (either automatically by the analyzers, or manually). The concentration of the diluted sample must be > 10 µIU/mL.

After manual dilution, multiply the result by the dilution factor.

After dilution by the analyzers, the software automatically takes the dilution into account when calculating the sample concentration.

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TSH

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Expected values

0.270–4.20 µIU/mL

These values correspond to the 2.5th and 97.5th percentiles of results obtained from a total of 516 healthy test subjects examined.

For detailed information about reference intervals in children, adolescents and pregnant women, refer to the brochure "Reference Intervals for Children and Adults", English: REF 04640292, German: REF 04625889.

This booklet also contains results of a detailed study about influencing factors on thyroid parameters in a well characterized reference group of adults. Different inclusion and exclusion criteria were applied (e.g. sonographic results (thyroid volume and density) as well as criteria according to the guidelines of the National Academy of Clinical Biochemistry - NACB).

Each laboratory should investigate the transferability of the expected values to its own patient population and if necessary determine its own reference ranges.

Specific performance data

Representative performance data on the analyzers are given below. Results obtained in individual laboratories may differ.

Precision

Precision was determined using Elecsys reagents, pooled human sera and controls in a modified protocol (EP5-A) of the CLSI (Clinical and Laboratory Standards Institute): 6 times daily for 10 days (n = 60); repeatability on MODULAR ANALYTICS E170 analyzer, n = 21. PreciControl TSH was determined once daily for 10 days (n = 10). The following results were obtained:

cobas e 411 analyzer					
		Repeatability		**Intermediate precision**	
Sample	Mean µIU/mL	SD µIU/mL	CV %	SD µIU/mL	CV %
Human serum 1	0.034	0.003	8.6	0.003	8.7
Human serum 2	0.91	0.02	2.1	0.03	3.3
Human serum 3	3.96	0.07	1.8	0.14	3.6
PC[b] Universal 1	2.45	0.05	1.9	0.05	2.2
PC Universal 2	10.67	0.16	1.5	0.19	1.8
PreciControl TSH	0.084	-	-	0.005	5.4

b) PC = PreciControl

MODULAR ANALYTICS E170, **cobas e** 601 and **cobas e** 602 analyzers						
	Repeatability			**Intermediate precision**		
Sample	Mean µIU/mL	SD µIU/mL	CV %	Mean µIU/mL	SD µIU/mL	CV %
Human serum 1	0.040	0.001	3.0	0.035	0.003	7.2
Human serum 2	0.092	0.002	2.7	0.151	0.005	3.2
Human serum 3	9.37	0.102	1.1	3.66	0.120	3.3
PC Universal 1	0.959	0.014	1.5	0.915	0.031	3.5
PC Universal 2	8.13	0.098	1.2	7.52	0.316	4.2

Method comparison

A comparison of the Elecsys TSH assay (y) with the Enzymun-Test TSH method (x) using clinical samples gave the following correlations:

Number of samples measured: 109

Passing/Bablok[9]	Linear regression
y = 1.01x + 0.01	y = 0.98x + 0.04
r = 0.944	r = 0.993

The sample concentrations were between approximately 0 and 19 µIU/mL.

Analytical specificity

For the monoclonal antibodies used, the following cross-reactivities were found:

LH 0.038 %, FSH 0.008 %; hGH and hCG no cross-reactivity.

Functional sensitivity

0.014 µIU/mL

The functional sensitivity is the lowest analyte concentration that can be reproducibly measured with an intermediate precision CV of ≤ 20 %.

References

1 Wheeler MH, Lazarus JH. Diseases of the Thyroid. London, Glascow, Weinheim, New York, Tokyo, Melbourne, Madras: Chapman and Hall Medical, 1994:109-115.

2 Pfannenstiel P, Saller B. Schilddrüsenkrankheiten Diagnose und Therapie. Berliner Medizinische Verlagsanstalt GmbH 1995;2:43-54.

3 Surks MI, Chopra IJ, Mariash CN, et al. American Thyroid Association Guidelines for the Use of Laboratory Tests in Thyroid Disorders. JAMA 1990;263:1529-1532.

4 Kefler JH. Preanalytical Considerations in Testing Thyroid Function. Clin Chem 1996;42(1):125-135.

5 Ladenson PW. Optimal laboratory testing for diagnosis and monitoring of thyroid nodules, goiter and thyroid cancer. Clin Chem 1996;42:(1):183-187.

6 Nicoloff JT, Spencer CA. The use and misuse of the sensitive thyrotropin assays. J Clin Endocr Metab 1990;71:553-558.

7 Tietz NW. Clinical Guide to Laboratory Tests, 3rd edition. Philadelphia, Pa. WB Saunders Co. 1995:594.

8 Sakai H, Fukuda G, Suzuki N, et al. Falsely Elevated Thyroid-Stimulating Hormone (TSH) Level Due to Macro-TSH. Endocr J 2009;56(3):435-440.

9 Passing H, Bablok W, Bender R et al. A general regression procedure for method transformation. J Clin Chem Clin Biochem 1988;26:783-790.

For further information, please refer to the appropriate operator's manual for the analyzer concerned, the respective application sheets, the product information and the Method Sheets of all necessary components (if available in your country).

A point (period/stop) is always used in this Method Sheet as the decimal separator to mark the border between the integral and the fractional parts of a decimal numeral. Separators for thousands are not used.

Symbols

Roche Diagnostics uses the following symbols and signs in addition to those listed in the ISO 15223-1 standard (for USA: see https://usdiagnostics.roche.com for definition of symbols used):

CONTENT	Contents of kit
SYSTEM	Analyzers/Instruments on which reagents can be used
REAGENT	Reagent
CALIBRATOR	Calibrator
→	Volume after reconstitution or mixing
GTIN	Global Trade Item Number

CE

Roche Diagnostics GmbH, Sandhofer Strasse 116, D-68305 Mannheim
www.roche.com

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Appendix 9: SOP for fT4

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Elecsys FT4 III

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REF	Σ	SYSTEM
07976836 190	200	MODULAR ANALYTICS E170 **cobas e** 411 **cobas e** 601 **cobas e** 602

English

System information

For **cobas e** 411 analyzer: test number 1610
For MODULAR ANALYTICS E170, **cobas e** 601 and **cobas e** 602 analyzers: Application Code Number 309

Intended use

Immunoassay for the in vitro quantitative determination of free thyroxine in human serum and plasma.

The **e**lectrochemiluminescence **i**mmuno**a**ssay "ECLIA" is intended for use on Elecsys and **cobas e** immunoassay analyzers.

Summary

Thyroxine (T4) is the main thyroid hormone secreted into the bloodstream by the thyroid gland. Together with triiodothyronine (T3) it plays a vital role in regulating the body's metabolic rate, influences the cardiovascular system, growth and bone metabolism, and is important for normal development of gonadal functions and nervous system.[1]

T4 circulates in the bloodstream as an equilibrium mixture of free and serum bound hormone. Free T4 (fT4) is the unbound and biologically active form, which represents only 0.03 % of the total T4. The remaining T4 is inactive and bound to serum proteins such as thyroxine binding globulin (TBG) (75 %), pre-albumin (15 %), and albumin (10 %).[2,3,4,5]

The determination of free T4 has the advantage of being independent of changes in the concentrations and binding properties of these binding proteins; additional determination of a binding parameter (T-uptake, TBG) is therefore unnecessary. Thus free T4 is a useful tool in clinical routine diagnostics for the assessment of the thyroid status. It should be measured together with TSH if thyroid disorders are suspected and is also suitable for monitoring thyrosuppressive therapy.[1,6,7]

A variety of methods are available for estimating the free thyroid hormone levels. The direct measurement of fT4 and fT3 via equilibrium dialysis or ultrafiltration is mainly used as a reference method for standardizing, the immunological procedures generally used for routine diagnostic purposes.[6,7]

In the Elecsys FT4 III assay a specific anti-T4 antibody labeled with a ruthenium complex[a)] is used to determine the free thyroxine.

a) Tris(2,2'-bipyridyl)ruthenium(II)-complex ($Ru(bpy)_3^{2+}$)

Test principle

Competition principle. Total duration of assay: 18 minutes.

- 1st incubation: 15 µL of sample and a T4-specific antibody labeled with a ruthenium complex.
- 2nd incubation: After addition of biotinylated T4 and streptavidin-coated microparticles, the still-free binding sites of the labeled antibody become occupied, with formation of an antibody-hapten complex. The entire complex becomes bound to the solid phase via interaction of biotin and streptavidin.
- The reaction mixture is aspirated into the measuring cell where the microparticles are magnetically captured onto the surface of the electrode. Unbound substances are then removed with ProCell/ProCell M. Application of a voltage to the electrode then induces chemiluminescent emission which is measured by a photomultiplier.
- Results are determined via a calibration curve which is instrument-specifically generated by 2-point calibration and a master curve provided via the reagent barcode or e-barcode.

Reagents - working solutions

The reagent rackpack is labeled as FT4 III.

M Streptavidin-coated microparticles (transparent cap), 1 bottle, 12 mL:
Streptavidin-coated microparticles 0.72 mg/mL; preservative.

R1 Anti-T4-Ab-$Ru(bpy)_3^{2+}$ (gray cap), 1 bottle, 18 mL:
Polyclonal anti-T4-antibody (sheep) labeled with ruthenium complex 75 ng/mL; phosphate buffer 100 mmol/L, pH 7.0; preservative.

R2 T4-biotin (black cap), 1 bottle, 18 mL:
Biotinylated T4 2.5 ng/mL; phosphate buffer 100 mmol/L, pH 7.0; preservative.

Precautions and warnings

For in vitro diagnostic use.
Exercise the normal precautions required for handling all laboratory reagents.
Disposal of all waste material should be in accordance with local guidelines.
Safety data sheet available for professional user on request.

Avoid foam formation in all reagents and sample types (specimens, calibrators and controls).

Reagent handling

The reagents in the kit have been assembled into a ready-for-use unit that cannot be separated.

All information required for correct operation is read in from the respective reagent barcodes.

Storage and stability

Store at 2-8 °C.

Do not freeze.

Store the Elecsys reagent kit **upright** in order to ensure complete availability of the microparticles during automatic mixing prior to use.

Stability:	
unopened at 2-8 °C	up to the stated expiration date
after opening at 2-8 °C	84 days
on the analyzers	28 days onboard or 56 days when stored alternatively in the refrigerator and on the analyzer, with the total time onboard on the analyzer not exceeding 120 hours

Specimen collection and preparation

Only the specimens listed below were tested and found acceptable.

Serum collected using standard sampling tubes or tubes containing separating gel.

Li-heparin, K_2-EDTA and K_3-EDTA plasma.

Plasma tubes containing separating gel can be used.

Criterion: Slope 0.9-1.1 + intercept within ≤ ± 0.6 pmol/L + coefficient of correlation ≥ 0.95.

Stable for 5 days at 20-25 °C, 7 days at 2-8 °C, 30 days at -20 °C (± 5 °C). Freeze only once.

The sample types listed were tested with a selection of sample collection tubes that were commercially available at the time of testing, i.e. not all available tubes of all manufacturers were tested. Sample collection systems from various manufacturers may contain differing materials which could affect the test results in some cases. When processing samples in primary tubes (sample collection systems), follow the instructions of the tube manufacturer.

Centrifuge samples containing precipitates before performing the assay.

Do not use heat-inactivated samples.

Do not use samples and controls stabilized with azide.

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Ensure the samples, calibrators and controls are at 20-25 °C prior to measurement.

Due to possible evaporation effects, samples, calibrators and controls on the analyzers should be analyzed/measured within 2 hours.

Materials provided

See "Reagents – working solutions" section for reagents.

Materials required (but not provided)

- REF 07976879190, CalSet FT4 III, 4 x 1.0 mL
- REF 11731416190, PreciControl Universal, for 4 x 3.0 mL
- General laboratory equipment
- MODULAR ANALYTICS E170 or **cobas e** analyzer

Accessories for **cobas e** 411 analyzer:

- REF 11662988122, ProCell, 6 x 380 mL system buffer
- REF 11662970122, CleanCell, 6 x 380 mL measuring cell cleaning solution
- REF 11930346122, Elecsys SysWash, 1 x 500 mL washwater additive
- REF 11933159001, Adapter for SysClean
- REF 11706802001, AssayCup, 60 x 60 reaction cups
- REF 11706799001, AssayTip, 30 x 120 pipette tips
- REF 11800507001, Clean-Liner

Accessories for MODULAR ANALYTICS E170, **cobas e** 601 and **cobas e** 602 analyzers:

- REF 04880340190, ProCell M, 2 x 2 L system buffer
- REF 04880293190, CleanCell M, 2 x 2 L measuring cell cleaning solution
- REF 03023141001, PC/CC-Cups, 12 cups to prewarm ProCell M and CleanCell M before use
- REF 03005712190, ProbeWash M, 12 x 70 mL cleaning solution for run finalization and rinsing during reagent change
- REF 03004899190, PreClean M, 5 x 600 mL detection cleaning solution
- REF 12102137001, AssayTip/AssayCup, 48 magazines x 84 reaction cups or pipette tips, waste bags
- REF 03023150001, WasteLiner, waste bags
- REF 03027651001, SysClean Adapter M

Accessories for all analyzers:

- REF 11298500316, ISE Cleaning Solution/Elecsys SysClean, 5 x 100 mL system cleaning solution

Assay

For optimum performance of the assay follow the directions given in this document for the analyzer concerned. Refer to the appropriate operator's manual for analyzer-specific assay instructions.

Resuspension of the microparticles takes place automatically prior to use. Read in the test-specific parameters via the reagent barcode. If in exceptional cases the barcode cannot be read, enter the 15-digit sequence of numbers (except for the **cobas e** 602 analyzer).

MODULAR ANALYTICS E170, **cobas e** 601 and **cobas e** 602 analyzers: PreClean M solution is necessary.

Bring the cooled reagents to approximately 20 °C and place on the reagent disk (20 °C) of the analyzer. Avoid foam formation. The system automatically regulates the temperature of the reagents and the opening/closing of the bottles.

Calibration

Traceability: This method has been standardized against the Elecsys FT4 II method. The Elecsys FT4 II assay is traceable to the Enzymun-Test which was standardized using equilibrium dialysis.[5,8]

Every Elecsys reagent set has a barcoded label containing specific information for calibration of the particular reagent lot. The predefined master curve is adapted to the analyzer using the relevant CalSet.

Calibration frequency: Calibration must be performed once per reagent lot using fresh reagent (i.e. not more than 24 hours since the reagent kit was registered on the analyzer).

Calibration interval may be extended based on acceptable verification of calibration by the laboratory.

Renewed calibration is recommended as follows:

- after 1 month (28 days) when using the same reagent lot
- after 7 days (when using the same reagent kit on the analyzer)
- as required: e.g. quality control findings outside the defined limits

Quality control

For quality control, use PreciControl Universal.

In addition, other suitable control material can be used.

Controls for the various concentration ranges should be run individually at least once every 24 hours when the test is in use, once per reagent kit, and following each calibration.

The control intervals and limits should be adapted to each laboratory's individual requirements. Values obtained should fall within the defined limits. Each laboratory should establish corrective measures to be taken if values fall outside the defined limits.

If necessary, repeat the measurement of the samples concerned.

Follow the applicable government regulations and local guidelines for quality control.

Calculation

The analyzer automatically calculates the analyte concentration of each sample either in pmol/L, ng/dL or ng/L.

Conversion factors:

pmol/L x 0.077688 = ng/dL
ng/dL x 12.872 = pmol/L
pmol/L x 0.77688 = ng/L

Limitations - interference

The effect of the following endogenous substances and pharmaceutical compounds on assay performance was tested. Interferences were tested up to the listed concentrations and no impact on results was observed.

Endogenous substances

Compound	Concentration tested
Bilirubin	≤ 701 µmol/L or ≤ 41 mg/dL
Hemoglobin	≤ 0.621 mmol/L or ≤ 1000 mg/dL
Intralipid	≤ 2000 mg/dL
Biotin	≤ 409 nmol/L or ≤ 100 ng/mL
Rheumatoid factors	≤ 1200 IU/mL
IgG	≤ 7 g/dL
IgA	≤ 1.6 g/dL
IgM	≤ 1 g/dL

Criterion: Recovery of ≤ ± 0.6 pmol/L of initial value ≤ 6 pmol/L and ± 10 % of initial value > 6 pmol/L.

Samples should not be taken from patients receiving therapy with high biotin doses (i.e. > 5 mg/day) until at least 8 hours following the last biotin administration.

Any influence that might affect the binding behavior of the binding proteins can alter the result of the fT4 tests (e.g. drugs, NTIs (Non-Thyroid-Illness) or patients suffering from FDH (Familial Dysalbuminemic Hyperthyroxinemia)).[9,10]

The test cannot be used in patients receiving treatment with lipid-lowering agents containing D-T4. If the thyroid function is to be checked in such patients, the therapy should first be discontinued for 4-6 weeks to allow the physiological state to become re-established.[11]

Autoantibodies to thyroid hormones can interfere with the assay.[7]

Pharmaceutical substances

In vitro tests were performed on 16 commonly used pharmaceuticals. No interference with the assay was found.

In addition, the following special thyroid drugs were tested. No interference with the assay was found.

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Special thyroid drugs

Drug	Concentration tested µg/mL
Iodide	0.2
Carbimazole	6
Thiamazole	80
Propylthiouracil	300
Perchlorate	2000
Propranolol	240
Amiodarone	200
Prednisolone	100
Hydrocortisone	200
Fluocortolone	100
Octreotide	0.3

In in vitro studies the drugs Furosemide and Levothyroxine caused elevated fT4 findings at the daily therapeutic dosage level.

In rare cases, interference due to extremely high titers of antibodies to analyte-specific antibodies, streptavidin or ruthenium can occur. These effects are minimized by suitable test design.

For diagnostic purposes, the results should always be assessed in conjunction with the patient's medical history, clinical examination and other findings.

Limits and ranges

Measuring range

0.5-100 pmol/L (defined by the Limit of Detection and the maximum of the master curve). Values below the Limit of Detection are reported as < 0.5 pmol/L. Values above the measuring range are reported as > 100 pmol/L.

Lower limits of measurement

Limit of Blank, Limit of Detection and Limit of Quantitation

Limit of Blank = 0.3 pmol/L

Limit of Detection = 0.5 pmol/L

Limit of Quantitation = 1.3 pmol/L

The Limit of Blank, Limit of Detection and Limit of Quantitation were determined in accordance with the CLSI (Clinical and Laboratory Standards Institute) EP17-A2 requirements.

The Limit of Blank is the 95th percentile value from n ≥ 60 measurements of analyte-free samples over several independent series. The Limit of Blank corresponds to the concentration below which analyte-free samples are found with a probability of 95 %.

The Limit of Detection is determined based on the Limit of Blank and the standard deviation of low concentration samples. The Limit of Detection corresponds to the lowest analyte concentration which can be detected (value above the Limit of Blank with a probability of 95 %).

The Limit of Quantitation is the lowest analyte concentration that can be reproducibly measured with an intermediate precision CV of ≤ 20 %.

Dilution

Samples for fT4 determinations cannot be diluted, as T4 in the blood is present in free and protein-bound forms which are in equilibrium. A change in the concentration of the binding proteins alters this equilibrium.

Expected values

Euthyroid: 12-22 pmol/L (0.93-1.7 ng/dL)

These values correspond to the 2.5th and 97.5th percentile of results from a total of 801 healthy test subjects studied.

Status: MCE Reference Range Thyroid, Status 1st quarter 1998.

For detailed information about reference intervals in children, adolescents and pregnant women, refer to the brochure "Reference Intervals for Children and Adults", English: REF 04640292, German: REF 04625889.

This booklet also contains results of a detailed study about influencing factors on thyroid parameters in a well characterized reference group of adults.

Different inclusion and exclusion criteria were applied (e.g. sonographic results (thyroid volume and density) as well as criteria according to the guidelines of the National Academy of Clinical Biochemistry - NACB).

Each laboratory should investigate the transferability of the expected values to its own patient population and if necessary determine its own reference ranges.

Specific performance data

Representative performance data on the analyzers are given below. Results obtained in individual laboratories may differ.

Precision

Precision was determined using Elecsys reagents, samples and controls in a protocol (EP05-A3) of the CLSI (Clinical and Laboratory Standards Institute): 2 runs per day in duplicate each for 21 days (n = 84). The following results were obtained:

cobas e 411 analyzer					
		Repeatability		**Intermediate precision**	
Sample	Mean pmol/L	SD pmol/L	CV %	SD pmol/L	CV %
Human serum 1	1.55	0.089	5.7	0.166	10.7
Human serum 2	13.6	0.239	1.8	0.475	3.5
Human serum 3	23.9	0.411	1.7	0.729	3.0
Human serum 4	57.8	1.11	1.9	2.26	3.9
Human serum 5	92.0	2.24	2.4	5.44	5.9
PreciControl U[a]1	15.8	0.258	1.6	0.474	3.0
PreciControl U2	41.0	0.867	2.1	1.39	3.4

b) U = Universal

MODULAR ANALYTICS E170, **cobas e** 601 and **cobas e** 602 analyzers					
		Repeatability		**Intermediate precision**	
Sample	Mean pmol/L	SD pmol/L	CV %	SD pmol/L	CV %
Human serum 1	1.63	0.070	4.3	0.138	8.4
Human serum 2	13.0	0.198	1.5	0.390	3.0
Human serum 3	22.7	0.348	1.5	0.742	3.3
Human serum 4	54.7	0.925	1.7	2.16	3.9
Human serum 5	92.7	2.37	2.6	4.76	5.1
PreciControl U1	15.3	0.173	1.1	0.399	2.6
PreciControl U2	39.0	0.695	1.8	1.30	3.3

Method comparison

A comparison of the Elecsys FT4 III assay (y) with the Elecsys FT4 II assay (x) using clinical samples gave the following correlations:

Number of samples measured: 141

Passing/Bablok[12]	Linear regression
y = 1.03x + 0.178	y = 1.02x + 0.610
τ = 0.957	r = 0.999

The sample concentrations were between approximately 2.3 and 92 pmol/L.

Analytical specificity

The following cross-reactivities were found, tested with fT4 concentrations of approximately 13 pmol/L and 39 pmol/L:

Cross-reactant	Concentration tested ng/dL	Cross-reactivity %
L-T3	50000	0.005
D-T3	50000	0.002

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Cross-reactant	Concentration tested ng/dL	Cross-reactivity %
rT3	190000	0.007
3-iodo-L-tyrosine	10000000	0.000
3,5-diiodo-L-tyrosine	10000000	0.000
3,3',5-triiodothyroacetic acid	100000	0.000
3,3',5,5'-tetraiodothyroacetic acid	100000	0.001

References

1 Kronenberg HM, Melmed S, Polonsky KS, et al. Williams Textbook of Endocrinology. Saunders Elsevier, Philadelphia, 12th edition, 2011, chapter 10, p. 301-311.

2 Robbins J, Rall JE. The interaction of thyroid hormones and protein in biological fluids. Recent Prog Horm Res 1957;13:161-208.

3 Oppenheimer JH. Role of plasma proteins in the binding, distribution and metabolism of the thyroid hormones. N Engl J Med 1968;278(21):1153-1162.

4 DeGroot LJ, Larsen PR, Hennemann G. Transport of thyroid hormone and cell uptake. The thyroid and its diseases. Wiley and Sons, New York, 1984:62-65.

5 Ekins RP. Measurement of free hormones in blood. Endocr Rev 1990;11(1):5-46.

6 Wu AHB. Tietz Clinical Guide To Laboratory Tests. Saunders Elsevier, Philadelphia, 4th edition, 2006, section II, p. 1076-1077.

7 Brent GA. Thyroid Function Testing. Springer, Berlin, 1st edition, 2010, chapter 5, p. 86-88.

8 Ekins RP, Ellis SM. The radioimmunoassay of free thyroid hormones in serum. In Robbins J, Braverman LE (eds). Thyroid research, Proceedings of the Seventh International Thyroid Conference, Boston. Amsterdam, Excerpta Medica 1975:597.

9 Wada N, Chiba H, Shimizu C, et al. A novel missense mutation in codon 218 of the albumin gene in a distinct phenotype of familial dysalbuminemic hyperthyroxinemia in a Japanese kindred. J Clin Endocrinol Metab 1997;82(10):3246-3250.

10 Arevalo G. Prevalence of familial dysalbuminemic hyperthyroxinemia in serum samples received for thyroid testing. Clin Chem 1991;37(8):1430-1431.

11 Bantle JP, Hunninghake DB, Frantz ID, et al. Comparison of effectiveness of thyrotropin-suppressive doses of D- and L-thyroxine in treatment of hypercholesterolemia. Am J Med 1984;77(3):475-481.

12 Bablok W, Passing H, Bender R, et al. A general regression procedure for method transformation. Application of linear regression procedures for method comparison studies in clinical chemistry, Part III. J Clin Chem Clin Biochem 1988 Nov;26(11):783-790.

For further information, please refer to the appropriate operator's manual for the analyzer concerned, the respective application sheets, the product information and the Method Sheets of all necessary components (if available in your country).

A point (period/stop) is always used in this Method Sheet as the decimal separator to mark the border between the integral and the fractional parts of a decimal numeral. Separators for thousands are not used.

Symbols

Roche Diagnostics uses the following symbols and signs in addition to those listed in the ISO 15223-1 standard (for USA: see https://usdiagnostics.roche.com for definition of symbols used):

CONTENT	Contents of kit
SYSTEM	Analyzers/Instruments on which reagents can be used
REAGENT	Reagent
CALIBRATOR	Calibrator
→	Volume after reconstitution or mixing
GTIN	Global Trade Item Number

CE

Roche Diagnostics GmbH, Sandhofer Strasse 116, D-68305 Mannheim
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Appendix 10:SOP for LDL-C

AMPATH Reference laboratory SOP
SOP for HDL/LDL Determination (Cobas)
SOP #: ARL424 *Version 4.0*

1. **Purpose**
   To describe the procedure of performing LDL-C and HDL-C testing, using Cobas Intergra 400 plus.

2. **Introduction**
   LDL-cholesterol
   Low density lipoproteins (LDL) play a key role in causing and influencing the progression of atherosclerosis and coronary sclerosis in particular. The LDLs are derived from VLDLs rich in triglycerides by action of lipolytic enzymes and are synthesized in the liver. The majority of cholesterol stored in atherosclerotic plaques originates from LDL.
   The LDL-C content is usually calculated from the difference between total cholesterol and the cholesterol in the remainder after precipitation with polyvinyl sulfate and dextran sulfate.

   HDL-cholesterol
   High density lipoprotein (HDL) is considered protective against artherosclerosis. The method for the direct determination of HDL-cholesterol uses PEG-modified enzymes and dextran sulfate. When choleserol esterase and cholesterol oxidase enzymes are modified by PEG, they show selective catalytic activities toward lipoproptein fractions, with the reactivity increasing in the order: LDL < VLDL = chylomicrons HDL.

3. **Principle**
   a. **Principle for HDL**
      Homogenous enzymatic colorimetric test.
      Sample and addition of R1 (buffer)
      In the presence of magnesiium sulfate, dextran sulfate selectively forms water-soluble complexes with LDL, VLDL and chylomicrons which are resistant to PEG-modifeid enzymes.
      Addition of R2 (PEG-modified enzymes/4-amini-antipyrine/buffer) and start of reaction:
      The cholesterol concerntration of HDL-cholesterol is determined enzymatically by cholesterol esterase and cholesterol oxidase coupled with PEG to the amino group.
      In the prescence of oxygen, cholesterol is oxidised by cholesterol oxidase to delta 4 –cholestenone and hydrogen peroxide.
      In the presence of peroxidase, the hydrogen peroxide generated reacts with 4-amino-antipyrine and HSDA to form a purple-blue dye. The color intensity is directly propotional to the cholesterol concentration and is measured photometrically.

   b. **Principle For LDL**
      LDLC ester is hydrolysed in the presence of cholesterol esterase to cholesterol and free fatty acid.
      LDLC in the presence of oxygen and cholesterol oxidase forms 4-cholestenone and hydrogen peroxide.

Hydrogen peroxide and 4-aminoantipyrine plus HDSA and H+,Hydrogen peroxide in the presence of peroxidase forms a purple blue pigment and 5 molecules of water.
The colour intensity of the blue quinoneime dye formed is directly proportional to the LDL-cholesterol concentration. It is determined by measuring the increase in absorbance at 583nm.

4. **Responsibility**
All laboratory technologists assigned to work at the AMPATH – Modular Routine Laboratory who have documented training and competency for this test.

5. **Scope**
The SOP covers procedure of Triglycerides done at modularLaboratory.

6. **Abbreviations**
   a. AMPATH - Academic Model Providing Access to Healthcare.
   b. SOP – Standard Operating Procedure
   c. CFAS- Calibrator for Automated system
   d. EQA- External Quality Assurance
   e. QC- Quality control

7. **Procedure**
   a. **Precautions**
      i. General laboratory precautions are required for handling reagents, calibrators and bleach.
      ii. Treat all samples and controls as infectious agents.
      iii. Comply with Laboratory Safety Policy ARL201
      iv. Dispose off laboratory waste in line with SOP ARL213

   b. **Reagents**
      i. HDLC and LDLC Cassette
      ii. Calibrators: C.f.a.s. lipids
      iii. Manufacturers recommended two levels of controls
      iv. Normal saline

   c. **Reagent stability**
      i. Unopened kit components are stable up to expiration date at $2\text{-}8^0$ C.
      ii. On board, reagents are stable for 12 weeks.

   d. **Equipment/Materials Required**
      i. COBAS INTEGRA 400 PLUS Chemistry Analyzer
      ii. Computerized Workstation
      iii. Printer
      iv. Centrifuge for separating blood samples
      v. Pipettors
      vi. Cobas cups
      vii. Centrifuge.

e. Specimen Collection And Preparation

i. Serum is the specimen of choice and is collected from clotted blood using standard sampling tubes or plasma in EDTA/ Li-Heparin as described in the SOP for Venepuncture ARL702.as described in the SOP for V*enepuncture ARL702*.
ii. Serum/Plasma may be stored for 7 day at 20-25ºC and up to 7 days at 4-8 ºC when separated from erythrocytes and stored tightly stoppered.
iii. Serum/plasma may be stored up to 4months at -20ºC
iv. Centrifuge samples containing precipitates before performing the assay.

f. Calibration.

i. Calibrate the machine, when reagent lot is changed or when internal quality controls give repeatedly out-of-range values.
ii. Each new lot number of calibrators needs to be registered on the analyser, either by barcode reader or manually.
iii. Check that the values of the calibrators are adjusted in line with the new lot number.
iv. Verify the calibration of a new calibrator lot by running calibrators as samples.

g. Quality Control

i. Manufacturers recommended two levels of controls and other suitable control are run once daily for the internal quality control.
ii. The EQA samples are run as scheduled
iii. In case of QC failure investigate and document a corrective action

h. Sample Testing

i. Samples are verified with the work list for its completeness and quality
ii. Accept samples in accordance with ARL828. sop for Laboratory Information System
iii. Proceed and run the samples as described in the SOP for the COBAS INTEGRA 400 PLUS Chemistry Analyser ARL806 and in the SOP for Laboratory Information system ARL 828.

i. Specimen Rejection

i. Refer to ARL 206 for sample rejection and acceptance criteria
ii. Whole Blood more than 24 hours old, hemolysed or lipaemic has to be rejected and redraw requested

j. Results

i. The machine automatically calculates the analyte activity in each sample. and the results are transmitted to the Laboratory Information System
ii. Refer to SOP ARL 828 for completion and authentication
iii. Interpretation of results is based on comparison with the normal ranges provided.
iv. Critical values are telephoned to the clinician as soon as possible. Log all phone calls reporting critical values.

k. Repeat Criteria

i. High values above AMR (analytical measurable range): Dilute sample and multiply result with the dilution factor
ii. Low values below AMR: If low on repeat, report result as "Below lowest detectable limit"
iii. Critical values: Re-run to confirm
iv. Flags for blockages, slope, or calibration: Confirm quality of sample and reagents and re-run the sample

l. Limitation

i. Hemolysis; No significant interference upto a hemoglobin level of 0.62mmol/l(10g/l).
ii. Icterus; No significant interference up to bilirubin level of 684umol/l (40mg/dl).
iii. Lipemia; No siqnificant interference upto an intralipid level of 400mg/dl.
iv. No significant interference from triglycerides up to 1200mg/dl. There is poor correlation between turbidity and triglycerides concentration.
v. Others;In rare cases, elevated immunoglobulin concentration
    1. Can lead to falsely increased LDL-cholesterol results.
    2. In very rare cases gammopathy may cause unreliable results.

m. Measuring Range

i. Refer to Analytical Measurement Range (AMR) verified summary report.

n. Reference Values

i. Refer to verified Reference ranges summary report.

8. Reference

Roche Cobas Integra 400 Operators Manual, Indianapolis 2004
Roche Cobas Integra 400 Test Manual, Indianapolis 2004
Roche Cobas Benchtop Guide

Appendix 11: IREC Approval

INSTITUTIONAL RESEARCH AND ETHICS COMMITTEE (IREC)

MOI TEACHING AND REFERRAL HOSPITAL
P.O. BOX 3
ELDORET
Tel: 33471/2/3

MOI UNIVERSITY
COLLEGE OF HEALTH SCIENCES
P.O. BOX 4606
ELDORET

Reference: IREC/2017/158
Approval Number: 0001981

9th November, 2017

Dr. Grace Wandia,
Moi University
School of Medicine,
P.O. Box 4606,
ELDORET-KENYA.

INSTITUTIONAL RESEARCH & ETHICS COMMITTEE
09 NOV 2017
APPROVED
P. O. Box 4606 - 30100 ELDORET

Dear Dr. Wandia,

RE: FORMAL APPROVAL

The Institutional Research and Ethics Committee has reviewed your research proposal titled:-

"Thyroid Dysfunction in Type 2 Diabetes Patients in Moi Teaching and Referral Hospital Eldoret, Kenya."

Your proposal has been granted a Formal Approval Number: ***FAN: IREC 1981*** on 9th November, 2017. You are therefore permitted to begin your investigations.

Note that this approval is for 1 year; it will thus expire on 8th November, 2018. If it is necessary to continue with this research beyond the expiry date, a request for continuation should be made in writing to IREC Secretariat two months prior to the expiry date.

You are required to submit progress report(s) regularly as dictated by your proposal. Furthermore, you must notify the Committee of any proposal change (s) or amendment (s), serious or unexpected outcomes related to the conduct of the study, or study termination for any reason. The Committee expects to receive a final report at the end of the study.

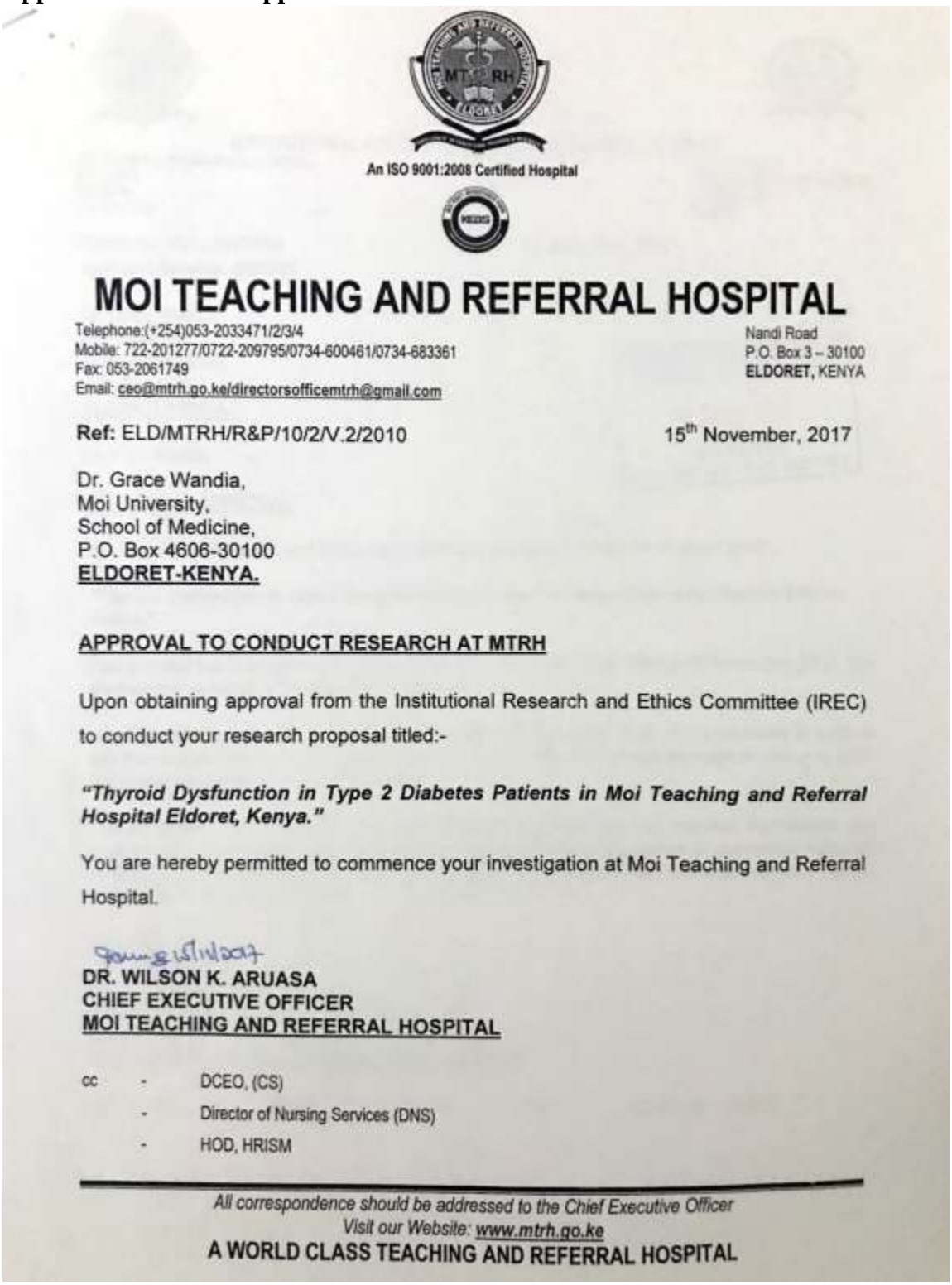
Sincerely,

For

PROF.E.O.WERE
CHAIRMAN
INSTITUTIONAL RESEARCH AND ETHICS COMMITTEE

cc CEO - MTRH Principal - CHS Chairman - COBES

Appendix 12:MTRH Approval



An ISO 9001:2008 Certified Hospital

MOI TEACHING AND REFERRAL HOSPITAL

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Nandi Road
P.O. Box 3 – 30100
ELDORET, KENYA

Ref: ELD/MTRH/R&P/10/2/V.2/2010

15th November, 2017

Dr. Grace Wandia,
Moi University,
School of Medicine,
P.O. Box 4606-30100
ELDORET-KENYA.

APPROVAL TO CONDUCT RESEARCH AT MTRH

Upon obtaining approval from the Institutional Research and Ethics Committee (IREC) to conduct your research proposal titled:-

"Thyroid Dysfunction in Type 2 Diabetes Patients in Moi Teaching and Referral Hospital Eldoret, Kenya."

You are hereby permitted to commence your investigation at Moi Teaching and Referral Hospital.

15/11/2017

DR. WILSON K. ARUASA
CHIEF EXECUTIVE OFFICER
MOI TEACHING AND REFERRAL HOSPITAL

cc - DCEO, (CS)
- Director of Nursing Services (DNS)
- HOD, HRISM

All correspondence should be addressed to the Chief Executive Officer
Visit our Website: www.mtrh.go.ke
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