EVALUATION OF SERUM THYROID HORMONES AND LIPID LEVELS IN HIV-INFECTED PATIENTS ON HIGHLY ACTIVE ANTIRETROVIRAL THERAPY AT THE JARAMOGI OGINGA ODINGA TEACHING AND REFERRAL HOSPITAL IN KISUMU COUNTY, WESTERN KENYA

BY

JUMA OMONDI ALFRED

A THESIS SUBMITTED IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE DEGREE OF MASTER OF SCIENCE IN MEDICAL PHYSIOLOGY

SCHOOL OF MEDICINE

MASENO UNIVERSITY

March 2021

DECLARATION

I declare that this thesis is my original work and has never been presented to any other University or institution for a degree or any other award.

Juma Omondi Alfred

MSC/SM/00011/2016

Department of Medical Physiology, Maseno University, Kenya

Signature ….…………………… Date: ……………….

This thesis has been submitted for examination with our approval as supervisors:

Prof. Gideon Ng'wena Magak, PhD

Department of Medical Physiology,

Maseno University, Kenya

Signature …………………………... Date ………………………

Dr. Michael Owiti George

Department of Medical Physiology,

Maseno University, Kenya

Signature………………………………… Date ……………………

ACKNOWLEDGEMENT

First, I wish to thank the Almighty God for the good health and strength to pursue this Master's degree in Medical Physiology. I am deeply indebted to Prof. Ng'wena Magak who has been a pillar in my academic life especially in Medical Physiology since the undergraduate studies. He has literally nurtured and mentored me to take up Medical Physiology as an area of specialty. His scholarly input and contributions in the research world has been a cornerstone for formulation of this research. His technical guidance has been an eye opener in literally every step I throughout the study period. I am fully indebted to Dr. George Owiti for his invaluable contributions particularly on the clinical aspects of the study. His positive contribution unlocked many hidden vital concepts that were valuable for this study. He brought to the fore the current clinical practices and updates pertaining to management of HIV infected patients. His guidance on ethical requirements of this work was outstanding. I cheerfully thank my all-time academic friend Dr. Elly Munde for always sparing his time to read and critique this work whenever I called upon him. His technical guidance on and the referencing of the literatures cited and analysis of the data cannot be gainsaid. He was a source of motivation to have this work completed within the strict deadlines. Special acknowledgement goes to Mathew Siguda, the laboratory technologist who was extremely instrumental during blood sample collection and running of the assays. His insights on quality control during the assays contributed immensely in generation of reliable data. I deeply appreciate the overwhelming support from the Health Department of County Government of Kisumu headed by Dr. Dickens Onyango and the staff of Jaramogi Oginga Odinga, teaching and Referral Hospital where this study was premised.

Finally, I acknowledge the overwhelming support and motivation from my lovely wife Courine Opot who is the constant source of motivation towards completion of this study. She was the special force and pivot around which my inspiration revolved.

DEDICATION

This work is dedicated to my loving mom, Margaret Awino Juma. My mom has been the oasis of peace and hope when things got extremely difficult during my entire academic life. Her charming smiles and humor made it possible for me to defy all the odds for this milestone achievement. Her power of prayers through Rosary was a constant source of renewed hope and energy to complete this work.

The previous scholarly work of Prof. Makawiti (deceased) was inspirational during the conceptualization stage of this work. His earlier contribution in the scholarly work that attempted to determine the effects on HAART on thyroid hormones was the cornerstone for the current study.

ABSTRACT

While Highly Active Antiretroviral Therapy (HAART) has significantly reduced HIV-associated mortalities, they are not devoid of adverse effects including endocrine and metabolic disorders like thyroid dysfunctions and dyslipidemias. However, Alterations in serum thyroid hormones and lipid levels among HAART users in Sub-Saharan population like Kenya is yet to be elucidated. This study determined the serum lipid levels; Total cholesterol (TC), Triglycerides (TG), High Density Lipoproteins (HDL), Low Density Lipoproteins (LDL) and the serum Thyroid hormones levels; Thyroid Stimulating Hormone (TSH), Thyroxine (T4) and Triiodothyronine (T3) levels among the HAART-treated and HAART-naïve subjects. Using cross study design, patients (n=104) attending Jaramogi Oginga Teaching and Referral Hospital (JOOTRH) in Kisumu County, Western Kenya were recruited. The study participants were categorized into two groups; HAART-treated (84) and HAART-nave (20). Demographic, medical history and anthropometric data were collected after which fasting state blood was collected and centrifuged to obtain serum. Colorimetric method was used to determine the serum TC, TG, HDL, and LDL levels while immunofluorescence method was used to determine the serum TSH, T4 and T3 levels. The difference in serum lipid and thyroid hormones levels between HAART-treated and HAART-naïve groups were determined using Mann Whitney U test while Spearman correlation test was used to determine the association between serum lipids and serum thyroid hormones levels. Although the thyroid hormones were found to be within normal ranges in both groups, TSH levels were significantly higher among the HAART-treated patients than in HAART-naïve counterparts, [median (IQR) 1.72 (1.71) nmo/L vs median (IQR), 0.87 (1.07) nmo/L, P=0.001]. However, the serum T4 and T3 levels were similar in both the HAART-treated and HAART-naïve patients, [median (IQR) 144.39 (67.53.64) nmo/L vs median (IQR), 136.99 (134.22) nmo/L, P=0.426] and [median (IQR) 2.43 (2.73) nmo/L vs median (IQR) 3.10 (2.62) nmo/L, (P=0.147) respectively. The serum lipids levels, were all within normal ranges, and did not differ significantly between the HAART-treated patients and the HAART-naïve counterparts; TC, [median (IQR) 186.00 (91.50) mg/dl vs median (IQR) 149.50 (70.30) mg/dl, (P=0.092)]; HDL, [median (IQR) 52.50 (24.00) mg/dl vs median (IQR) 44.00 (34.00) mg/dl, (P=0.280)], LDL, [138.00 (86.30) vs 117.00(46.50), (P=0.476)] as well as TG [median (IQR) 82.00 (62.80) mg/dl vs median (IQR) 94.50 (55.80) mg/dl, (P=0.504)]. While a positive association was found between T4 and LDL levels, (ρ =0.240, P=0.014), no association was established between serum TSH as well as T3 levels and serum lipid profiles. This study shows that there is varying serum TSH levels HAART-treated and HAART-naive patients and that serum LDL levels increases with increase in serum T4 levels. Routine monitoring of serum lipids and thyroid hormones levels for the HIV-infected patients particularly those on HAART treatment is therefore recommended.

TABLE OF CONTENTS

DECLARATION ii
ACKNOWLEDGEMENT iii
DEDICATION iv
ABSTRACT v
LIST OF TABLES x
1.0 CHAPTER ONE: INTRODUCTION 1
1.1. Background of the study 1
1.2 Statement of the problem 3
1.3 Objectives 4
1.3.1 General objective 4
1.3.2 Specific objectives 4
1.4 Null Hypothesis 4
1.5 Significance of the study 5
2.0 CHAPTER TWO: LITERATURE REVIEW 6
2.1 The Hypothalamic-Pituitary-Thyroid Axis 6
2. 2 Role of Thyroid hormones in regulation of lipid metabolism 7
2. 3 Effects of Thyroid disorders on lipid metabolism 9
2.4 HIV infection and HIV treatment among HIV infected patients 10
2.5 Thyroid function among HIV-infected patients 11
2.5.1 Thyroid function among HAART-naïve patients 11
2.5.2 Thyroid function in HAART-treated patients 12
2.6 Lipids profile among HIV infected patients 13
2.6.1 Lipids profile among HAART-naïve patients 13
2.6.2 Lipids profile among the HAART-treated patients 14
2.7 Association between Thyroid function and Lipids profile among HIV infected patients 15
3.0 CHAPTER THREE: METHODS AND MATERIALS 17
3.1. Study design 17
3.2 Study site 17
3.3 Study population 17
3.4 Inclusion criteria 18

3.5 Exclusion criteria 18
3.6 Sample size determination 18
3.7 Sampling techniques and study procedure 19
3.8 Collection of Demographic, Medical and Anthropometric data 20
3.9 Collection and processing of blood samples 21
3.10 Determination of serum lipids levels 22
3.11 Determination of serum Thyroid Function Hormone levels 22
3.12 Data analysis. 23
3.13 Ethical Considerations 23
4.0 CHAPTER FOUR: RESULTS 27
4.1 General Demographics, Medical and Anthropometric characteristics of HAART-treated and HAART-naïve HIV patients 27
4.2 Serum thyroid hormones levels in HAART-treated and HAART-naïve HIV patients 30
4.3 Serum lipid levels of the HAART-treated and HAART-naïve HIV patients 31
4.4 Association between the serum Thyroid hormone levels and serum lipid levels among the HAART-treated and HAART-naïve patients 32
5.0 CHAPTER FIVE: DISCUSSION 35
5.1 Introduction 35
5.2 Serum Thyroid hormone levels among the HAART-treated and HAART-naïve patients 35
5.3 Serum lipids levels among the HAART-treated and HAART-naïve HIV patients. 37
5.4 Association between the serum Thyroid hormones and lipid levels among the HAART-treated and HAART-naïve patients 40
6.0 CHAPTER SIX: SUMMARY OF FINDINGS, CONCLUSION AND RECOMMENDATIONS 43
6.1 Summary of findings 43
6.2 Conclusions 43
6.3 Recommendations from the current study 43
6.4 Recommendations for future studies 44
REFERENCES 45
APPENDICES 52
Appendix 1: Consent Form 52
Appendix 2: Questionnaire for data collection 55
Appendix 3: ichroma TSH protocol 57

Appendix 4: ichroma T3 60
Appendix 5: ichoma T4 protocol 64
Appendix 6: Ethical Approval 67

LIST OF FIGURES

Figure 2.1: Hypothalamic-Pituitary-Thyroid axis.. 7

LIST OF TABLES

Table 4. 1: General Demographic, Medical and Anthropometric data of the HAART-treated and HAART-naive HIV patients 29

Table 4.2: Level of Thyroid function hormones among the HAART-treated and HAART-naïve participants 31

Table 4.3: Level of Serum lipid profile among the HAART-treated and HAART-naïve participants 32

Table 4.4: Spearman correlations between lipid profiles and thyroid hormones levels among HAART-treated and HAART-naïve subjects 34

LIST OF ABBREVIATIONS AND ACRRONYMS

3TC: Lamivudine

ABC: Abacavir

AIDS: Acquired Immunodeficiency Syndrome

ApoAV: Apolipoprotein AV

ATP: Adenosine Triphosphate

AZT: Zidovudine

BMI: Body Mass Index

cAMP: Cyclic Adenosine Monophosphate

CCC: Comprehensive Care Centre

CCR5: Chemokine receptor 5

CD4: Cluster of Differentiation

CETP: Cholesteryl ester transfer protein

CVD: Cardiovascular diseases

CYP3A4: Cytochrome P450 3A4

d4T: Stavudine

DNA: Deoxyribonucleic acid

DTG:	Dolutegravir
FFAs:	Free fatty acids
fT3:	free T3
fT4:	free T4
FTC:	Emtricitabine
HAART:	Highly Active Antiretroviral Therapy
HDL:	High-density lipoprotein
HDLC:	High Density Lipoprotein Cholesterol
HIV-1:	Human Immunodeficiency Virus -1
HL:	Hepatic lipase
HMG-CoA:	Hydroxy-3-methyl-glutaryl-CoA reductase
IHD:	Ischemic heart disease
INSTIs:	Integrase strand transfer inhibitors
JOOTRH:	Jaramogi Oginga Teaching and Referral Hospital
KNH:	Kenyatta National Hospital
LDL:	Low-density lipoprotein
LDLR:	low density lipoprotein receptor

LPL: Lipoprotein lipase

LPV/r: Lopinavir/Ritonavir

LRP1: LDL-receptor-related protein type 1

MCT8 Monocarboxylate transporter 8

MI: Myocardial infarction

MUERC: Maseno University Ethics Review Committee

NNRTIs: Non-Nucleoside reverse transcriptase inhibitors

NRTIs: Nucleoside reverse transcriptase inhibitors

NTI: Non-thyroidal illness

NVP: Nevirapine

PI: Protease Inhibitor

PLHIV: People Living with HIV

rT3: reverse triiodothyronine

RXR-PPARγ: Retinoid X receptor-peroxisome proliferator-activated receptor γ

SCH: Subclinical hypothyroidism

SPSS: Statistical Package for Social Sciences

SREBP-2: Sterol regulatory element-binding protein-2

T3:	Triiodothyronine
T4:	Thyroxine
TBG:	Thyroid binding globulin
TC:	Total cholesterol
TDF:	Tenofovir disoproxil fumarate
TFT:	Thyroid Function Test
TG:	Triglycerides
TH:	Thyroid Hormone
TNF:	Tumor Necrosis Factor
TRH:	Thyroid Releasing Hormone
TSH:	Thyroid Stimulating Hormone
VDLD	Very low density lipoprotein
VLDL:	Very low density lipoprotein
WHO:	World Health Organization

1.0 CHAPTER ONE: INTRODUCTION

1.1. Background of the study

The rise in global HIV-related morbidity and mortality prompted the discovery and introduction of HAART (Arts & Hazuda, 2012), whose use has exponentially grown particularly among the Sub-Saharan population (Crunkhorn & Patti, 2008). Despite the dramatic reduction in immunodeficiency conditions and related deaths, the use of HAART has been associated with significant rise in metabolic complications alongside other disorders (Crunkhorn & Patti, 2008; Glesby, 2016). The most prominent metabolic complications among HIV-infected patients on HAART include dyslipidemias and thyroid dysfunctions (Glesby, 2016). These complications have been implicated for increased propensity for cardiovascular Disease (CVD) which is a top global killer disease (Nsagha *et al.*, 2015)

Use of HAART has been associated with thyroid dysfunction (Abbiyesuku *et al.*, 2014). The commonest form of thyroid dysfunction reported among HAART users is Sub-Clinical hypothyroidism (SCH) (Jain *et al.*, 2009). SCH, is characterized by isolated elevated thyroid-stimulating hormone (TSH) levels, and isolated low free thyroxine (T4) levels (Abelleira *et al.*, 2014). It has also been previously reported that low levels of Thyroid Stimulating Hormone (TSH) levels occur among HAART users (Abbiyesuku *et al.*, 2014; Beltran *et al.*, 2006; Thongam *et al.*, 2015). In clinical practice, determination of serum TSH levels is critical for suspected cases of hypothyroidism or hyperthyroidism (Eghtedari & Correa, 2019; Hoffmann & Brown, 2007).

Additionally, reduced levels of serum Triiodothyronine (T3) levels has been reported among the HAART-treated and HAART-naïve patients(Idowu & Adesegun, 2019; Ji & Jin, 2016). Similarly, decline in Free T4 hormone levels has also been reported among HAART users

(Thaimuta *et al.*, 2010). It is thought that reduced Thyroid hormone levels among HAART users are thought to be an adaptive response to spare calories and protein by inducing hypothyroidism (De Groot, 1999; Jiskra *et al.*, 2007).

There is evidence from studies conducted among populations in developed countries suggesting possible alterations in thyroid function occasioned by use of HAART (Abelleira *et al.*, 2014; Verma *et al.*, 2017). However, there is paucity of data on serum thyroid hormone levels among patients on HAART in Kisumu County which is one of regions leading in HIV prevalence in Kenya (Harklerode *et al.*, 2020). To bridge this gap, the current study determined the serum TSH, T3 and T4 among the HAART-treated and HAART-naïve patients attending Comprehensive Care Centre (CCC) Jaramogi Oginga Odinga Teaching and Referral Hospital (JOOTRH), in Kisumu County, Western Kenya.

Dyslipidemia has been reported among HIV-infected patients including those enrolled on (Nsagha *et al.*, 2015) HAART-associated dyslipidemia is characterized by increased serum concentrations of TC, TG, LDL and low levels of HDL (Quirino *et al.*, 2004; Toft & Beckett, 2003). HAART treatment is thought to impair hydrolysis of triglyceride-rich lipoproteins by plasma and tissue lipases, disrupting normal post-prandial free fatty acid and lipoprotein catabolism (Thongam *et al.*, 2015).

Even though enrolment of HIV-infected patients on HAART has continued to grow exponentially in the Sub Saharan population (Wamalwa *et al.*, 2007), the extent to which the use of HAART affects lipid metabolism is yet to be clearly elucidated among HIV-infected patients enrolled on HAART in Kenya. In an attempt to understand how the serum lipid levels are affected in HIV-infected patients using HAART, the current study determined the serum Total Cholesterol (TC), Triglycerides (TG), High Density Lipoproteins (LDL) and Low Density Lipoproteins (LDL) levels among the HAART-treated patients and compared with that among

the HAART-naïve counterparts seeking CCC services at JOOTRH in Kisumu County, Western Kenya.

Thyroid hormones like TSH have been reported to regulate lipid metabolism (Berti *et al.*, 2001; Duntas, 2002; Gagnon *et al.*, 2010; Jiskra *et al.*, 2007). Clear understanding of the relationship between thyroid function and lipid levels among the HIV-infected patients on HAART would be critical step towards improving clinical outcomes. Studies conducted in developed countries have reported findings that suggest that there could be association between thyroid hormones and lipid profiles (Ahi *et al.*, 2017; Asvold *et al.*, 2007; Bongiovanni *et al.*, 2006; Chin *et al.*, 2014; Jung *et al.*, 2017; Santos-Palacios *et al.*, 2013). However, similar studies conducted in the Sub Saharan population did not concomitantly determine the thyroid hormones and lipid levels among HIV-infected patients on HAART hence missing the opportunity to explore the possible influence thyroid hormone functions on lipid metabolism (Duntas & Brenta, 2012; Thongam *et al.*, 2015). The current study therefore, determined the association between the serum TSH, T4 and T3 levels and serum TC, TG, HDL and LDL levels among HAART-treated and HAART-naïve patients at JOOTRH, Western Kenya.

1.2 Statement of the problem

Even though HAART has significantly reduced AIDS related morbidity and mortality among HIV infected patients, various metabolic complications have increased among HAART users. Dyslipidaemias and endocrine complications including thyroid dysfunction have risen among the Sub Saharan population due to exponential increase in HAART enrolment. These complications are known predisposing factors for Cardiovascular Diseases (CVD) which are the leading cause of deaths globally. Despite the high prevalence of HIV in Kenya and enhanced enrolment on HAART, there paucity of data on state of thyroid function as well as lipid profiles among the HAART users. Elucidation of the serum thyroid hormones and lipid levels among HIV-infected

patients on HAART in Kisumu County, Western Kenya is therefore essential in achieving desired clinical outcomes in HIV management.

1.3 Objectives

1.3.1 General objective

To evaluate the serum Thyroid hormones and lipid levels among the HIV-infected patients on HAART at Jaramogi Oginga Odinga Teaching and Referral Hospital in Kisumu County, Western Kenya.

1.3.2 Specific objectives

The specific objectives for this study included:

i) To determine the serum levels of Thyroid Stimulating Hormone, Triiodothyronine and Thyroxine among HAART-treated and HAART-naïve patients at JOOTRH in Kisumu County, Western Kenya.

ii) To compare the serum Triglycerides, total cholesterol, High Density Lipoproteins, and Low Density Lipoproteins levels between the HAART-treated and HAART-naïve patients at JOOTRH in Kisumu County, Western Kenya.

iii) To determine the association between the serum Thyroid Stimulating Hormone, Triiodothyronine and Thyroxine and Triglycerides, total cholesterol, High Density Lipoproteins, and Low Density Lipoproteins among the HAART-treated and HAART-naïve patients at JOOTRH in Kisumu County, Western Kenya.

1.4 Null Hypothesis

i) The serum Thyroid Stimulating Hormone, Triiodothyronine and Thyroxine levels among HAART-treated patients is lower than in HAART-naïve patients at JOOTRH in Kisumu County, Western Kenya.

ii) The serum Triglycerides, Total cholesterol, High Density Lipoproteins, and Low Density Lipoproteins levels is lower among the HAART-treated patients than in HAART-naïve patients at JOOTRH in Kisumu County, Western Kenya.

iii) There is no association between the serum Thyroid Stimulating Hormone, Triiodothyronine and Thyroxine levels and serum Triglycerides, total cholesterol, High Density Lipoproteins, and Low Density Lipoproteins levels among the HAART-treated and HAART-naïve patients at JOOTRH in Kisumu County, Western Kenya.

1.5 Significance of the study

Enrolment of HIV-infected patients on HAART without routine assessment of thyroid hormones may result in various fatal metabolic disorders given that thyroid dysfunction affects almost all major metabolic pathways. Evaluation of the serum thyroid hormones levels study is important in assessing the possibility occurrence of thyroid dysfunction which is a fatal disorder among HIV-infected patients on HAART.

Atherogenic lipids associated with HAART use is a potential risk factor for development of cardiovascular diseases which are top killers globally. Continued use of HAART without proper assessment of serum lipid levels among the HAART-users may result in poor clinical outcomes due to derangement of serum lipid levels. Evaluation of serum TC, TG, LDL and HDL levels and determination of association of the lipid levels with thyroid levels is critical in ensuring safety of HIV-infected patients while on HAART.

2.0 CHAPTER TWO: LITERATURE REVIEW

2.1 The Hypothalamic-Pituitary-Thyroid Axis

Production and release of the thyroid hormones is under the regulation of the hypothalamic-pituitary-thyroid axis (Ortiga-Carvalho *et al.*, 2016) as shown in Figure 2.1. Thyroid Releasing Hormone (TRH), secreted from the hypothalamus, acts on the anterior pituitary gland by binding to G protein-coupled TRH receptors on the thyrotrope, resulting in an increase in intracellular cAMP, and subsequent thyrotropin (TSH) release (Penzak & Chuck, 2000). TSH binds to a G protein-coupled TSH receptor on the thyroid follicular cell thereby stimulating the production and release of thyroid hormones ((Tuncel, 2017)).

The thyroid gland releases two important hormones called thyroxine (T4) and triiodothyronine (T3) that exert their physiological effects through the nuclear receptors (Chung, 2014; Ketsamathi *et al.*, 2006). Local conversion of T_4 to T_3 provides negative feedback at the level of both thyrotrophs in the anterior pituitary gland and tanycytes in the hypothalamus (Ortiga-Carvalho *et al.*, 2016) . Tight regulation of this feedback loop is the reason for using a serum TSH measurement for the diagnosis and management of primary thyroid disease, since small changes in serum T_4 are amplified by changes in serum TSH (Calsolaro *et al.*, 2019; Eghtedari & Correa, 2019). In primary disease, the thyroid gland itself is affected resulting in either overproduction or underproduction of T3 and T4 due to inappropriate secretion of TSH (Calsolaro *et al.*, 2019; Jannin *et al.*, 2019).

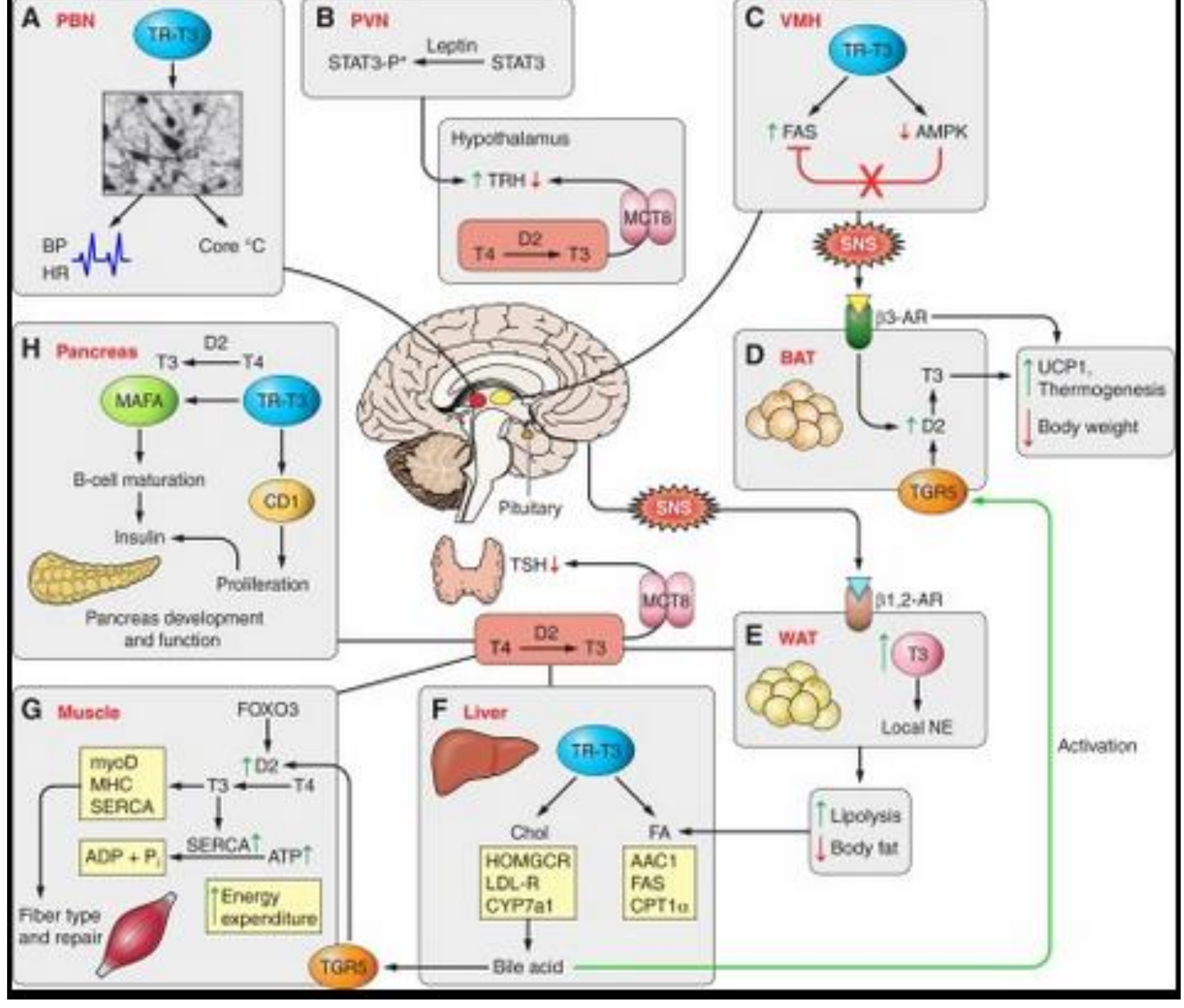


Figure 2.1: Hypothalamic-Pituitary-Thyroid axis.

Adopted with permission from (Mullur *et al.*, 2014)

2. 2 Role of Thyroid hormones in regulation of lipid metabolism

There are four basic parameters that define lipid profile namely Total Cholesterol (TC), Triglycerides (TG), Low Density Lipoproteins (LDL) and High Density Liproteins (HDL) (Enas *et al.*, 2007; Nery *et al.*, 2011). Abnormal lipid levels is known as dyslipidemia (Calsolaro *et al.*, 2019), which is a risk factor for Cardiovascular disease. Dyslipidemia is defined by elevated high either TC, TG, LDL or low level of HDL (Franssen *et al.*, 2011). Dyslipidemia is characterized by TC $\geq$ 220 mg/dL, LDL $\geq$ 150 mg/dL, TG > 200 mg/dl and or HDL < 40 mg/dL (Nery *et al.*,

2011). Thyroid hormones have influence on all major metabolic pathways including lipid metabolism (Pucci *et al.*, 2000).

Literature reports that thyroid hormones influence lipid metabolism during synthesis, mobilization and degradation stages with degradation influenced more than synthesis. Under the influence of thyroid hormones, lipid metabolism is enhanced through utilization of lipid substrates, increased synthesis and mobilization of triglycerides stored in adipose tissue, increased concentration of non-esterified fatty acids (NEFA) and increase in lipoprotein-lipase activity (Pucci *et al.*, 2000). According to Rizos et al., thyroid hormones induce 3-hydroxy-3-methylglutarylcoenzyme A (HMGCoA) reductase, which is the first step in cholesterol biosynthesis. Triiodothyronine (T3) causes an upregulation of LDL receptors, controls the sterol regulatory element-binding protein-2 (SREBP-2), which in turn regulates LDL receptor's gene expression and protects Low Density Lipoproteins (LDL) from oxidation (Rizos *et al.*, 2011).

Thyroid hormones is said to influence High Density Lipoproteins (HDL) metabolism by increasing cholesteryl ester transfer protein (CETP) activity, which exchanges cholesteryl esters from HDL2 to the very low density lipoproteins (VLDL) and Triglycerides (TGs) to the opposite direction (Kaliaperumal *et al.*, 2014). The hormones stimulate lipoprotein lipase (LPL), which catabolizes the TG-rich lipoproteins, and the hepatic lipase (HL), which hydrolyzes HDL2 to HDL3 and contributes to the conversion of intermediate- density lipoproteins (Buchacz *et al.*, 2008), to LDL and in turn LDL to sdLDL.

Another effect of T3 is the up-regulation of apolipoprotein AV (ApoAV), which plays a major role in TG regulation (Asvold *et al.*, 2007). Beyond their effect on lipid profile, thyroid hormones also affect a number of other metabolic parameters related to CVD risk like as the metabolism and production of adipokines, insulin sensitivity, oxidative stress and BMI or waist circumference (Crunkhorn & Patti, 2008).

2. 3 Effects of Thyroid disorders on lipid metabolism

Thyroid diseases are one of the major endocrine disorders worldwide (Taylor *et al.*, 2018). Thyroid disorders that include the overt and subclinical hypothyroidism alter lipid profile and promote cardiovascular disease. Thyroid hormones affect lipoprotein metabolism as well as some CVD risk factors, hence influencing the overall CVD risk (Duntas, 2002; Khan *et al.*, 2020). Evidence shows that high thyroid-stimulating hormone (TSH) is associated with a non-favorable lipid profile, although TSH has no cutoff threshold for its association with lipids (Duntas & Brenta, 2012). It has been observed that Thyroid dysfunction has a great impact on lipids as well as a number of other cardiovascular risk factors (Crunkhorn & Patti, 2008; L. H. Duntas & Brenta, 2018).

Hypothyroidism is relatively common and is associated with an unfavorable effect on lipids (Quirino *et al.*, 2004). A linear positive association has been established between TSH values (within the reference range) and concentrations of total serum cholesterol, LDL cholesterol, non-HDL cholesterol and TG, and a linear negative association with HDL cholesterol (Asvold *et al.*, 2007). Low HDL has been established to be the most frequent abnormality followed by high TG (Bibiloni *et al.*, 2016). A study by Nerry et al (2011) reported that patients on a Protease Inhibitor (PI) regimen were even more prone to dyslipidemia (Nery *et al.*, 2011)

Hypothyroidism therefore accelerates the process of atherogenesis and increases cardiovascular risk (Khan *et al.*, 2020). In manifest hypothyroidism, the number of LDL receptors in the liver decreases and there is an increase in levels of overall cholesterol, LDL-cholesterol and apolipoprotein B in the blood (Jiskra *et al.*, 2007). Levels of HDL particles remain normal or even rise slightly as a result of reduced activity of the Cholesterol ester transfer protein (CETP) and hepatic lipase (Rastgooye Haghi *et al.*, 2017). This leads to a reduction in the transport of cholesterol esters from HDL-(2) to VLDL and LDL. Subclinical hypothyroidism also has a

negative effect on the lipid profile, but is more likely to lead to pro-atherogenic changes in the proportion of lipid particles than to a reduction in overall cholesterol. Subclinical hypothyroidism leads to the manifestation of certain risk factors of atherosclerosis such as high cholesterol, high blood pressure among others (Jiskra *et al.*, 2007).

2.4 HIV infection and HIV treatment among HIV infected patients

There are two main types of Human immunodeficiency Virus (HIV), HIV-1 and HIV-2 (German Advisory Committee Blood, 2016). The HIV-1 is well-known for its extensive genetic diversity with four different lineages including M, N, O, and P. The most commonly reported HIV virus across the globe is group M. HIV-2 was first reported in Africa in 1985 and is significantly different from HIV-1 (Buchacz *et al.*, 2008). HIV-2 is most commonly reported in West Africa, with Guinea-Bissau and Senegal having the highest prevalence (Sousa *et al.*, 2016). Eight different types of HIV-2 exist, labeled HIV-A to HIV-H. Group A is reported throughout the sub-Saharan region (de Silva *et al.*, 2008).

The HIV-1 infection occurs by attacking the CD4 T-lymphocytes (CD4 cells) of the immune system. Once infected, the virus gradually and silently overpowers the host's immune system, resulting in opportunistic infections and cancers. Activated and differentiated CD4 cells have a pivotal role in the activation of cell-mediated and humoral immune systems (de Silva *et al.*, 2008; Jiskra *et al.*, 2007; Rastgooye Haghi *et al.*, 2017) resulting in the depletion of CD4 cells in the peripheral blood (Brenchley *et al.*, 2004). Virtually every endocrine organ is involved in the course of HIV infection (Wong & Yukl, 2016). Endocrine and metabolic disturbances occur in the course of HIV infection. Pathogenesis includes direct infection of endocrine glands by HIV or opportunistic infections, infiltration by neoplasms and side effects of drugs.

The current HIV treatment guidelines recommend using Highly Active Antiretroviral Therapy (HAART) treatment for all patients, irrespective of the CD4 cell count, to prevent or prolong the

progression of disease to AIDS (Crabtree-Ramírez *et al.*, 2016). The First-line ART treatment for adults consists of two NRTIs and one NNRTI. Tenofovir disoproxil fumarate (TDF) + lamivudine (3TC) or emtricitabine (FTC) + efavirenz (EFV) as a fixed dose is the favored choice for this type of ART (Ghate *et al.*, 2013). When this drug combination is contraindicated or is unavailable, zidovudine (AZT) + 3TC + EFV, 2) AZT + 3TC + nevirapine (NVP), or 3) TDF + 3TC (or FTC) + NVP is used.

It is advised that when adults (including pregnant and breastfeeding patients) experience failure on first-line treatment of ART, a second-line ART should be utilized. The second-line ART is comprises primarily of two NRTIs and a ritonavir-boosted PI. The recommended option for second-line ART includes AZT and 3TC as the NRTI. After the failure of AZT or stavudine (d4T) + 3TC-based first-line regimen, TDF + 3TC (or FTC) as the NRTI should be considered. When first-line NNRTI-based treatment fails, two NRTIs + a boosted PI are suggested (Jaoko & Kredo, 2017).

If first- and second-line ART fails, the WHO recommends inclusion of new medicines with the least amount of risk for development of cross-resistance towards previously used drugs e.g. integrase inhibitors and second-generation NNRTIs and PIs (Chimbetete *et al.*, 2018).

2.5 Thyroid function among HIV-infected patients

2.5.1 Thyroid function among HAART-naïve patients

There are contradicting reports about thyroid function among the HIV-1 infected patients (Dobs *et al.*, 1988; Ghate *et al.*, 2013; Low *et al.*, 2018). Whereas abnormal Thyroid Function Tests (TFTs) have often been reported in HIV positive individuals (Low *et al.*, 2018; Nelson *et al.*, 2009), other studies have maintained that during HIV-1 infection, overt clinical or biochemical thyroid dysfunctions are rare (Lambert, 1994; Zanoni *et al.*, 2012). Whether thyroid screening should be compulsory for HIV-1 infected individuals therefore remains an area of controversy.

Some studies have reported that thyroid dysfunction is a common endocrinopathy among the HIV infected patients. Changes in pituitary-thyroid function occur in patients with virtually all illnesses and those undergoing major surgical procedures (Roos *et al.*, 2007).

Patients infected with HIV-1 have a higher prevalence of thyroid dysfunction when compared with the general population (Abelleira *et al.*, 2014). The most common change is a decrease in extra-thyroidal conversion of T4 to T3, the active form of thyroid hormone (Moura & Zantut-Wittmann, 2016), a reaction which is responsible for the production of 75 to 80 percent of the circulating T4 in normal patients. In disease, the production and serum concentrations of T3 reduces as a result either of decreased delivery of T4 to the widely distributed intracellular deiodinases that catalyze the conversion or of decreases in the activity of the enzymes (Pasupathi *et al.*, 2010).

2.5.2 Thyroid function in HAART-treated patients

While some studies have established that thyroid dysfunction is more common among HAART-treated patients(Abbiyesuku *et al.*, 2014; Calza *et al.*, 2002), some have reported similar prevalence among the HAART-treated and HAART-naïve patients (S. Thongam *et al.*, 2015). The most commonly observed manifestations of thyroid dysfunction in HIV infection is euthyroidism (Garduno-Garcia Jde *et al.*, 2010). Subclinical hypothyroidism (SCH), characterized by isolated elevated thyroid-stimulating hormone (TSH) levels, and isolated low free thyroxine (T4) levels) is increased while in Graves' disease, by low TSH and elevated T4 levels, may occur during immune reconstitution (Abelleira *et al.*, 2014).

Among the Indian population, it was determined that Subclinical hypothyroidism and overt hypothyroidism are the commonest thyroid abnormalities seen especially among the females. The study revealed that thyroid abnormalities vary with the type and duration of HAART regimen (Verma *et al.*, 2017). Contrary to the Indian study, a study conducted in France found

that there was high prevalence of subclinical hypothyroidism among HIV-infected men than among HIV-infected women (Beltran *et al.*, 2006), as supported by findings from Agentina (Abelleira *et al.*, 2014). A previous study conducted in Kenya, at Kenyatta National Hospital and Mbangathi Hospital established that progressive use of HAART caused decline in Free T4 hormone levels and that the TSH levels were not altered with HAART treatment (Thaimuta *et al.*, 2010). It is speculated that low thyroid hormone levels is an adaptive response by the body to spare calories and protein by inducing hypothyroidism (De Groot, 1999; Jiskra *et al.*, 2007).

Despite the efforts to elucidate the effects of HAART on thyroid function among the HAART users in developed regions, it remains to be established whether the use of HAART causes decrease or increase in thyroid hormones levels in the Kenyan population where enrollment on HAART has grown exponentially in the recent past. The current study determined the serum TSH, T3 and T4 among the HAART-treated and HAART-naïve patients at JOOTRH in Kisumu County, Western Kenya.

2.6 Lipids profile among HIV infected patients

2.6.1 Lipids profile among HAART-naïve patients

The level of lipids among HAART-naïve patients is not clear just like among the HAART-treated counterparts (da Cunha *et al.*, 2015). Some authors have argued that dyslipidemia was discovered in HIV-infected patients even before introduction of HAART characterized by elevated plasma triglyceride and free fatty acid levels whereas HIV-infected patients without AIDS had decreased total cholesterol and HDL-cholesterol (Grunfeld, 2010). In agreement with that finding, a study by Abelleira et al (2014) reported that HIV virus associated dyslipidemia is characterized by decreased TC, LDL, and HDL cholesterol (Abelleira *et al.*, 2014), and later elevated plasma triglyceride (Nelson *et al.*, 2009).

Similarly, hyperalphalipoproteinemia among the HAART-naïve patients have been associated with the HIV virus itself (Rose *et al.*, 2006). Elevated levels of lipids has been reported among the HAART-naïve patients (Adewole *et al.*, 2010), observations also made by other studies (Onyedum *et al.*, 2014). The difference in lipids profile has been noted between the HAART-naïve patients and HIV negative patients (Baza Caraciolo *et al.*, 2007; Denue *et al.*, 2013). However, following HIV sero-conversion, a study established that there was a decline in total cholesterol, HDL-cholesterol, and LDL-cholesterol levels (Padmapriyadarsini *et al.*, 2017; Riddler *et al.*, 2003).

2.6.2 Lipids profile among the HAART-treated patients

There is paucity of data on the relative risk of dyslipidemia among the patients on HAART despite fears that it increases the risk of cardiovascular Disease (CVD) among the users. Use of HAART has been associated with atherogenic lipid profile among the Cameroonian population (Nsagha *et al.*, 2015). Literature has revealed that HAART-associated dyslipidemia is characterized by increased serum concentrations TC, TG, LDL and low levels of HDL, changes that occur within three months of HAART initiation and peaks after six to nine months (Quirino *et al.*, 2004; Toft & Beckett, 2003). It is also thought that HAART treatment impairs hydrolysis of triglyceride-rich lipoproteins by plasma and tissue lipases and disrupts normal post-prandial free fatty acid and lipoprotein catabolism (da Cunha *et al.*, 2015; Thongam *et al.*, 2015).

The World Health Organization (WHO) recommends for selection of lipid-friendly ART for patients in Sub Saharan Africa due to widespread reports of increased dyslipidemias among the HAART users, further justifying the need for routine monitoring of lipids among HIV-infected patients on first line HAART (Ombeni & Kamuhabwa, 2016). The Protease inhibitor (PI)-based regimens particularly, have been associated with dyslipidemia which is a common metabolic complication among HAART-treated patients (Penzak & Chuck, 2000).

Despite the efforts to elucidate the effects of HAART on thyroid function among the HAART users in developed regions, it remains to be established whether the use of HAART causes decrease or increase in thyroid hormones levels in the Kenyan population where enrollment on HAART has grown exponentially. The current study therefore determined the serum TSH, T3 and T4 among the HAART-treated and HAART-naïve patients at JOOTRH in Kisumu County, Western Kenya.

2.7 Association between Thyroid function and Lipids profile among HIV infected patients

Thyroid hormones play an important role in the regulation of lipid metabolism (Zaid & Greenman, 2019). Even though dyslipidemia and thyroid dysfunction continues to be reported among the patients on HAART, the association between lipid profile and thyroid function remains to be determined understood. The mechanism underlying the occurrence of thyroid dysfunctions to explain the occurrence of dyslipidemia among HIV infected on HAART is not clear (Beltran *et al.*, 2006; Jung *et al.*, 2017). It has been speculated that there might be a link though not yet proven, between thyroid dysfunction and atherosclerosis (Duntas & Brenta, 2012).

Changes in lipid profile has been commonly observed in patients with thyroid dysfunction, even though the relationship between lipids and thyroid hormone levels in euthyroid patientsis poorly understood (Chin *et al.*, 2014). It is thought that TSH stimulates adipocyte lipolysis, (Gagnon *et al.*, 2010). In vivo studies have linked reduced HDL levels owing to increased metabolism accelerated by thyroid hormones in Cholesteryl ester transfer protein (CETP). Hypothyroid status however, was found not to change CETP activity and HDL metabolism (Berti *et al.*, 2001; Skoczyńska *et al.*, 2016). It can be inferred that hypothyroidism accelerates the process of atherogenesis and increases cardiovascular risk (Jiskra *et al.*, 2007). In fact, high TSH

particularly has been reported to be associated with; a non-favorable lipids profile (Duntas & Brenta, 2012). A thorough understanding of the association between lipid profile and thyroid function is warranted.

Positive and significant association has been determined between TSH and TG levels, and between FT4 level and cholesterol levels among the euthyroid Malaysian men, (Chin *et al.*, 2014). Just like in Italy, occurrence of subclinical hypothyroidism in HIV-positive patients was reported with increase in total cholesterol serum levels occurring after initiation of HAART (Bongiovanni *et al.*, 2006). Similarly, changes in thyroid function have been associated with alterations in the concentrations of various plasma lipid components and with changes in HDL function among the Koreans (Jung *et al.*, 2017). Within the normal range of TSH it has been reported that an increase in the level of TSH was associated with less favorable lipid concentrations among the Norwegian population, (Asvold *et al.*, 2007)) just like among Spanish population (Santos-Palacios *et al.*, 2013). A study in Iran attributed dyslipidemia in thyroid dysfunction to varying TSH levels (Ahi *et al.*, 2017).

Most of those association studies between thyroid hormones and lipid levels were conducted in other regions in the developed world. However, in regions like Kenya where HIV has remained prevalent occasioning an increase in enrolment on HAART, abnormal lipid levels may not be clearly be associated with thyroid dysfunction among the HAART users due to scarcity of data. The current study therefore, determined the association between lipid profile and thyroid hormones among patients on HAART at Kisumu, Western Kenya.

3.0 CHAPTER THREE: METHODS AND MATERIALS

3.1. Study design

This was a hospital-based cross-sectional study. Data was required to determine the serum levels of thyroid function hormones and lipid profile among HAART-treated and HAART-naïve patients at a particular point in time hence the choice of study design (Denue *et al.*, 2013).

3.2 Study site

This study was premised at the Comprehensive Care Centre (CCC) at Jaramogi Oginga Odinga Teaching and Referral Hospital (JOOTRH) located in Kisumu County, Western Kenya. The hospital serves a population largely drawn from the expansive western Kenya region where HIV prevalence remains high at 17.5 % compared to the national prevalence at 4.9 % (Harklerode *et al.*, 2020). Kisumu County, lies within longitudes 33° 20'E and 35° 20'E and latitudes 0° 20'South and 0° 50'South. Kisumu County neighbors Siaya County to the West, Vihiga County to the North, Nandi County to the North East, Kericho County to the East, to the South is Nyamira County and Homa Bay County is to the South West. The facility acts as the regional referral center to most of these counties. Likelihood of ease of access of the participants with desired characteristics was considered high hence the choice of the study area.

3.3 Study population

Study population refers to the aggregate of individuals, cases or observation units that are of interest and remain the focus of investigation upon which the study outcome would be extrapolated (Riddler *et al.*, 2003). Patients on HAART attending CCC of JOOTRH who had been on HAART for more than 6 months were considered eligible for the study as tests. It is speculated that HAART exert physiological effects like reduced viral load on the HIV-infected patients after about 6 months hence justification for selection of HAART-treated patients who

had been enrolled on HAART for at least six months (Søgaard *et al.*, 2012). Newly diagnosed HIV-1 infected patients, yet to be initiated on HAART otherwise called HAART-naïve, were used as the controls.

3.4 Inclusion criteria

HIV infected patients on HAART (HAART-treated) for period ≥ 6 months; HIV infected patients yet to be initiated on HAART (HAART-naive); both males and females of age ≥18 years; willing and able to consent to the study; willing to keep the study schedule and appointment; attending Comprehensive Care Centre at JOOTRH were included in the study.

3.5 Exclusion criteria

HAART-treated and HAART-naïve individuals below 18 years; Patients with history of disease like thyroid, liver, kidney, cardio vascular disorders; Pregnant and lactating mothers; patients on drugs like anabolic steroids, corticosteroids, immune-modulating therapy, anti-dyslipidemic drug, and thyroid illness drugs; patients with risky lifestyle like excessive alcohol intake and cigarette smoking are all confounding factors on metabolic outcomes were excluded from the study.

3.6 Sample size determination

Using Epi Info statistical calculator (Charan & Biswas, 2013), the total sample size was generated to be 104 as shown below.

$$\text{Sample Size} = \frac{Z^2 P (1-P)}{d^2}$$

Where:

Z^2= Confidence level, given as 95%

P = Prevalence, given as 17.5% for HIV prevalence in Kisumu County in 2018 (Harklerode *et al.*, 2020)

d = Absolute error or precision, (0.05)

$$\text{Sample size} = \frac{0.95^2 \times 0.175\ (1 - 0.175)}{0.05^2}$$

$$= 52^*$$

* To enhance the power of the study, the calculated sample size was doubled. The final sample size used for the study was 104.

Distribution of HAART-treated and HAART-naïve

Adult ART coverage in Kisumu approximated to be below 90% in 2018 at about 80.5% (Borgdorff *et al.*, 2018). The number of HAART-treated patients selected for the study was approximated as below:

HAART-treated = 80.5% * 104

= 84 participants

HAART-naïve = 104- 84

= 20 participants

3.7 Sampling techniques and study procedure

The hospital, JOOTRH was purposively selected because of its capacity and history in the provision of highly active antiretroviral therapy among a homogenous population. It had a well-equipped laboratory owing to its status as a Teaching and Referral Hospital. Moreover, the

hospital has a well-equipped laboratory with capacity to perform highly specialized assays like lipid panel and thyroid function tests among others.

The eligible participants who consented to the study were recruited by simple random sampling to take part in the study. In case a patient declined to consent for the study then the next patient in a row was recruited. Recruited participants had their demographic, medical and anthropometric data taken thereafter requested to report to the clinic the following day in fasting state for collection of blood for laboratory assay of serum levels of thyroid hormones and lipids profile. Fasting blood was preferred in the current study because it improves test accuracy. For instance assay of TG and LDL using non-fasting blood results to inaccurate results (Cartier *et al.*, 2018).

3.8 Collection of Demographic, Medical and Anthropometric data

Participants' demographic and medical data including age, sex, and HAART regimen were collected and recorded for both the HAART-treated and HAART-naïve subjects.

Weight was measured for all the study participants using a medical weighing scale (Weigh AWS 160 Adult Height and Weight Scale). Calibration was done at the beginning and end of each examining day. Participants were asked to remove their heavy outer garments and shoes. The participant stood in the center of the platform to ensure that weight distributed evenly to both feet. The weight was recorded to the resolution of the scale (nearest 0.5 kg).

Height was measured for all the study participants using height meter (Weigh AWS 160 Adult Height and Weight Scale). At the beginning and end of each examination day, the height meter was checked with standardized rods and corrected if the error was greater than 2 mm. Participants were asked to remove their shoes, heavy outer garments, and hair ornaments. The participant was asked to stand with his/her back to the height rule. The back of the head, back,

buttocks, calves and heels should be touching the upright, feet together. The top of the external auditory meatus (ear canal) was in line with the inferior margin of the bony orbit (cheek bone). The participant was asked to look straight. Height was recorded to the resolution of the height rule (0.5m).

The BMI was calculated from weight and height using the formula; BMI= Weight (kg) divided by height (m^2). BMI categories, based on international standards (Fryar *et al.*, 2018), were classified as underweight (BMI < 18.5kg/m2); normal (BMI between 18.5 to below 24.9kg/m2); overweight (BMI between 25 to 29.9kg); Obese (BMI > 30kg/m^2 and above). Determination of BMI was important for this study because previous studies have established its association with plasma lipids(Toft & Beckett, 2003).

3.9 Collection and processing of blood samples

These tests were performed on fasting blood sample taken from the vein. The blood sample (about 3ml) was collected using 4ml red top vacutainers by a qualified phlebotomist. Once all the equipment needed for the procedures were assembled within a safe and reachable place, the phlebotomist welcomed the patient and explained the procedure to be performed. The patient's arm, while seated, was extended and the ante-cubital fossa or forearm was inspected for vein of good size that was visible, straight and clearly was located.

The site was cleaned with a 70% alcohol swab for 30 seconds and allowed to dry completely (30 seconds). A tourniquet was applied a few inches above the desired puncture site. The patient was asked to form a fist so the veins were more prominent. The vein was punctured swiftly at a 30 degree angle or less, and the needle introduced along the vein at the easiest angle of entry. Once sufficient blood was collected (about 3ml), the tourniquet was released before withdrawing the needle. The needle was withdrawn carefully and gentle pressure applied to the site with a clean gauze or dry cotton-wool ball. The patient was asked to hold the gauze or cotton wool in

place, with the arm extended and elevated. The blood was introduced into the vacutainer blood bottle gently without causing hemolysis. The blood vacutainer bottles were labeled using unique patent codes. Collected blood samples was centrifuged to obtain serum and stored in a deep freezer for later assays the levels of lipid (TG, TC, HDL, LDL) and thyroid function hormones (TSH, T3, and T4).

3.10 Determination of serum lipids levels

Lipids profile (TG, TC, HDL and LDL) was done using Clinical chemistry analyzer (Name-FujiFilm, Model- FujiDry-Chem NX500i). Briefly, about 1000uL of serum was loaded on the sample cuvette, machine set as per the manufacturer's instruction for the result to be read for all the lipid profile parameters including TG, TC, HDL. The LDL was determined using Friedewald formula calculated by subtracting HDL from TC (Choukem *et al.*, 2018). To ensure that assay of serum lipid levels were valid, quality control procedures were conducted immediately after opening every new batch/ lot of reagents as prescribed by the protocols.

3.11 Determination of serum Thyroid Function Hormone levels

Serum thyroid hormones levels (TSH, T3 and T4) were determined using i-CHROMA^{TM} Reader (BODITECH MED INC.) from Germany according to manufacturers' protocols as briefly described below:

i) To assay TSH levels 150uL of serum was pipetted into sample bottle and mixed thoroughly with 75uL of buffer after which 75uL of the mixture was picked and incubated for 12 minutes before being loaded onto the machine for reading and recording (appendix 3)

ii) To assay T3 levels, about 75uL of serum was mixed with solution A and mixed 10 times. 75uL of solution B was further be added and the resultant mixture was incubated for

8 minutes. 75uL of the mixture was pipetted into cartridge and further incubated for 8 minutes at room temperature then loaded on the machine for reading and recording following the procedure protocol (appendix 4).

iii) Moreover, to assay the T4 levels, about 75uL of serum was mixed with solution A and mixed 10 times. 75uL of solution B was further added and the resultant mixture was incubated for 8 minutes. 75uL of the mixture was pipetted into cartridge and further incubated for 8 minutes then loaded on the machine for reading and recording following the procedure protocol (appendix 5).

To ensure that assay of thyroid hormone levels were valid, quality control procedures were conducted immediately after opening every new batch/ lot of reagents as prescribed by the protocols.

3.12 Data analysis.

The differences in proportions in demographic, medical and anthropometric characteristics of the participants were determined using Chi-square (χ^2) analysis as well as Fisher's Exact test. Mann Whitney U test was used to determine the differences in the serum TSH, T3 and T4 levels between the HAART-treated and HAART-naïve groups as well as the difference in serum TC, TG, LDL and HDL levels between the two groups. The association between the serum TSH, T3 and T4 levels and serum TC, TG, LDL and HDL levels was determined using the Pearson correlation test. P- Values were considered significant at $P \leq 0.05$.

3.13 Ethical Considerations

Approval process

Ethical approval to conduct this study was provided by the Maseno University Ethics Review Committee (MUERC) through the Ethical Approval letter (Appendix 6). Administrative

approval was obtained from Kisumu County Department of Health and further authorization by the management of JOOTRH to conduct the study at their CCC and laboratory sections of the hospital.

Informed Consent

Potential participants were given consent forms that comprehensively address their rights and welfare as participants in the study. They were permitted to ask questions about the study for clarification. Each participant were required to confirm their acceptance to participate in the study by either appending a signature or a finger print. Participants were informed before the start of the interviews and sample collection and that if they would feel uncomfortable, they were free to stop the interview or procedure at any time. All information collected during the study will be kept confidential. The guidelines for the protection of human patientswere be followed as prescribed by the Maseno University Ethics Review Committee (MUERC).

The research procedures were explained to the participants and written informed consent obtained before any participant are screened for eligibility. These consents were sought after written and verbal information about the purpose and procedures of the study are given in English or Kiswahili as appropriate. In addition, participants were informed that participation was voluntary and that they could decline participation at any time. Given that this study only include patients aged 18 years and above, they were expected to consent themselves directly.

Confidentiality

Study participants were not identified by name during and after the research process, instead study codes were used. Any tools or documents containing identifying information were destroyed after the data entry. Data collection tools were kept in lockable shelves to ensure confidentiality. Data were maintained in electronic database that is password-protected and to

which only authorized study personnel (Principle and Co-investigators) have access. A copy of the informed consent documents were given to the participants for their records. The rights and welfare of the participants were protected by emphasizing to them that their attendance of clinic were not be affected if they declined to participate in this study.

Risks to Participants

Blood samples were collected in this study from the ante-cubetal fosa. Aseptic techniques were considered to avoid any form of infections using sterile needles and any other materials that was used. Highly qualified laboratory were used in to perform phlebotomy procedures.

Benefits to Participants

Patients with elevated lipid profile and other laboratory values were referred to the clinician for appropriate treatment. Those with risky lifestyle like smoking, excessive alcohol intake were referred to the counselor for appropriate lifestyle modification counseling. The benefit of participation in this study is likely to outweigh the risks.

Vulnerable Populations

No direct harm was anticipated in this study. The vulnerable population like pregnant women and children below 18 years were excluded from this study.

Compensation and Incentives

No compensation or incentives were given to participants for willingly taking part in the study. Participants who were not able to make return trip to the hospital for laboratory assays the following day were reimbursed fare. This was at minimal rates since it was assumed that majority came from the catchment area.

Expected Application of Results

Findings of this study are available for sharing with study participants, lead clinician and supporting partners for purposes of prompt decision for better clinical care. They are also available for presentation also be presented at professional conferences, and in peer-reviewed journals. Publication of the results of this study shall be governed by Maseno University research policies.

4.0 CHAPTER FOUR: RESULTS

4.1 General Demographics, Medical and Anthropometric characteristics of HAART-treated and HAART-naïve HIV patients

A total of 104 participants were enrolled into the study. They were divided into two groups, HAART-treated 84 (80.8%) and HAART-naïve 20 (19.2%). There was no significant difference in distribution of participants by sex in both groups (*P*=0.504) with HAART-treated group having 31 (36.9%) males and 53 (63.1%) compared to (45%) males and 11 (55%) females among the HAART-naïve group as shown in Table 4.1

There was no significant difference in the distribution of the participants by age between the HAART-treated and HAART-naive groups (*P*= 0.061). The proportions in age distributions were such that 18- 30 years was 12 (14.3%) vs 8 (40%) ,above 30-40 years was 29 (34.5%) vs 7 (35.5%), above 40- 50 years was 23 (27.4%) vs 4 (20%), above 50-60 years was 10 (11.9%) vs 0(0%) and above 60years was 10 (11.9%) vs 1 (5%) had 60 years between the HAART-treated and HAART-naive patients respectively.

Determination of the HAART regimens among the HAART-treated group revealed that majority were on TDF/3TC/EFV, 43 (51.7%); followed by TDF/3TC/NVP, 17 (20.2%); then AZT/3TC/NVP, 13 (15.5%); and finally TDF/3TC/DTG, 11 (13.1%). All regimens included 3TC drug combination.

Finally, it was established that there was no significant differences in the mean BMI (kg/m^2) of the HAART-treated (22.6 kg/m^2) and the HAART-naïve (22.4kg/m^2) groups, (*P*=0.571). In terms of proportion in BMI distributions, no significant statistical difference was observed between the two the HAART-treated and HAART-naïve groups, *P*= 0.671. Participants with BMI < 18.5 kg/m^2 were 2 (2.4%) vs 0; those with BMI 18.5- 24.9 kg/m^2 were 61 (72.6%) vs

16(80.0%); BMI of 25.0 – 29.9 kg/m^2 were 4 (4.8%) vs 0; and finally BMI of 30.0 kg/m^2 and above were 22.6 (3.6%) vs 22.4 (2.5%) among the HAART-treated and HAART-naïve groups respectively as shown in Table 4.1

Table 4. 1: General Demographic, Medical and Anthropometric data of the HAART-treated and HAART-naive HIV patients

Variables	HAART-treated (n=84)	HAART-naïve (n=20)	*P*
	Gender		
Male n (%)	31 (36.9)	9 (45.0)	0.504[a]
Female n (%)	53 (63.1)	11 (55.0)	
	Age		
18- 30 years n (%)	12 (14.3)	8 (40.0)	
>30- 40 years n (%)	29 (34.5)	7 (35.0)	
>40- 50 years n (%)	23 (27.4)	4 (20.0)	0.061[b]
>50- 60 years n (%)	10 (11.9)	0	
>60 years n (%)	10 (11.9)	1 (5.0)	
	HAART regimen		
AZT/3TC/NVP n (%)	13 (15.5)		
TDF/3TC/NVP n (%)	17 (20.2)	N/A	N/A
TDF/3TC/EFV n (%)	43 (51.2)		
TDF/3TC/DTG n (%)	11 (13.1)		
	BMI		
Underweight (BMI <18.5 kg/m^2) n (%)	2 (2.4)	0	
Normal (BMI = 18.5 – 24.9 kg/m^2) n (%)	61 (72.6)	16 (80.0)	0.671[b]
Overweight (BMI = 25 – 29.9 kg/m^2) n (%)	17 (20.2)	4 (20.0)	
Obese (BMI above 30kg/ m^2) n (%)	4 (4.8)	0	
Mean (kg/m^2), SD	22.6 (3.8)	22.4 (2.5)	0.571[a]

Data is presented as n (%) of the participants within the study groups. The study participants were categorized into HAART-treated (n=84) and HAART-naïve (n=20). [a]Statistical differences in proportions were determined by Chi-Square analysis. [b]Statistical differences in proportions were determined by Fisher's Exact Test. *P*- Values were considered significant at $P \leq 0.05$. Abbreviations; BMI; Body Mass Index, AZT: Zidovudine, TDF: Tenofovir Disoproxil Fumarate 3TC: Lamivudine, NVP: Nevirapine, DTG: Dolutegravir, EVF: Efeverenz

4.2 Serum thyroid hormones levels in HAART-treated and HAART-naïve HIV patients

The serum thyroid hormones levels; Thyroid stimulating Hormone (TSH), thyroxine (T4) and Triiodothyronine (T3) were determined among the HAART-treated and HAART-naïve groups as shown in Table 4.2. The serum TSH median (IQR) levels were significantly higher among the HAART-treated patients than in HAART-naïve subjects, [median (IQR) 1.72 (1.71) nmo/L vs median (IQR) 0.87 (1.07) nmo/L, *P*=0.001)]. The serum TSH levels were however within the reference ranges (0.4 – 4.4 nmo/L) in both the HAART-treated and HAART-naïve groups.

On assay of serum T3 levels (nmol/L), it was observed that there was no significant difference in serum T3 levels among the HAART-treated patients and HAART-naïve counterparts, [median (IQR) 2.43 (2.73) nmo/L vs median (IQR) 3.10 (2.62) nmo/L, (*P*=0.147). In both groups however, the serum T3 levels were within normal physiological ranges (0.78-7.7 nmol/L).

Additionally, determination of the serum T4 levels (nmol/L) revealed that while the serum T4 levels were with normal ranges (57.9-150.6 nmol/L) in both groups, there was no significant difference in serum T4 levels between HAART-treated and HAART-naïve counterparts , [median (IQR) 144.39 (67.53.64) nmo/L vs median (IQR) 136.99 (134.22) nmo/L, (*P*=0.426)].

Table 4.2: Level of Thyroid function hormones among the HAART-treated and HAART-naïve participants

Variables	HAART-treated (n=84)	HAART-naïve (n=20)	*P*
TSH	1.72 (1.71)	0.87 (1.07)	**0.01**
T3	2.43 (2.73)	3.10 (2.62)	0.147
T4	144.39 (67.53)	136.99 (134.22)	0.426

Data is presented as the Median (IQR) values unless stated otherwise. The study participants were categorized as HAART-treated (n=84) and HAART-naïve (n=20). Statistical difference was determined by Mann-Whitney test at $P \leq 0.05$. Abbreviations; TSH: Thyroid Stimulating Hormone, T3: Triiodothyronine, T4: Thyroxine, IQR: Interquartile range.

4.3 Serum lipid levels of the HAART-treated and HAART-naïve HIV patients

The serum lipids levels were determined among the HAART-treated and HAART-naïve groups as shown in Table 4.3. Determination of the serum TC levels (mg/dl) revealed that the serum levels were slightly elevated among the HAART-treated, median (IQR), 186.00 (±91.50) compared to the HAART-naïve group, median (IQR), 149.50 (±70.30). However, there was no significant difference in the serum TC levels between the two groups, *P*=0.092 and the levels were within normal physiological ranges, 0-220 mg/dl.

The serum levels of HDL (mg/dl) among the HAART-treated group, median (IQR), 52.50 (±24.00) were determined to be slightly high compared to the levels among the HAART-naive counterparts median (IQR) 44.00 (±34.00). However, the difference in serum HDL levels between the HAART-treated and HAART-naïve groups was not statistically significant, *P*= 0.280. In both groups, the HDL levels were within normal physiological ranges (0-150mg/dl).

Additionally, the serum LDL levels (mg/dl) were determined to be slightly elevated among the HAART-treated group, median (IQR), 138.00 (±86.30) compared to the HAART-naïve group median (IQR), 117.00 (±46.50), *P*=0.476). Again the difference in the serum LDL levels

between the HAART-treated and HAART-naïve groups was not statistically significant and the LDL levels among both groups were within normal physiological ranges (0-150 mg/dl).

Moreover, the TG levels (mg/dl) were evaluated to be slightly lower among the HAART-treated group, median (IQR), 82.00 (±62.80) than in the HAART-naïve group, median, (IQR), 94.50 (±55.80), *P*=0.504. However, there was no significant difference in serum TG levels between the HAART-treated and HAART-naïve patients. The serum TG levels among both groups were within normal ranges, 0-200 mg/dl.

Table 4.3: Level of Serum lipid profile among the HAART-treated and HAART-naïve participants

Variables	**HAART-treated (n=84)**	**HAART-naïve (n=20)**	***P***
TC, Median (IQR)	186.00 (91.50)	149.50 (70.30)	0.092
TG, Median (IQR)	82.00 (62.80)	94.50 (55.80)	0.504
HDL, Median (IQR)	52.50 (24.00)	44.00 (34.00)	0.280
LDL, Median (IQR)	138.00 (86.30)	117.00 (46.50)`	0.476

Data is presented as the Medians (IQR) values unless stated otherwise. The study participants were categorized as HAART-treated (n=84) and HAART-naïve (n=20). Statistical difference was determined by Mann-Whitney test at $P \leq 0.05$. Abbreviations: TC: Total Cholesterol, TG: Triglycerides, HDL: High Density Lipoprotein, LDL: Low Density Lipoprotein, IQR: Interquartile range

4.4 Association between the serum Thyroid hormone levels and serum lipid levels among the HAART-treated and HAART-naïve patients

Correlation between the thyroid hormones levels and lipid profile was determined by spearman bivariate correlation methods. This study established that there was significant association between the T4 levels and LDL levels (ρ= 0.240, *P*=0.014). However, no association was established between the T4 and HDL levels (ρ= 0.134, *P*=0.174), T4 and TG levels (ρ= -0.039, *P*=0.693), T4 and HDL levels (ρ= 0.134, *P*=0.174), T4 and TC levels (ρ= 0.145, *P*=0.143) as shown in Table 4.4

Further, correlation test run between T3 and lipids profile and results revealed that there was no association between T3 and TC, TG, HDL and LDL. The correlation coefficient between T3 and TC levels was $\rho = 0.019$ and $P=0.897$, between T3 and TG levels the $\rho = -0.094$ and $P=0.342$, between T3 and HDL levels the $\rho = 0.074$ while $P=0.458$, and finally between T3 and LDL levels the $\rho = 0.037$ and $P=0.706$.

Additionally, no association was found between TSH and lipid profiles. It was determined that the correlation coefficient between TSH and TC levels, $\rho = 0.013$ and $P=0.897$, TSH and TG levels had $\rho = -0.085$, $P=0.392$, TSH and HDL levels had $\rho = -0.126$, $P=0.201$, and finally TSH and LDL levels had $\rho = -0.016$, $P=0.871$.

Table 4.4: Spearman correlations between lipid profiles and thyroid hormones levels among HAART-treated and HAART-naïve subjects

Variables	Spearman's rho (ρ)	*P*- value
TSH		
TC	0.013	0.897
TG	0.085	0.392
HDL	-0.126	0.201
LDL	-0.016	0.871
T3		
TC	0.019	0.849
TG	-0.094	0.342
HDL	0.074	0.458
LDL	0.037	0.706
T4		
TC	0.145	0.143
TG	-0.039	0.693
HDL	0.134	0.174
LDL	0.240	**0.014**

The correlation was determined through spearman correlation test. *P*- Values and spearman correlation coefficient were considered significant at $P \leq 0.05$. *P*- Values in bold are statistically significant.

5.0 CHAPTER FIVE: DISCUSSION

5.1 Introduction

Changes in pituitary-thyroid axis occur in patients with virtually all illnesses (Roos *et al.*, 2007; Zaid & Greenman, 2019). While it has been suggested that HIV infection itself could be associated with altered thyroid function, (Ghate *et al.*, 2013) other studies have maintained that during HIV-1 infection, biochemical thyroid dysfunctions are rare (Rastgooye Haghi *et al.*, 2017; Zanoni *et al.*, 2012). The alterations in serum thyroid hormones following use of HAART in high HIV prevalent regions in Sub Saharan regions like Kenya remains to be established (Abbiyesuku *et al.*, 2014; Calza *et al.*, 2002). The current study therefore evaluated the serum TSH, T4 and T3 among the HAART-treated and HAART-naïve patients.

Furthermore, there is lack of reliable data on possible serum lipid changes occasioned by use of HAART among the HIV-infected population in countries with high HAART enrolment like Kenya. Consequently, the risk for development of lipid abnormalities like dyslipidemias (Nsagha *et al.*, 2015) may not be clearly determined hence posing dilemma among the HAART-givers and users.

Moreover, the thyroid function influences almost all the major metabolic pathways including but not limited to lipid metabolism. Alterations in serum thyroid hormones may be associated with thyroidal and lipid metabolism disorders among the HAART users. Clear understanding of changes in serum thyroid hormones and lipid levels would be important is assessing of the safety of HIV-infected patients while using HAART.

5.2 Serum Thyroid hormone levels among the HAART-treated and HAART-naïve patients

The current study established that the serum TSH levels among the HAART-treated patients was significantly higher than the in HAART-naïve patients even though the levels were within the

normal physiological range (0.4 -4.4 ul/dl). These findings were in concurrence with previous studies that equally established elevated levels of TSH among the HAART users (Beltran *et al.*, 2006; Thongam *et al.*, 2015). Elevated serum TSH levels among the HAART-treated patients could be related to autoimmune reconstitution inflammatory syndrome following initiation and use of HAART (Hoffmann & Brown, 2007; Wang *et al.*, 2012). On the other hand, low serum TSH levels observed among the HAART-naïve group could be attributed to high viral loads before initiation of on HAART causing depletion of TSH levels (Abbiyesuku *et al.*, 2014; De Groot, 1999; Thongam *et al.*, 2015). Determination of serum TSH levels is critical, therefore is not surprising that it is an accepted first-line screening test for patients suspected of having hypothyroidism or hyperthyroidism (Eghtedari & Correa, 2019; Hoffmann & Brown, 2007).

In as much as the current findings agree with some of the observations made previously, the results should be interpreted in the context of the limitations of the current study that non-HIV infected patients were not included to help determine whether the changes in serum TSH levels observed were attributable to use of HAART and or HIV infection itself. Further studies should consider including non HIV-infected patients in the study while determining the serum thyroid hormone levels in the HAART-treated and HAART-naïve patients.

While the current study did not establish any significant difference in the serum T_3 levels between the HAART-treated and HAART-naïve groups, the levels were slightly lower among the HAART-treated patients than the HAART-naïve counterparts. The serum T_3 levels were within normal physiological range (0.78 -7.7 nmol/L). These findings were in conformity with results of previous studies conducted that also reported comparable serum T3 levels between the two groups and that the values were within the reference range (Ji & Jin, 2016). In HAART-naïve patients, T_3 concentrations are thought to be initially maintained in the normal range just as observed in the current study by increased peripheral conversion of T_4 to T_3 (Mayer *et al.*, 2007).

Findings from the current study however, were at variance with observation's from a previous study that reported significantly high serum TSH levels among the HAART-treated patients than the HAART-naïve counterparts (Hoffmann & Brown, 2007). The inconsistency in the findings could be due to differences in demographics and medical history of the study populations. It not a coincidence therefore that measurement of serum T_3 levels in isolation from other thyroid function hormones is not advisable in detection of thyroidal dysfunctions (Eghtedari & Correa, 2019). Future studies therefore could consider conducting free T3 assays alongside T_3 to further approve or disapprove whether there could be significant difference in the serum level of the HAART-treated and HAART-naïve patients. The strength in the current study however, is the assay of serum TSH levels which is first line test in screening for detection of alterations in thyroid dysfunctions.

There was no difference in the serum Thyroxine hormone levels (T_4) between the HAART-treated patients and HAART-naïve counterparts. These findings were inconsistent with those from a previous study conducted in Kenya that reported increased T4 levels among the HAART users (Thaimuta *et al.*, 2010).The inconsistencies in the findings could be attributed to the difference in HAART-regimens on which the study populations were enrolled. Whereas the current study assayed the levels of bound thyroxine hormones through determination of serum T4 levels which is the abundant form of T4 in the body, future studies should consider including assay of free T4 to help estimate the level of unbound T4 in blood.

5.3 Serum lipids levels among the HAART-treated and HAART-naïve HIV patients.

In the present study, there was no difference in the serum Total Cholesterol (TC) levels between the HAART-treated and HAART-naïve patients even though the levels were within reference range. The level of cholesterol plays a vital role in cardiovascular diseases process since a high level of cholesterol may to a higher risk of developing atherosclerotic cardiovascular disease (CVD) (Gregg *et al.*, 2005). The current findings contradicted those from previous studies that

among the HAART-naïve patients, there was decrease in serum TC levels while after HAART initiation, increase in TC was noted (Riddler *et al.*, 2003). In the current study, the TC levels were assayed at a particular point in time while the TC levels reported from the previous study were measured after follow-up for 9 years hence a possible reason for the variations in TC levels in the studies.

The current study further revealed that the there was no difference in serum Triglycerides (TG) levels between the HAART-treated patients and the HAART-naïve counterparts even though the levels were within the normal ranges. Contrary to these findings, a similar study conducted among HIV-infected females reported that the TG levels were elevated in both HAART-treated and HAART-naïve counterparts with higher TG elevations observed in the former than the later (Anastos *et al.*, 2007). While the current study recruited both males and females, Anastos et al., (2007), exclusively enrolled female HIV-infected patients. These variations in demographics could account for the differences observed since females tend to experience more lipid derangements than males (Scheers *et al.*, 2008). Serum TG levels within reference range is a good clinical indicator since elevated TG levels increases propensity for development of cardiovascular complications (Denue *et al.*, 2013). Evaluation of serum TG levels is therefore an important risk measure for development of cardiovascular disease especially (Gregg *et al.*, 2005).

The current study reported high levels of HDL among the HAART-treated group than in the HAART-naïve patients even though the difference was not statistically significant and the observed HDL levels were within the normal range (0-150 mg/dl). These findings were at variance with a previous study that established that the HDL levels were clinically low among the HAART-naïve in the Nigerian population (Adewole *et al.*, 2010). The variations observed could be due to difference in study population characteristics.

High Density Lipoprotein (HDL) is considered as a good cholesterol for its atheroprotective properties. (Karalis & Jukema, 2018). It is involved in reverse transport of lipids through promotion of Cholesterol efflux and inhibition of inflammatory activities (Chapman *et al.*, 2011). HDL is said to promote the regression of atherosclerosis when the levels of functional particles are increased from endogenous or exogenous sources. The regression results from a combination of reduced lipid plaque lipid and macrophage contents, as well as from a reduction in its inflammatory state (Rajagopal *et al.*, 2012). The slight serum HDL elevations among the HAART-treated patients in the current study, although not significant could be attributable to the beneficial effects of HAART by reducing the viral load.

Determination of the serum LDL levels revealed that there was slight increase in serum LDL levels among the HAART-treated patients than the HAART-naïve counterparts even though the difference was significant and that levels were within reference ranges. In agreement with this findings, it has been previously reported that LDL levels gets elevated after HAART initiation (Idowu & Adesegun, 2019; Nelson *et al.*, 2009). The current study further corroborates findings from previous studies that established that HIV infection is associated with substantial decrease in serum LDL-C levels but the levels increases after HAART initiation since use of HAART is associated with improved immunity/ CD4 count (Riddler *et al.*, 2003)The slight elevations in LDL levels among the HAART-treated patients could be due to inhibitory cytokines effects on lipid metabolism, (de Silva *et al.*, 2008; Grunfeld, 2010; Idowu & Adesegun, 2019). It is worth noting that that elevated serum LDL levels are the most critical lipid risk factor for cardiovascular abnormalities (Baigent *et al.*, 2005).

While the current study adopted a cross-sectional determined the serum lipid levels between HAART-treated and HAART-naïve counterparts at a particular point in time, it remains to seen how the serum levels would vary when a follow up is made on the serum TC, TG, LDL and

HDL of the HAART-treated and HAART-naïve over a longer period of time through longitudinal studies.

5.4 Association between the serum Thyroid hormones and lipid levels among the HAART-treated and HAART-naïve patients

The mechanism underlying the occurrence of dyslipidemia in HIV infected patients with thyroid dysfunctions especially those on HAART is yet to be clearly elucidated (Beltran *et al.*, 2006). Changes in lipids profile has been commonly observed in patients with thyroid dysfunction, even though the relationship between lipids and thyroid hormone levels is ill understood (Chin *et al.*, 2014). It is speculated that there might be a link between thyroid dysfunction and atherosclerosis though this is yet to be proven (Duntas & Brenta, 2012). The present study determined the association between thyroid hormones and lipids profile among the HAART-treated and HAART-naïve patients using Spearman Correlation tests

The current study revealed that there was a significant positive association between the T4 and LDL levels. The current finding was in tandem with the earlier studies that reported a rise in LDL levels occasioned by elevations in T4 levels (Chin *et al.*, 2014; Kim *et al.*, 2009). As the T_4 levels rises, the LDL is expected to rise as well. It is thought that the activity of LDL receptors is under hormonal control which can be influenced by both HIV virus and HAART (Petit *et al.*, 2002). Thyroid hormones are known to regulate LDL receptors by binding directly to thyroid hormone responsive elements and controlling sterol regulatory element binding protein (Pucci *et al.*, 2000). The hormones are also involved in hepatic expression of hydorxymethyl glutalyl coenzyme areductase which enhance cholesterol synthesis (Petit *et al.*, 2002).

Interestingly, the current study did not find any association between the T4 and TC levels, HDL levels and TG levels, and it is contrary to findings from other studies (Chin *et al.*, 2014; Maugeri *et al.*, 1999). Thyroid hormones are thought to influence HDL metabolism by increasing

cholesteryl ester transfer protein (CETP) activity, which exchanges cholesteryl esters from HDL2 to the very low density lipoproteins (VLDL) and TG levels to the opposite direction (Buchacz *et al.*, 2008). Differences among the study populations might account for the difference in the correlations observed in the current and previous studies.

In addition, no correlation was found between T3 and lipid profiles, TC, TG, HDL and LDL, contrary to findings by other studies that reported that there was a significant negative correlation between T3 and cholesterol (Chin *et al.*, 2014). Even though the relationship between T3 and lipid profile was not apparent from this present study, T3 actions are mediated via modulation of gene expression and cell signaling pathways which in turn regulates the cholesterol synthesis (Hughes et al., 2011). The previous studies mentioned were conducted among populations outside of Sub Saharan Africa hence could account for the variations in association relationship between T3 and the lipid profile parameters possibly due to genetic, environmental, socio-cultural or other yet unidentified factors.

TSH is thought to stimulate adipocyte lipolysis (Gagnon *et al.*, 2010), and has been particularly associated with a non-favorable lipids profile (Duntas & Brenta, 2012). The need to understand the correlation between TSH and lipid profiles is strongly justified. However, the present study did not establish any correlation between TSH and any of the lipid profiles. Lack of correlation between TSH and TC levels according to the present study could be an indication that production of TSH from the pituitary gland did not affect the levels of TC levels circulating in blood. This observation was in tandem with that an earlier study, which also established insignificant correlations between TSH and cholesterol levels (Ruhla *et al.*, 2010).

The present study found no correlation between TSH and TG levels unlike the previous studies that established positive correlation between TSH and TG (Garduno-Garcia Jde *et al.*, 2010; Santos-Palacios *et al.*, 2013). The present study might have failed to establish any correlation

because the patients for the study were not known Sub Clinical Hypothyroid cases as it were in the previous studies where TSH had been found to have regulatory effects on TG (Wang *et al.*, 2012).

Further correlation tests revealed that there was no correlation between TSH and HDL levels, as well as between TSH and LDL, findings that were similar to those of other other studies (Maugeri *et al.*, 1999; Wang *et al.*, 2012). On the contrary, increasing levels of TSH within the normal range was associated with rising level of LDL among the Norwegian population (Asvold *et al.*, 2007), suggesting a positive correlation between TSH and LDL. The correlation between TSH and LDL might not have been apparent in the current study because the TSH that seem to regulate other lipid levels was not adversely affected by the HAART use and HIV infection among the population under study hence the levels were within normal physiological ranges.

The findings from this current study which was cross-sectional in design should inspire future studies to adopt longitudinal study designs to determine the association between the serum thyroid hormones and lipid levels among the HAART-treated and HAART-naïve counterparts over a longer period of time.

6.0 CHAPTER SIX: SUMMARY OF FINDINGS, CONCLUSION AND RECOMMENDATIONS

6.1 Summary of findings

The study revealed that the serum TSH, levels were higher among the HAART-treated patients than the HAART-naïve counterparts. However, there was no difference in the serum T4 and T3 levels between the HAART-treated patients and HAART-naïve counterparts. The serum TSH, T3 and T4 well all within reference range in both groups.

Further, there was no difference in the serum lipids levels between the HAART-treated and HAART-naïve patients and the levels were within normal physiological range.

Additionally, a positive correlation was found between serum T4 and LDL levels. However, no association was found between serum TSH as well as T3 and the serum TC, TG, HDL and LDL.

6.2 Conclusions

i) The current study determined that serum TSH levels are higher among the HAART-treated than HAART-naïve group. However, the serum T4 and T3 levels are similar in the two groups.

ii) The serum lipid levels in both HAART-treated and HAART-naïve patients are similar.

iii) Increase in serum T4 levels is associated with increase in LDL levels. However, no association exist between serum TSH as well as T3 and the serum lipids (TC, TG, HDL and LDL).

6.3 Recommendations from the current study

i) HIV-infected patients on HAART should have their Thyroid function monitored through routine assessment of serum thyroid hormones levels.

ii) HIV-infected patients on HAART should be screened periodically for potential alterations in serum levels

iii) Whenever there occurs abnormalities in serum lipid levels among the HAART-treated patients, further thyroid hormone assays should be conducted to rule out possible thyroid dysfunctions.

6.4 Recommendations for future studies

i) Future studies should consider determining the Free T4 (FT4), Free T3 (FT3) and Thyroid binding globulin (TBG) for further describe the thyroid function among patients on HAART.

ii) Longitudinal studies should be conducted to infer causal relationship which was not possible in the current cross-sectional study.

iii) Further research should consider bigger sample size to sufficiently generalize the study findings to the larger population groups

REFERENCES

1. Abbiyesuku, F. M., Osuji, K. C., Kuti M, O., & Atiba, A. S. (2014). Thyroid function tests in nigerian hiv sero- positive patients on highly active antiretroviral therapy (HAART). *International Journal of Medical and Clinical Research, 5*(1), 277.
2. Abelleira, E., De Cross, G. A., & Pitoia, F. (2014). [Thyroid dysfunction in adults infected by human immunodeficiency virus]. *Medicina (B Aires), 74*(4), 315-320.
3. Adewole, O. O., Eze, S., Betiku, Y., Anteyi, E., Wada, I., Ajuwon, Z., & Erhabor, G. (2010). Lipid profile in HIV/AIDS patients in Nigeria. *African health sciences, 10*(2), 144-149.
4. Ahi, S., Amouzegar, A., Gharibzadeh, S., Kazempour-Ardebili, S., Delshad, H., Tohidi, M., & Azizi, F. (2017). The Association Between Normal Range TSH and Lipid Profile. *Horm Metab Res, 49*(6), 424-429. doi:10.1055/s-0043-110142
5. Anastos, K., Lu, D., Shi, Q., Tien, P. C., Kaplan, R. C., Hessol, N. A., . . . Justman, J. (2007). Association of serum lipid levels with HIV serostatus, specific antiretroviral agents, and treatment regimens. *J Acquir Immune Defic Syndr, 45*(1), 34-42. doi:10.1097/QAI.0b013e318042d5fe
6. Arts, E. J., & Hazuda, D. J. (2012). HIV-1 antiretroviral drug therapy. *Cold Spring Harbor perspectives in medicine, 2*(4), a007161-a007161. doi:10.1101/cshperspect.a007161
7. Asvold, B. O., Vatten, L. J., Nilsen, T. I., & Bjoro, T. (2007). The association between TSH within the reference range and serum lipid concentrations in a population-based study. The HUNT Study. *Eur J Endocrinol, 156*(2), 181-186. doi:10.1530/eje.1.02333
8. Baigent, C., Keech, A., Kearney, P. M., Blackwell, L., Buck, G., Pollicino, C., . . . Simes, R. (2005). Efficacy and safety of cholesterol-lowering treatment: prospective meta-analysis of data from 90,056 participants in 14 randomised trials of statins. *Lancet, 366*(9493), 1267-1278. doi:10.1016/s0140-6736(05)67394-1
9. Baza Caraciolo, B., Perez de Oteyza, C., Carrio Montiel, D., Carrio Montiel, J. C., Salguero Aparicio, M., & Del Romero Guerrero, J. (2007). [Lipid profile in untreated HIV positive patients. HIV infection: cardiovascular risk factor?]. *An Med Interna, 24*(4), 160-167.
10. Beltran, S., Lescure, F. X., El Esper, I., Schmit, J. L., & Desailloud, R. (2006). Subclinical hypothyroidism in HIV-infected patients is not an autoimmune disease. *Horm Res, 66*(1), 21-26. doi:10.1159/000093228
11. Berti, J. A., Amaral, M. E., Boschero, A. C., Nunes, V. S., Harada, L. M., Castilho, L. N., & Oliveira, H. C. (2001). Thyroid hormone increases plasma cholesteryl ester transfer protein activity and plasma high-density lipoprotein removal rate in transgenic mice. *Metabolism, 50*(5), 530-536. doi:10.1053/meta.2001.22514
12. Bibiloni, M. D. M., Salas, R., De la Garza, Y. E., Villarreal, J. Z., Sureda, A., & Tur, J. A. (2016). Serum Lipid Profile, Prevalence of Dyslipidaemia, and Associated Risk Factors Among Northern Mexican Adolescents. *Journal of pediatric gastroenterology and nutrition, 63*(5), 544-549. doi:10.1097/MPG.0000000000001325
13. Bongiovanni, M., Adorni, F., Casana, M., Tordato, F., Tincati, C., Cicconi, P., . . . d'Arminio Monforte, A. (2006). Subclinical hypothyroidism in HIV-infected subjects. *J Antimicrob Chemother, 58*(5), 1086-1089. doi:10.1093/jac/dkl360
14. Borgdorff, M. W., Kwaro, D., Obor, D., Otieno, G., Kamire, V., Odongo, F., . . . De Cock, K. M. (2018). HIV incidence in western Kenya during scale-up of antiretroviral therapy and voluntary medical male circumcision: a population-based cohort analysis. *Lancet HIV, 5*(5), e241-e249. doi:10.1016/s2352-3018(18)30025-0

15. Brenchley, J. M., Schacker, T. W., Ruff, L. E., Price, D. A., Taylor, J. H., Beilman, G. J., . . . Douek, D. C. (2004). CD4+ T cell depletion during all stages of HIV disease occurs predominantly in the gastrointestinal tract. *The Journal of experimental medicine, 200*(6), 749-759. doi:10.1084/jem.20040874
16. Buchacz, K., Weidle, P. J., Moore, D., Were, W., Mermin, J., Downing, R., . . . Brooks, J. T. (2008). Changes in lipid profile over 24 months among adults on first-line highly active antiretroviral therapy in the home-based AIDS care program in rural Uganda. *J Acquir Immune Defic Syndr, 47*(3), 304-311.
17. Calsolaro, V., Niccolai, F., Pasqualetti, G., Calabrese, A. M., Polini, A., Okoye, C., . . . Monzani, F. (2019). Overt and Subclinical Hypothyroidism in the Elderly: When to Treat? *Front Endocrinol (Lausanne), 10*, 177. doi:10.3389/fendo.2019.00177
18. Calza, L., Manfredi, R., & Chiodo, F. (2002). Subclinical hypothyroidism in HIV-infected patients receiving highly active antiretroviral therapy. *J Acquir Immune Defic Syndr, 31*(3), 361-363.
19. Cartier, L. J., Collins, C., Lagacé, M., & Douville, P. (2018). Comparison of fasting and non-fasting lipid profiles in a large cohort of patients presenting at a community hospital. *Clin Biochem, 52*, 61-66. doi:10.1016/j.clinbiochem.2017.11.007
20. Chapman, M. J., Ginsberg, H. N., Amarenco, P., Andreotti, F., Boren, J., Catapano, A. L., . . . Watts, G. F. (2011). Triglyceride-rich lipoproteins and high-density lipoprotein cholesterol in patients at high risk of cardiovascular disease: evidence and guidance for management. *Eur Heart J, 32*(11), 1345-1361. doi:10.1093/eurheartj/ehr112
21. Charan, J., & Biswas, T. (2013). How to calculate sample size for different study designs in medical research? *Indian journal of psychological medicine, 35*(2), 121-126. doi:10.4103/0253-7176.116232
22. Chimbetete, C., Katzenstein, D., Shamu, T., Spoerri, A., Estill, J., Egger, M., & Keiser, O. (2018). HIV-1 Drug Resistance and Third-Line Therapy Outcomes in Patients Failing Second-Line Therapy in Zimbabwe. *Open forum infectious diseases, 5*(2), ofy005-ofy005. doi:10.1093/ofid/ofy005
23. Chin, K.-Y., Ima-Nirwana, S., Mohamed, I. N., Aminuddin, A., Johari, M. H., & Ngah, W. Z. W. (2014). The Relationships between Thyroid Hormones and Thyroid-stimulating Hormone with Lipid Profile in Euthyroid Men. *International Journal of Medical Sciences, 11*(4), 349-355. doi:10.7150/ijms.7104
24. Choukem, S. P., Manases, T., Nda-Mefoo, J. P., Dimala, C. A., Mboue-Djieka, Y., Sobngwi, E., & Kengne, A. P. (2018). Validation of the Friedewald formula for the estimation of low density lipoprotein cholesterol in a sub-Saharan African population. *Clin Biochem, 53*, 25-30. doi:10.1016/j.clinbiochem.2017.12.008
25. Chung, H. R. (2014). Iodine and thyroid function. *Annals of pediatric endocrinology & metabolism, 19*(1), 8-12. doi:10.6065/apem.2014.19.1.8
26. Crabtree-Ramírez, B., Caro-Vega, Y., Shepherd, B. E., Grinsztejn, B., Wolff, M., Cortes, C. P., . . . South America Network for Hiv Epidemiology, o. t. I. E. D. t. E. A. P. (2016). Time to HAART Initiation after Diagnosis and Treatment of Opportunistic Infections in Patients with AIDS in Latin America. *PloS one, 11*(6), e0153921-e0153921. doi:10.1371/journal.pone.0153921
27. Crunkhorn, S., & Patti, M. E. (2008). Links between thyroid hormone action, oxidative metabolism, and diabetes risk? *Thyroid, 18*(2), 227-237. doi:10.1089/thy.2007.0249
28. da Cunha, J., Maselli, L. M. F., Stern, A. C. B., Spada, C., & Bydlowski, S. P. (2015). Impact of antiretroviral therapy on lipid metabolism of human immunodeficiency virus-infected patients: Old and new drugs. *World journal of virology, 4*(2), 56-77. doi:10.5501/wjv.v4.i2.56

29. De Groot, L. J. (1999). Dangerous Dogmas in Medicine: The Nonthyroidal Illness Syndrome. *The Journal of Clinical Endocrinology & Metabolism, 84*(1), 151-164. doi:10.1210/jcem.84.1.5364
30. de Silva, T. I., Cotten, M., & Rowland-Jones, S. L. (2008). HIV-2: the forgotten AIDS virus. *Trends Microbiol, 16*(12), 588-595. doi:10.1016/j.tim.2008.09.003
31. Denue, B. A., Alkali, M. B., Abjah, A. U., Kida, I. M., Ajayi, B., & Fate, B. Z. (2013). Changes in Lipid Profiles and Other Biochemical Parameters in HIV-1 Infected Patients Newly Commenced on HAART Regimen. *Infectious Diseases, 6*, 7-14. doi:10.4137/IDRT.S10044
32. Dobs, A. S., Dempsey, M. A., Ladenson, P. W., & Polk, B. F. (1988). Endocrine disorders in men infected with human immunodeficiency virus. *Am J Med, 84*(3 Pt 2), 611-616.
33. Duntas. (2002). Thyroid disease and lipids. *Thyroid, 12*(4), 287-293. doi:10.1089/10507250252949405
34. Duntas, & Brenta, G. (2012). The effect of thyroid disorders on lipid levels and metabolism. *Med Clin North Am, 96*(2), 269-281. doi:10.1016/j.mcna.2012.01.012
35. Duntas, L. H., & Brenta, G. (2018). A Renewed Focus on the Association Between Thyroid Hormones and Lipid Metabolism. *Front Endocrinol (Lausanne), 9*, 511-511. doi:10.3389/fendo.2018.00511
36. Eghtedari, B., & Correa, R. (2019). Levothyroxine *StatPearls*. Treasure Island (FL): StatPearls Publishing LLC.
37. Enas, E. A., Chacko, V., Pazhoor, S. G., Chennikkara, H., & Devarapalli, H. P. (2007). Dyslipidemia in South Asian patients. *Curr Atheroscler Rep, 9*(5), 367-374.
38. Franssen, R., Monajemi, H., Stroes, E. S., & Kastelein, J. J. (2011). Obesity and dyslipidemia. *Med Clin North Am, 95*(5), 893-902. doi:10.1016/j.mcna.2011.06.003
39. Fryar, C. D., Kruszon-Moran, D., Gu, Q., & Ogden, C. L. (2018). Mean Body Weight, Height, Waist Circumference, and Body Mass Index Among Adults: United States, 1999-2000 Through 2015-2016. *Natl Health Stat Report*(122), 1-16.
40. Gagnon, A., Antunes, T. T., Ly, T., Pongsuwan, P., Gavin, C., Lochnan, H. A., & Sorisky, A. (2010). Thyroid-stimulating hormone stimulates lipolysis in adipocytes in culture and raises serum free fatty acid levels in vivo. *Metabolism, 59*(4), 547-553. doi:10.1016/j.metabol.2009.08.018
41. Garduno-Garcia Jde, J., Alvirde-Garcia, U., Lopez-Carrasco, G., Padilla Mendoza, M. E., Mehta, R., Arellano-Campos, O., . . . Aguilar-Salinas, C. A. (2010). TSH and free thyroxine concentrations are associated with differing metabolic markers in euthyroid subjects. *Eur J Endocrinol, 163*(2), 273-278. doi:10.1530/eje-10-0312
42. German Advisory Committee Blood, S. A. o. P. T. b. B. (2016). Human Immunodeficiency Virus (HIV). *Transfusion medicine and hemotherapy : offizielles Organ der Deutschen Gesellschaft fur Transfusionsmedizin und Immunhamatologie, 43*(3), 203-222. doi:10.1159/000445852
43. Ghate, M., Tripathy, S., Gangakhedkar, R., Thakar, M., Bhattacharya, J., Choudhury, I., . . . Paranjape, R. (2013). Use of first line antiretroviral therapy from a free ART programme clinic in Pune, India - a preliminary report. *The Indian journal of medical research, 137*(5), 942-949.
44. Glesby, M. J. (2016). Cardiovascular Complications of HIV Infection. *Topics in antiviral medicine, 24*(4), 127-131.
45. Gregg, E. W., Cheng, Y. J., Cadwell, B. L., Imperatore, G., Williams, D. E., Flegal, K. M., . . . Williamson, D. F. (2005). Secular trends in cardiovascular disease risk factors

according to body mass index in US adults. *Jama, 293*(15), 1868-1874. doi:10.1001/jama.293.15.1868
46. Grunfeld, C. (2010). Dyslipidemia and its Treatment in HIV Infection. *Top HIV Med, 18*.
47. Harklerode, R., Waruiru, W., Humwa, F., Waruru, A., Kellogg, T., Muthoni, L., . . . Zielinski-Gutierrez, E. (2020). Epidemiological profile of individuals diagnosed with HIV: results from the preliminary phase of case-based surveillance in Kenya. *AIDS care, 32*(1), 43-49. doi:10.1080/09540121.2019.1612021
48. Hoffmann, C. J., & Brown, T. T. (2007). Thyroid function abnormalities in HIV-infected patients. *Clin Infect Dis, 45*(4), 488-494. doi:10.1086/519978
49. Idowu, A., & Adesegun, O. (2019). *Thyroid Function Abnormalities among Patients with HIV/AIDS in South-Western Nigeria*.
50. Jain, G., Devpura, G., & Gupta, B. S. (2009). Abnormalities in the thyroid function tests as surrogate marker of advancing HIV infection in infected adults. *J Assoc Physicians India, 57*, 508-510.
51. Jannin, A., Peltier, L., d'Herbomez, M., Defrance, F., Marcelli, S., Ben Hamou, A., . . . Espiard, S. (2019). Lesson from inappropriate TSH-receptor antibody measurement in hypothyroidism: case series and literature review. *Clin Chem Lab Med*. doi:10.1515/cclm-2019-0090
52. Jaoko, W., & Kredo, T. (2017). Boosted protease inhibitor (PI) monotherapy for treating HIV/AIDS. *The Cochrane Database of Systematic Reviews, 2017*(8), CD008171. doi:10.1002/14651858.CD008171.pub2
53. Ji, S., & Jin, C. (2016). Prevalence and Influencing Factors of Thyroid Dysfunction in HIV-Infected Patients. *2016*, 3874257. doi:10.1155/2016/3874257
54. Jiskra, J., Limanova, Z., & Antosova, M. (2007). [Thyroid diseases, dyslipidemia and cardiovascular risk]. *Vnitr Lek, 53*(4), 382-385.
55. Jung, K. Y., Ahn, H. Y., Han, S. K., Park, Y. J., Cho, B. Y., & Moon, M. K. (2017). Association between thyroid function and lipid profiles, apolipoproteins, and high-density lipoprotein function. *J Clin Lipidol, 11*(6), 1347-1353. doi:10.1016/j.jacl.2017.08.015
56. Kaliaperumal, R., William, E., Selvam, T., & Krishnan, S. M. (2014). Relationship between Lipoprotein(a) and Thyroid Hormones in Hypothyroid Patients. *Journal of clinical and diagnostic research : JCDR, 8*(2), 37-39. doi:10.7860/JCDR/2014/7817.4001
57. Karalis, I., & Jukema, J. W. (2018). HDL Mimetics Infusion and Regression of Atherosclerosis: Is It Still Considered a Valid Therapeutic Option? *Curr Cardiol Rep, 20*(8), 66. doi:10.1007/s11886-018-1004-9
58. Ketsamathi, C., Jongjaroenprasert, W., Chailurkit, L.-o., Udomsubpayakul, U., & Kiertiburanakul, S. (2006). Prevalence of thyroid dysfunction in Thai HIV-infected patients. *Current HIV research, 4*(4), 463-467. doi:10.2174/157016206778560036
59. Khan, R., Sikanderkhel, S., Gui, J., Adeniyi, A.-R., O'Dell, K., Erickson, M., . . . Patel, D. (2020). Thyroid and Cardiovascular Disease: A Focused Review on the Impact of Hyperthyroidism in Heart Failure. *Cardiology research, 11*(2), 68-75. doi:10.14740/cr1034
60. Kim, B. J., Kim, T. Y., Koh, J. M., Kim, H. K., Park, J. Y., Lee, K. U., . . . Kim, W. B. (2009). Relationship between serum free T4 (FT4) levels and metabolic syndrome (MS) and its components in healthy euthyroid subjects. *Clin Endocrinol (Oxf), 70*(1), 152-160. doi:10.1111/j.1365-2265.2008.03304.x
61. Lambert, M. (1994). Thyroid dysfunction in HIV infection. *Baillieres Clin Endocrinol Metab, 8*(4), 825-835.

62. Low, Y. S., Islahudin, F., Razali, K. A. M., & Adnan, S. (2018). Modification of Initial Highly Active Antiretroviral Therapy (HAART) Regimen in Paediatric HIV Patients. *The open AIDS journal, 12*, 11-19. doi:10.2174/1874613601812010011
63. Maugeri, D., Santangelo, A., Barbagallo, P., Bonanno, M. R., Malaguarnera, M., Rizza, I., . . . Panebianco, P. (1999). Thyroid hormones and lipid metabolism in a group of patients over seventy. *Eur Rev Med Pharmacol Sci, 3*(5), 211-216.
64. Mayer, K. H., Hoffmann, C. J., & Brown, T. T. (2007). Thyroid Function Abnormalities in HIV-Infected Patients. *Clinical Infectious Diseases, 45*(4), 488-494. doi:10.1086/519978
65. Moura, N. A., & Zantut-Wittmann, D. E. (2016). Abnormalities of Thyroid Hormone Metabolism during Systemic Illness: The Low T3 Syndrome in Different Clinical Settings. *International journal of endocrinology, 2016*, 2157583-2157583. doi:10.1155/2016/2157583
66. Mullur, R., Liu, Y.-Y., & Brent, G. A. (2014). Thyroid hormone regulation of metabolism. *Physiological reviews, 94*(2), 355-382. doi:10.1152/physrev.00030.2013
67. Nelson, M., Powles, T., Zeitlin, A., Sen, P., Scourfield, A., Bower, M., . . . Stebbing, J. (2009). Thyroid dysfunction and relationship to antiretroviral therapy in HIV-positive individuals in the HAART era. *J Acquir Immune Defic Syndr, 50*(1), 113-114. doi:10.1097/QAI.0b013e31818ce835
68. Nery, M. W., Martelli, C. M. T., & Turchi, M. D. (2011). Dyslipidemia in AIDS patients on highly active antiretroviral therapy. *Brazilian Journal of Infectious Diseases, 15*, 151-155.
69. Nsagha, D. S., Weledji, E. P., Assob, N. J. C., Njunda, L. A., Tanue, E. A., kibu, O. D., . . . Ngowe, M. N. (2015). Highly active antiretroviral therapy and dyslipidemia in people living with HIV/AIDS in Fako Division, South West Region of Cameroon. *BMC Cardiovasc Disord, 15*(1), 95. doi:10.1186/s12872-015-0090-5
70. Ombeni, W., & Kamuhabwa, A. R. (2016). Lipid Profile in HIV-Infected Patients Using First-Line Antiretroviral Drugs. *J Int Assoc Provid AIDS Care, 15*(2), 164-171. doi:10.1177/2325957415614642
71. Onyedum, C. C., Young, E. E., Iroezindu, M. O., Chukwuka, C. J., & Nwagha, U. I. (2014). Atherogenic index of plasma in highly active antiretroviral therapy-naïve patients with human immunodeficiency virus infection in Southeast Nigeria. *Indian J Endocrinol Metab, 18*(5), 631-636. doi:10.4103/2230-8210.139217
72. Ortiga-Carvalho, T. M., Chiamolera, M. I., Pazos-Moura, C. C., & Wondisford, F. E. (2016). Hypothalamus-Pituitary-Thyroid Axis. *Compr Physiol, 6*(3), 1387-1428. doi:10.1002/cphy.c150027
73. Padmapriyadarsini, C., Ramesh, K., Sekar, L., Ramachandran, G., Reddy, D., Narendran, G., . . . Swaminathan, S. (2017). Factors affecting high-density lipoprotein cholesterol in HIV-infected patients on nevirapine-based antiretroviral therapy. *The Indian journal of medical research, 145*(5), 641-650. doi:10.4103/ijmr.IJMR_1611_15
74. Pasupathi, P., Manivannan, P., M, U., & Deepa, M. (2010). *Thyroid Function, Cardiac Risk Assessment Profile and Hematological Changes During HIV Infection and AIDS Patients* (Vol. 11).

75. Penzak, S. R., & Chuck, S. K. (2000). Hyperlipidemia associated with HIV protease inhibitor use: pathophysiology, prevalence, risk factors and treatment. *Scand J Infect Dis, 32*(2), 111-123.
76. Petit, J. M., Duong, M., Duvillard, L., Florentin, E., Portier, H., Lizard, G., . . . Verges, B. (2002). LDL-receptors expression in HIV-infected patients: relations to antiretroviral therapy, hormonal status, and presence of lipodystrophy. *Eur J Clin Invest, 32*(5), 354-359.
77. Pucci, E., Chiovato, L., & Pinchera, A. (2000). Thyroid and lipid metabolism. *Int J Obes Relat Metab Disord, 24 Suppl 2*, S109-112.
78. Quirino, T., Bongiovanni, M., Ricci, E., Chebat, E., Carradori, S., Martinelli, C., . . . Bonfanti, P. (2004). Hypothyroidism in HIV-infected patients who have or have not received HAART. *Clin Infect Dis, 38*(4), 596-597. doi:10.1086/381442
79. Rajagopal, G., Suresh, V., & Sachan, A. (2012). High-density lipoprotein cholesterol: How High. *Indian J Endocrinol Metab, 16*(Suppl 2), S236-S238. doi:10.4103/2230-8210.104048
80. Rastgooye Haghi, A., Solhjoo, M., & Tavakoli, M. H. (2017). Correlation Between Subclinical Hypothyroidism and Dyslipidemia. *Iranian Journal of Pathology, 12*(2), 106-111.
81. Riddler, S. A., Smit, E., Cole, S. R., Li, R., Chmiel, J. S., Dobs, A., . . . Kingsley, L. A. (2003). Impact of HIV infection and HAART on serum lipids in men. *Jama, 289*(22), 2978-2982. doi:10.1001/jama.289.22.2978
82. Rizos, C. V., Elisaf, M. S., & Liberopoulos, E. N. (2011). Effects of Thyroid Dysfunction on Lipid Profile. *The Open Cardiovascular Medicine Journal, 5*, 76-84. doi:10.2174/1874192401105010076
83. Roos, A., Bakker, S. J., Links, T. P., Gans, R. O., & Wolffenbuttel, B. H. (2007). Thyroid function is associated with components of the metabolic syndrome in euthyroid subjects. *J Clin Endocrinol Metab, 92*(2), 491-496. doi:10.1210/jc.2006-1718
84. Rose, H., Woolley, I., Hoy, J., Dart, A., Bryant, B., Mijch, A., & Sviridov, D. (2006). HIV infection and high-density lipoprotein: the effect of the disease vs the effect of treatment. *Metabolism, 55*(1), 90-95. doi:10.1016/j.metabol.2005.07.012
85. Ruhla, S., Weickert, M. O., Arafat, A. M., Osterhoff, M., Isken, F., Spranger, J., . . . Mohlig, M. (2010). A high normal TSH is associated with the metabolic syndrome. *Clin Endocrinol (Oxf), 72*(5), 696-701. doi:10.1111/j.1365-2265.2009.03698.x
86. Santos-Palacios, S., Brugos-Larumbe, A., Guillen-Grima, F., & Galofre, J. C. (2013). A cross-sectional study of the association between circulating TSH level and lipid profile in a large Spanish population. *Clin Endocrinol (Oxf), 79*(6), 874-881. doi:10.1111/cen.12216
87. Scheers, T., Philippaerts, R., Van Langendonck, L., Duquet, W., Duvigneaud, N., Matton, L., . . . Lefevre, J. (2008). Lipid profile in men and women with different levels of sports participation and physical activity. *Public Health Nutr, 11*(11), 1098-1106. doi:10.1017/s1368980007001425
88. Skoczyńska, A., Wojakowska, A., Turczyn, B., Zatońska, K., Wołyniec, M., Rogala, N., . . . Bednarek-Tupikowska, G. (2016). Serum Lipid Transfer Proteins in Hypothyreotic Patients Are Inversely Correlated with Thyroid-Stimulating Hormone (TSH) Levels. *Medical science monitor : international medical journal of experimental and clinical research, 22*, 4661-4669. doi:10.12659/msm.898134
89. Søgaard, O. S., Lohse, N., Østergaard, L., Kronborg, G., Røge, B., Gerstoft, J., . . . Obel, N. (2012). Morbidity and risk of subsequent diagnosis of HIV: a population based case

control study identifying indicator diseases for HIV infection. *PloS one, 7*(3), e32538. doi:10.1371/journal.pone.0032538
90. Sousa, J. D., Temudo, M. P., Hewlett, B. S., Camacho, R. J., Müller, V., & Vandamme, A.-M. (2016). Male Circumcision and the Epidemic Emergence of HIV-2 in West Africa. *PloS one, 11*(12), e0166805-e0166805. doi:10.1371/journal.pone.0166805
91. Taylor, P. N., Albrecht, D., Scholz, A., Gutierrez-Buey, G., Lazarus, J. H., Dayan, C. M., & Okosieme, O. E. (2018). Global epidemiology of hyperthyroidism and hypothyroidism. *Nat Rev Endocrinol, 14*(5), 301-316. doi:10.1038/nrendo.2018.18
92. Thaimuta, Z. L., Sekadde-Kigondu, C., & Makawiti, D. W. (2010). Thyroid function among HIV/AIDS patients on highly active anti-retroviral therapy. *East Afr Med J, 87*(12), 474-480.
93. Thongam, Keithelakpam, S., Singh, T. Y., Singh, R. L., Singh, A. M., & Ranabir, S. (2015). Thyroid dysfunction in human immunodeficiency virus-infected children and its correlation with CD4(+) T lymphocyte count. *Indian J Endocrinol Metab, 19*(2), 272-276. doi:10.4103/2230-8210.149321
94. Thongam, S., Keithelakpam, S., Singh, T. Y., Singh, R. L., Singh, A. M., & Ranabir, S. (2015). Thyroid dysfunction in human immunodeficiency virus-infected children and its correlation with CD4(+) T lymphocyte count. *Indian J Endocrinol Metab, 19*(2), 272-276. doi:10.4103/2230-8210.149321
95. Toft, A. D., & Beckett, G. J. (2003). Thyroid function tests and hypothyroidism. *BMJ (Clinical research ed.), 326*(7384), 295-296.
96. Tuncel, M. (2017). Thyroid Stimulating Hormone Receptor. [Tiroid Stimulan Hormon Reseptörü]. *Molecular imaging and radionuclide therapy, 26*(Suppl 1), 87-91. doi:10.4274/2017.26.suppl.10
97. Verma, R. K., Giri, R., Gupta, C., & Srivastava, V. (2017). Study of thyroid profile in seropositive HIV adults patients on HAART regimen. *2017, 4*(4), 7. doi:10.18203/2349-3933.ijam20173238
98. Wamalwa, D. C., Farquhar, C., Obimbo, E. M., Selig, S., Mbori-Ngacha, D. A., Richardson, B. A., . . . John-Stewart, G. (2007). Early response to highly active antiretroviral therapy in HIV-1-infected Kenyan children. *J Acquir Immune Defic Syndr, 45*(3), 311-317. doi:10.1097/QAI.0b013e318042d613
99. Wang, F., Tan, Y., Wang, C., Zhang, X., Zhao, Y., Song, X., . . . Zhao, J. (2012). Thyroid-stimulating hormone levels within the reference range are associated with serum lipid profiles independent of thyroid hormones. *J Clin Endocrinol Metab, 97*(8), 2724-2731. doi:10.1210/jc.2012-1133
100. Wong, J. K., & Yukl, S. A. (2016). Tissue reservoirs of HIV. *Current opinion in HIV and AIDS, 11*(4), 362-370. doi:10.1097/COH.0000000000000293
101. Zaid, D., & Greenman, Y. (2019). Human Immunodeficiency Virus Infection and the Endocrine System. *Endocrinology and metabolism (Seoul, Korea), 34*(2), 95-105. doi:10.3803/EnM.2019.34.2.95
102. Zanoni, B. C., Sunpath, H., & Feeney, M. E. (2012). Pediatric response to second-line antiretroviral therapy in South Africa. *PloS one, 7*(11), e49591-e49591. doi:10.1371/journal.pone.0049591

APPENDICES

Appendix 1: Consent Form

Written consent for study participants

1. Introduction

Greetings, my name are Alfred Juma. I am conducting a study entitled "Evaluation of serum levels of lipids and Thyroid hormones in the serum of HIV infected patients on Highly Active Antiretroviral Therapy at JOOTRH in Kisumu County, Kenya" This is a necessary requirement for fulfillment of my postgraduate studies in MSc. Medical Physiology.

This study requires you to participate so that important information can be obtained from you regarding your health.

2. Purpose of research:

The purpose of the study is to determine Levels of thyroid function hormones and lipid profiles among patients at JOOTRH in Kisumu County, Kenya

3. What we will do:

If you agree to participate in the study, your demographic and medical history will be collected. Your anthropometric data for Body Mass Index (BMI) will be collected by measuring your height and weight. All the information will be secured in the lockable cabinets. The following day, you will be requested to report to the clinic in fasting state (do not eat anything in the morning) for laboratory investigations. Some blood sample (about 5 mls) will also be collected from you by a qualified laboratory technologist and will take less than 5 minutes.

4. Potential benefits:

Participants with elevated lipid profile and thyroid function hormones values will be referred to the clinician for immediate appropriate treatment. Those with risky lifestyle like smoking, excessive alcohol intake will be referred to the counselor for appropriate lifestyle modification counseling.

5. Potential risks:

Blood will be collected using aseptic techniques by a highly competent laboratory technologist. There might be minimal discomfort during blood collection process. Demographic, medical history and anthropometric data will be collected using questionnaires which will take limited time to fill-in.

6. Privacy and confidentiality:

The high level of privacy and confidentiality will be maintained throughout the research. The data collected will be stored in locked cabinets and on password-protected computers. The information collected will be used for research only. The name of participant will not appear anywhere in the research tools. At the end of the study, any identifiers will be removed from the data collection tools to avoid any form of identification

7. Your rights to participate or withdraw:

Participation in this study is completely voluntary. You can withdraw from this study at any time. Any participant who withdraws from the study or refuses to participate will not be disadvantaged in any way.

8. Contact information for questions and concerns:

If you have any questions about this study or feel that you have been harmed in this study, you can contact the researcher on 0723129682 or send an email to alfredjuma2015@yahoo.com or

post a letter to P.O. BOX 341, SONDU. If you want to talk about the study with someone who is not directly involved with this study or have questions about your rights, please contact the Secretary, Maseno University Ethics Review Committee, Private Bag, Maseno; Telephone numbers: 057-51622, 0722203411, 0721543976, 0733230878; Email address: muerc-secretariate@maseno.ac.ke; muerc-secretariate@gmail.com.

Do you agree? (Tick as appropriate)

Participant agrees………….. Participant does NOT agree……………………

That I have read/been told of the contents of this form and understood its meaning; hence, I do agree to participate in this study.

Participant ………………………………….. Sign ……………….. Date..........................

Researcher ………………………………. Sign ……………………Date..........................

Appendix 2: Questionnaire for data collection

Part 1: Introduction

This questionnaire is meant to collect data about the Lipids profile and Thyroid hormones levels in the serum of HIV-infected patients at JOOTRH in Kisumu County, Kenya. The respondents required are patients that are considered eligible for this study and have consented. The information will be treated with high level of confidentiality and used solely for academic purposes. Thank you in advance for taking your time to fill in this questionnaire.

Part 2: Demographic, Medical and Anthropometric data

1. Age
   a) 18-30 years b) above 30- 40 years c) above 40-50 years d) above 50-60 years e) above 61 years
2. Gender
   a) Male () b) Female ()
3. The HAART regimen options
   a) ZDV/3TC/EFV c) TDF/3TC/NVP e) TDF/3TC/DTG
   b) AZT/3TC/NVP d) TDF/3TC/EFV f) N/A (HAART-naive)
4. Anthropometric data

Height (cm)	Weight	BMI (calculated)

Part 3: Laboratory results

a) Lipid profile

Parameter (mg/dl)	TC	TG	HDL	LDL
Value				

b) Thyroid Function hormones

Parameter	TSH	T3	T4
Value			

Appendix 3: ichroma TSH protocol

Document No. : INS-TS-EN (Rev. 08)
Revision date : November 4, 2016

ichroma™ TSH

INTENDED USE

ichroma™ TSH is a fluorescence Immunoassay (FIA) for the quantitative determination of TSH in human serum/plasma. It is useful as an aid in management and monitoring of measurement in the assessment of thyroid function.

For *in vitro* diagnostic use only.

INTRODUCTION

The determination of serum or plasma levels of thyroid stimulating hormone (TSH or thyrotropin) is recognized as an important measurement in the assessment of thyroid function. Thyroid stimulating hormone is secreted by the anterior lobe of the pituitary gland, and induces the production and release of thyroxine (T4) and triiodothyronine (T3) from the thyroid gland. It is a glycoprotein with a molecular weight of approximately 28,000 daltons, consisting of two chemically different subunits, alpha and beta. Although the concentration of TSH in the blood is extremely low, it is essential in the maintenance of normal thyroid function. The release of TSH is regulated by a TSH-releasing hormone (TRH) produced by the hypothalamus. The levels of TSH and TRH are inversely related to the level of thyroid hormone. When there is a high level of thyroid hormone in the blood, less TRH is released by the hypothalamus, so less TSH is secreted by the pituitary. The opposite action will occur when there are decreased levels of thyroid hormones in the blood. This process, known as a negative feedback mechanism, is responsible for maintaining the proper blood levels of these hormones.

PRINCIPLE

The test uses a sandwich immunodetection method; the detector antibody in buffer binds to antigen in sample, forming antigen-antibody complexes, and migrates onto nitrocellulose matrix to be captured by the other immobilized-antibody on test strip.

The more antigen in sample forms the more antigen-antibody complex and leads to stronger intensity of fluorescence signal on detector antibody, which is processed by instrument for ichroma™ tests to show TSH concentration in sample.

COMPONENTS

ichroma™ TSH consists of 'Cartridges', 'Detection Buffer Vial', 'Sample Mixing Tubes' and an 'ID chip'.

- The cartridge contains a test strip, the membrane which has anti human TSH at the test line, while streptavidin at the control line.
- Each cartridge is individually sealed in an aluminum foil pouch containing a desiccant. 25 sealed cartridges are packed in a box which also contains an ID chip.
- The detection buffer contains anti human TSH-fluorescence conjugate, biotin-BSA-fluorescence conjugate, bovine serum albumin (BSA) as a stabilizer and sodium azide in phosphate buffered saline (PBS) as a preservative.
- The detection buffer is dispensed in a vial. Detection buffer vial is packed in a Styrofoam box with ice-pack for the shipment.

WARNINGS AND PRECAUTIONS

- For *in vitro* diagnostic use only.
- Carefully follow the instructions and procedures described in this 'Instructions for use'.
- Use only fresh samples and avoid direct sunlight.
- Lot numbers of all the test components (cartridge, ID chip and detection buffer) must match each other.
- Do not interchange the test components between different lots or use the test components after the expiration date, either of which might yield misleading of test result(s).
- Do not reuse. A detection buffer tube should be used for processing one sample only. So should a cartridge.
- The cartridge should remain sealed in its original pouch before use. Do not use the cartridge, if is damaged or already opened.
- Frozen sample should be thawed only once. For shipping, samples must be packed in accordance with the regulations. Sample with severe hemolytic and hyperlipidemia cannot be used and should be recollected.
- Just before use, allow the cartridge, detection buffer and sample to be at room temperature for approximately 30 minutes.
- ichroma™ TSH as well as the instrument for ichroma™ tests should be used away from vibration and/or magnetic field. During normal usage, it can be noted that instrument for ichroma™ tests may produce minor vibration.
- Used detection buffer vial, pipette tips and cartridges should be handled carefully and discarded by an appropriate method in accordance with relevant local regulations.
- An exposure to larger quantities of sodium azide may cause certain health issues like convulsions, low blood pressure and heart rate, loss of consciousness, lung injury and respiratory failure.
- ichroma™ TSH will provide accurate and reliable results subject to the following conditions.
  - Use ichroma™ TSH should be used only in conjunction with instrument for ichroma™ tests.
  - Any anticoagulants other than heparin sodium should be avoided.

STORAGE AND STABILITY

- The cartridge is stable for 20 months (while sealed in an aluminum foil pouch) if stored at 4-30 °C.
- The detection buffer dispensed in a vial is stable for 20 months if stored at 2-8 °C.
- After the cartridge pouch is opened, the test should be performed immediately.

LIMITATION OF THE TEST SYSTEM

- The test may yield false positive result(s) due to the cross-reactions and/or non-specific adhesion of certain sample components to the capture/detector antibodies.
- The test may yield false negative result. The non-responsiveness of the antigen to the antibodies is most common where the epitope is masked by some unknown components, so as not to be detected or captured by the antibodies. The instability or degradation of the antigen with time and/or temperature may cause the false negative as it makes antigen unrecognizable by the antibodies.
- Other factors may interfere with the test and cause erroneous results, such as technical/procedural errors, degradation of the test components/reagents or presence of interfering substances in the test samples.
- Any clinical diagnosis based on the test result must be supported by a comprehensive judgment of the concerned physician including clinical symptoms and other relevant test results.

MATERIALS SUPPLIED

REF CFPC-22

Components of ichroma™ TSH

- Cartridge Box:
  - Cartridges 25
  - ID Chip 1
  - Instruction For Use 1
  - Sample Mixing Tubes 25
- Detection Buffer Vial 1

양식-GE02-15 (Rev .03)

1 / 3

MATERIALS REQUIRED BUT SUPPLIED ON DEMAND

Following items can be purchased separately from ichroma™ TSH.
Please contact our sales division for more information.

- Instrument for ichroma™ tests
  - ichroma™ Reader REF FR203
  - ichroma™ II REF FPRR021
  - ichroma™ D REF 13303
- ichroma™ Printer REF FPRR007
- Boditech Hormone Control REF CFPO-95

SAMPLE COLLECTION AND PROCESSING

The sample type for ichroma™ TSH is human serum/plasma.

- It is recommended to test the sample within 24 hours after collection.
- The serum or plasma should be separated from the clot by centrifugation within 3 hours after the collection of whole blood. If longer storage is required, e.g. if the test could not be performed within 24 hours, serum or plasma should be immediately frozen below -20 °C. The freezing storage of sample up to 3 months does not affect the quality of results.
- Once the sample was frozen, it should be used one time only for test, because repeated freezing and thawing can result in the change of test values.

TEST SETUP

- Check the contents of ichroma™ TSH: Sealed Cartridge, Detection Buffer Vial, Sample Mixing Tubes and ID Chip.
- Ensure that the lot number of the cartridge matches that of the ID chip as well as the detection buffer.
- Keep the sealed cartridge (if stored in refrigerator) and the detection buffer tube at room temperature for at least 30 minutes just prior to the test. Place the cartridge on a clean, dust-free and flat surface.
- Turn on the instrument for ichroma™ tests.
- Insert the ID Chip into the ID chip port of the instrument for ichroma™ tests.
- Press the 'Select' button on the instrument for ichroma™ tests. (Please refer to the 'Instrument for ichroma™ tests Operation Manual' for complete information and operating instructions.)

TEST PROCEDURE

1) Transfer 150 μL (Human serum/plasma/control) of sample using a transfer pipette to a sample mixing tube.
2) Add 75 μL detection buffer to the sample mixing tube containing sample (serum/plasma/control).
3) Close the lid of the sample mixing tube and mix the sample thoroughly by shaking it about 10 times. (The sample mixture must be used immediately.)
4) Pipette out 75 μL of a sample mixture and load it into the sample well on the cartridge.
5) Leave the sample-loaded cartridge at room temperature for 12 minutes.
   ⚠ *Scan the sample-loaded cartridge immediately when the incubation time is over. If not, it will cause inexact test result.*
6) To scan the sample-loaded cartridge, insert it into the cartridge holder of the instrument for ichroma™ tests. Ensure proper orientation of the cartridge before pushing it all the way inside the cartridge holder. An arrow has been marked on the cartridge especially for this purpose.
7) Press 'Select' button on the instrument for ichroma™ tests to start the scanning process.
8) Instrument for ichroma™ tests will start scanning the sample-loaded cartridge immediately.
9) Read the test result on the display screen of the instrument for ichroma™ tests.

INTERPRETATION OF TEST RESULT

- Instrument for ichroma™ tests calculates the test result automatically and displays TSH concentration of the test sample in terms of μIU/mL.
- The cut-off (reference value)

		TSH(μIU/mL)
Gestation and Childhood	0 day	1.0-39.0
	5 days	1.7-9.1
	1 years	0.4-8.6
	2 years	0.4-7.6
	3 years	0.3-6.7
	4-19 years	0.4-6.2
Adults	20-54 years	0.4-4.2
	55-87 years	0.5-8.9
Pregnancy	1st Trimester	0.3-4.5
	2nd Trimester	0.5-4.6
	3rd Trimester	0.8-5.2

- Working range : 0.1-100 μIU/mL

QUALITY CONTROL

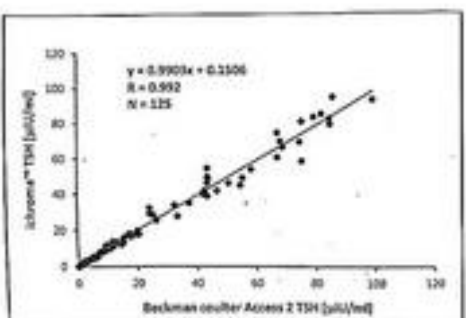
- Quality control tests are a part of the good testing practice to confirm the expected results and validity of the assay and should be performed at regular intervals.
- The control tests should be performed immediately after opening a new test lot to ensure the test performance is not altered.
- Quality control tests should also be performed whenever there is any question concerning the validity of the test results.
- Control materials are not provided with ichroma™ TSH. For more information regarding obtaining the control materials, contact Boditech Med Inc.'s Sales Division for assistance. (Please refer to the instruction for use of control material.)

PERFORMANCE CHARACTERISTICS

- **Specificity:** There, in test samples, are biomolecules such as LH (300 mIU/mL), FSH (200 mIU/mL), and hCG (200,000 mIU/mL) in higher concentration than their normal physiological levels. But this doesn't interfere with the ichroma™ TSH test measurements, nor occurs any significant cross-reactivity.
- **Hook Effect:** No high dose hook effect is observed in this assay at TSH concentrations up to 500 μIU/mL.
- **Precision:** The intra-assay precision was calculated by one evaluator, who tested different concentration of control standard ten times each with three different lots of ichroma™ TSH. The inter-assay precision was confirmed by 3 different evaluators with 3 different lots, testing five times each different concentration.

TSH (μIU/mL)	Intra-assay		Inter-assay	
	Mean	CV (%)	Mean	CV (%)
0.25	0.24	16.35	0.23	17.40
0.5	0.50	11.50	0.51	12.17
2	2.02	6.10	2.07	4.87
5	5.02	4.61	4.96	4.10
20	20.58	5.87	20.28	5.01
50	50.44	3.84	50.50	5.60

- **Comparability:** TSH concentrations of 125 serum samples were quantified independently with ichroma™ TSH and Beckman Coulter Access2 as per prescribed test procedures. Test results were compared and their comparability was investigated with linear regression and coefficient of correlation (R). Linear regression and coefficient of correlation between the two tests were Y=0.9903X + 0.1506 and R = 0.992 respectively.



REFERENCES

1. Marshaal, J.C.: Clinic. In Endocrinol. Metab, 1975, 4:545.
2. Burger, H. G., Patel, Y. C. Thyrotropin releasing hormone-TSH. J. Clinic. Endocrinol. Metab. 1977, 6:831-00.
3. Jeffcoate, S.L.: Clinic. In Endocrinol. Metab. 1975, 4:521.
4. Cohen, K.L.: Metabolism, 1977, 26:1165.
5. Pierce, J. G. Endocrinology. 1971, 89:1331-1344.
6. Berger, S. and Quinn, J.L., Fund. Clin. Chem., N. W. Tietz(ed.), W. B. Saunders Co., Phila., PA 14, 824-848(1976).
7. Lundy, L.E., Lee, S.G., Levy, W., et al. Obstet. Gynecol. 1974, 44:14
8. Utiger, R. D., The Thyroid, S.C. Werner and S. H. Ingbar(eds.), Harper and Row, Hagerstown, MD, 1978, 9:196-205.
9. Clinical Guide to Laboratory Tests. Ed. N.W. Tietz, 3rd Ed., W.B. Saunders Company, Philadelphia, PA 19106, 1995

Note: Please refer to the table below to identify various symbols

Symbol	Meaning
	Sufficient for <n> tests
	Read instruction for use
	Use by Date
	Batch code
	Catalog number
	Caution
	Manufacturer
	Authorized representative of the European Community
	In vitro diagnostic medical device
	Temperature limit
	Do not reuse
CE	This product fulfills the requirements of the Directive 98/79/EC on in vitro diagnostic medical devices

For technical assistance; please contact:
Boditech Med Inc.'s Technical Services
Tel: +82 33 243-1400
E-mail: sales@boditech.co.kr

Boditech Med Incorporated
43, Geodudanji 1-gil, Dongnae-myeon,
Chuncheon-si, Gang-won-do, 24398
Republic of Korea
Tel: +(82) -33-243-1400
Fax: +(82) -33-243-9373
www.boditech.co.kr

Obelis s.a
Bd. Général Wahis 53,
1030 Brussels, BELGIUM
Tel: +(32) -2-732-59-54
Fax: +(32) -2-732-60-03
E-Mail: mail@obelis.net

Appendix 4: ichroma T3

Document No. : INS-T3-EN (Rev. 09)
Revision date : February 21, 2017

ichroma™ T3

INTENDED USE

Ichroma™ T3 is a fluorescence Immunoassay (FIA) for the quantitative determination of triiodothyronine (total T3) in human serum/plasma. It is useful as an aid in management and monitoring of determination of thyroid disorders.

For *in vitro* diagnostic use only.

INTRODUCTION

3,5,3' Triiodothyronine (T3) is a thyroid hormone with a molecular weight of 651 daltons.[1]

T3 circulates in the blood as an equilibrium mixture of free and protein bound hormone.[2] T3 is bound to thyroxin binding globulin (TBG), prealbumin, and albumin. The actual distribution of T3 among these binding proteins is controversial as estimates range from 38-80 % for TBG, 9-27 % for prealbumin, and 11-35 % for albumin.[3]

T3 plays an important role in the maintenance of the euthyroid state. T3 measurements can be a valuable component in diagnosing certain disorders of thyroid function.[4] Most reports indicate that T3 levels distinguish clearly between euthyroid and hyperthyroid subjects, but *provide a less clear-cut separation between hypothyroid and euthyroid subjects.[5] Total T3 measurements may be valuable when* hyperthyroidism is suspected and the free T4 is normal.[6] For example, one recognized type of thyroid dysfunction is T3 thyrotoxicosis, associated with a decrease in serum thyroid stimulating hormone (TSH), increased T3 level, normal T4, normal free T4, and normal to increase in vitro Uptake results.[7-11]

T3 levels are affected by conditions which affect TBG concentration.[12-14] Slightly elevated T3 levels may occur in pregnancy or during estrogen therapy, while depressed levels may occur during severe illness, renal failure, myocardial infarction, alcoholism, inadequate nutritional intake, and during therapy with some medications such as dopamine, glucocorticoids, methimazone, propranolol, propylthiouracil, and salicylates.[6,15,16]

Numerous conditions unrelated to thyoid disease may cause abnormal T3 values.[5,17-20] Consequently, total T3 values should not be used on their own in establishing the thyroid status of an individual. The level of serum T4, TSH and other clinical findings must be considered as well.

PRINCIPLE

The test uses a competitive immunodetection method.

In this method, the target material in the sample binds to the fluorescence (FL)-labeled detection antibody in detection buffer, to form the complex as sample mixture. This complex is loaded to migrate onto the nitrocellulose matrix, where the covalent couple of T3 and bovine serum albumin (BSA) is immobilized on a test strip, and interferes with the binding of target material and FL-labeled antibody. If the more target material exists in blood, the less detection antibody is accumulated, resulting in the less fluorescence signal.

COMPONENTS

Ichroma™ T3 consists of 'Cartridges', 'Solution A Tubes', 'Solution B Vial 'and an 'ID chip'.

- The cartridge contains a test strip, the membrane which has T3-BSA at the test line, while chicken IgY at the control line.
- Each cartridge is individually sealed in an aluminum foil pouch containing a desiccant. 25 sealed cartridges are packed in a box which also contains an ID chip.
- The solution A pre-dispensed in a tube contains ANS and sodium azide in NaOH solution.
- The solution B is dispensed in a vial contains anti human T3-fluorescence conjugate, anti chicken IgY-fluorescence conjugate, bovine serum albumin (BSA) as a stabilizer and less than 0.1% sodium azide as a preservative in phosphate buffer.
- The solution A, B are packaged together in a single box. The box will be placed in a Styrofoam box with ice pack for shipping.

WARNINGS AND PRECAUTIONS

- For *in vitro* diagnostic use only.
- Carefully follow the instructions and procedures described in this 'Instruction for use'.
- Use only fresh samples and avoid direct sunlight.
- Lot numbers of all the test components (cartridge, ID chip and solution A & B) must match each other.
- Do not interchange the test components between different lots or use the test components after the expiration date, either of which might yield misleading of test result(s).
- Do not reuse. A solution A tube should be used for processing one sample only. So should a cartridge.
- The cartridge should remain sealed in its original pouch before use. Do not use the cartridge, if is damaged or already opened.
- Frozen sample should be thawed only once. For shipping, samples must be packed in accordance with the regulations. Sample with severe hemolytic and hyperlipidemia cannot be used and should be recollected.
- Just before use, allow the cartridge, solution A, solution B and sample to be at room temperature for approximately 30 minutes.
- Ichroma™ T3 as well as the instrument for ichroma™ tests should be used away from vibration and/or magnetic field. During normal usage, it can be noted that instrument for ichroma™ tests may produce minor vibration.
- Used solution A, B, pipette tips and cartridges should be handled carefully and discarded by an appropriate method in accordance with relevant local regulations.
- An exposure to larger quantities of sodium azide may cause certain health issues like convulsions, low blood pressure and heart rate, loss of consciousness, lung injury and respiratory failure.
- Ichroma™ T3 will provide accurate and reliable results subject to the following conditions.
  - Use ichroma™ T3 should be used only in conjunction with instrument for ichroma™ tests.
  - Any anticoagulants other than sodium heparin should be avoided.

STORAGE AND STABILITY

- The cartridge is stable for 20 months (while sealed in an aluminum foil pouch) if stored at 4-30 °C.
- The solution A pre-dispensed in a tube is stable for 20 months if stored at 2-8 °C.
- The solution B dispensed in a vial is stable for 20 months if stored at 2-8 °C.
- Opened Solution B is stable for 12 months at 2-8 °C if kept in the capped original container and free from contaminations.
- After the cartridge pouch is opened, the test should be performed immediately.

LIMITATION OF THE TEST SYSTEM

- The test may yield false positive result(s) due to the cross-reactions and/or non-specific adhesion of certain sample components to the capture/detector antibodies.
- The test may yield false negative result. The non-responsiveness of the antigen to the antibodies is most common where the

epitope is masked by some unknown components, so as not to be detected or captured by the antibodies. The instability or degradation of the antigen with time and/or temperature may cause the false negative as it makes antigen unrecognizable by the antibodies.

- Other factors may interfere with the test and cause erroneous results, such as technical/procedural errors, degradation of the test components/reagents or presence of interfering substances in the test samples.
- Any clinical diagnosis based on the test result must be supported by a comprehensive judgment of the concerned physician including clinical symptoms and other relevant test results.

MATERIALS SUPPLIED

REF CFPC-44

Components of ichroma™ T3

- Cartridge Box:
  - Cartridges 25
  - ID Chip 1
  - Instruction For Use 1
- Box containing Solution A, B
  - Solution A tubes 25
  - Solution B Vial (3 mL) 1

MATERIALS REQUIRED BUT SUPPLIED ON DEMAND

Following items can be purchased separately from ichroma™ T3.
Please contact our sales division for more information.

- Instrument for ichroma™ tests
  - ichroma™ Reader REF FR203
  - ichroma™ II REF FPRR021
  - ichroma™ D REF 13303
- ichroma™ Printer REF FPRR007
- Boditech Hormone Control REF CFPO-95

SAMPLE COLLECTION AND PROCESSING

The sample type for ichroma™ T3 is human serum/plasma.

- It is recommended to test the sample within 24 hours after collection.
- The serum or plasma should be separated from the clot by centrifugation within 3 hours after the collection of whole blood.
- Samples may be stored for up to a month at 2-8 °C prior to being tested. If testing will be delayed more than a month, samples should be frozen at -20 °C.
- Samples stored frozen at -20 °C for 2 months showed no performance difference.
- Once the sample was frozen, it should be used one time only for test, because repeated freezing and thawing can result in the change of test values.

TEST SETUP

- Check the contents of ichroma™ T3: Sealed Cartridge, Solution A Tubes, Solution B Vial and ID Chip.
- Ensure that the lot number of the cartridge matches that of the ID chip as well as the solution A & B.
- Keep the sealed cartridge (if stored in refrigerator), solution A and solution B at room temperature for at least 30 minutes just prior to the test. Place the cartridge on a clean, dust-free and flat surface.
- Turn on the instrument for ichroma™ tests.
- Insert the ID Chip into the ID chip port of the instrument for ichroma™ tests.
- Press the 'Select' button on the instrument for ichroma™ tests. (Please refer to the 'Instrument for ichroma™ tests Operation Manual' for complete information and operating instructions.)

CAUTION

- To minimize erroneous test results, we suggest that the ambient temperature of the cartridge should be 25 °C during the reaction time after loading sample mixture to the cartridge.
- To maintain the ambient temperature to 25 °C, you can use various devices such as an i-Chamber or an incubator and so on.

TEST PROCEDURE

1) Transfer 75 μl of sample (Human serum/plasma/control) using a transfer pipette to a tube containing the solution A (yellow tube).
2) Close the lid of the solution A tube and mix the sample thoroughly by shaking it about 10 times. (The sample mixture must be used immediately.)
3) Add 75 μl of Solution B using a transfer pipette with new tip to the tube containing the solution A and sample mixture.
4) Close the lid of the solution A tube and mix the sample thoroughly by shaking it about 10 times.
5) Incubate the solution A + Solution B + sample mixture at room temperature for 8 minutes.
6) Pipette out 75 μL of a sample mixture and load it into the sample well on the cartridge.
7) Insert the sample-loaded cartridge into the slot of the i-Chamber or an incubator (25 °C).
8) Leave the sample-loaded cartridge in the i-Chamber or an incubator for 8 minutes.
   ⚠ *Scan the sample-loaded cartridge immediately when the incubation time is over. If not, it will cause inexact test result.*
9) To scan the sample-loaded cartridge, insert it into the cartridge holder of the instrument for ichroma™ tests. Ensure proper orientation of the cartridge before pushing it all the way inside the cartridge holder. An arrow has been marked on the cartridge especially for this purpose.
10) Press 'Select' button on the instrument for ichroma™ tests to start the scanning process.
11) Instrument for ichroma™ tests will start scanning the sample-loaded cartridge immediately.

INTERPRETATION OF TEST RESULT

- Instrument for ichroma™ tests calculates the test result automatically and displays T3 concentration of the test sample in terms of ng/mL and nmol/L.
- The cut-off (reference range)

Age group of the subject			ng/mL	nmol/L (SI unit)
Adult			0.8–2.0	1.23–3.08
Pediatric Ranges	1-10 years		0.82–2.82	1.26–4.34
	11-15 years	Male	0.8–2.33	1.23–3.59
		Female	0.6–2.09	0.92–3.22
	16-17 years	Male	0.71–2.12	1.09–3.27
		Female	0.61–1.51	0.94–2.33

- Working range : 0.5-5.0 ng/mL (0.77-7.7 nmol/L)
- Conversion factor as unit of nmol/L
  - nmol/L (SI unit) = 1.54 × ng/mL
  - ng/dl = 100 × ng/mL

QUALITY CONTROL

- Quality control tests are a part of the good testing practice to confirm the expected results and validity of the assay and should be performed at regular intervals.
- The control tests should be performed immediately after opening a new test lot to ensure the test performance is not altered.
- Quality control tests should also be performed whenever there is any question concerning the validity of the test results.
- Control materials are not provided with ichroma™ T3. For more information regarding obtaining the control materials, contact Boditech Med Inc.'s Sales Division for assistance. (Please refer to the instruction for use of control material.)

PERFORMANCE CHARACTERISTICS

- **Analytical sensitivity**

Limit of Blank (LoB)	0.15 ng/mL
Limit of Detection (LoD)	0.25 ng/mL
Limit of Quantitation (LoQ)	0.50 ng/mL

- **Analytical specificity**

- Cross reactivity

There, in test samples, are biomolecules such as below the table were added to the test sample(s) at concentrations much higher than their normal physiological levels in blood. **ichroma™ T3** test results did not show any significant cross-reactivity with these biomolecules.

Cross reactants	Concentration	Cross reactivity (%)
D-thyroxine	300 ng/ml	0.19
L-thyroxine	300 ng/ml	0.19
Reverse T3	500 ng/ml	0.08
Salicylic acid	1,000,000 ng/ml	ND
Monoiodotyrosine	50,000 ng/ml	ND

* ND : Not Detected

- Interference

Study of interference from table below with **ichroma™ T3** showed following results. EDTA (K_2) and sodium citrate as an anticoagulants, have effects on **ichroma™** T3 test in the procedure.

Interference materials	Concentration	Interference (%)
D-glucose	60 mM/L	< 0.7
L-Ascorbic acid	0.2 mM/L	< 0.8
Bilirubin	0.4 mM/L	< 0.1
Hemoglobin	2 g/L	< 0.1
Cholesterol	13 mM/L	< 5.5
triglyceride	10 mg/ml	< 2.3
EDTA_K_2	10.8 mg/ml	< 16.2
Sodium Heparin	54 mg/ml	< 1.1
Sodium Citrate	40 mg/ml	< 14.8

- **Precision**

- Between Lot

One person tested three different lots of **ichroma™ T3**, twenty times at each concentration of the control standard.

- Between person

Three different persons tested **ichroma™ T3**, three times at each concentration of the control standard.

- Between Lot

One person tested **ichroma™ T3** during five days, three times at each concentration of the control standard.

- Between Lot

One person tested **ichroma™** T3 at three different sites, three times at each concentration of the control standard.

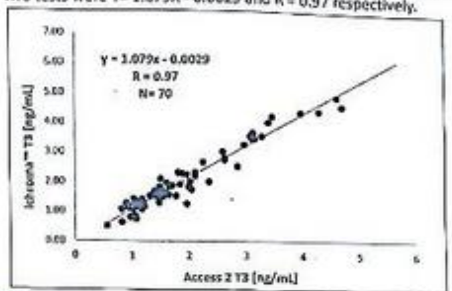
Conc. (ng/mL)	between Lot		between person		between day		between site	
	mean	CV (%)	mean	CV (%)	mean	CV (%)	mean	CV (%)
0.7	0.69	10.3	0.71	10.0	0.70	8.90	0.77	8.7
1.5	1.52	6.3	1.53	4.1	1.52	4.70	1.59	4.7
4.0	4.09	4.5	4.03	3.4	4.12	4.10	4.13	2.8

- **Accuracy**

The accuracy was confirmed by testing with 3 different lots of **ichroma™ T3**. The tests are repeated 3 times in each different concentration.

Conc. (ng/mL)	Lot 1	Lot 2	Lot 3	Mean (ng/mL)	Recovery (%)
4.0	4.19	3.98	4.17	4.11	102.8
3.67	3.52	3.56	3.54	3.54	96.4
3.34	3.26	3.26	3.31	3.27	98.0
3.01	3.13	3.18	2.89	3.07	102.1
2.68	2.67	2.75	2.71	2.71	101.2
2.35	2.24	2.52	2.44	2.40	102.5
2.02	2.1	1.92	1.98	2.00	98.8
1.6	1.75	1.6	1.73	1.70	100.5
1.36	1.42	1.44	1.38	1.41	106.4
1.03	1.07	0.99	1.05	1.04	101.7

- **Comparability:** T3 concentrations of 70 serum samples were quantified independently with **ichroma™** T3 and Access2 (Beckman Coulter Inc. USA) as per prescribed test procedures. Test results were compared and their comparability was investigated with linear regression and coefficient of correlation (R). Linear regression and coefficient of correlation between the two tests were Y= 1.079X - 0.0029 and R = 0.97 respectively.



REFERENCES

1. O'Neil MJ, editor. The Merck Index. 13th ed. Whitehouse Station, NJ: Merck & Co., Inc., 2001;987-988.
2. Ekins RP. Methods for the measurement of free thyroid hormones. In: Free Thyroid Hormones: Proceedings of the International Symposium Held in Venice, December 1978. Amsterdam: Excerpta Medica; 1979:72-92.
3. Robbins J, Rall JE. The iodine-containing hormones. In: Gray CH, James VHT, eds. Hormones in Blood. Vol 1. 3rd ed. London: Academic Press, 1979;632-667.
4. Demers LM, Spencer CA, eds. Laboratory medicine practice guidelines: laboratory support for the diagnosis and monitoring of thyroid disease. Thyroid. 2003;13:3-126.
5. Hollander CS, Shenkman L. Radioimmunoassay for triiodothyronine and thyroxine. In; Rothfeld B, editor. Nuclear medicine in vitro. Philadelphia: Lippincott, 1974;136-49.
6. Kaplan MM, Larsen PR, Crantz FR, Dzau VJ, Rossing TH, Haddow JE. Prevalence of abnormal thyroid function test results in patients with acute medical illnesses. Am J Med. 1982;72:9-16.
7. Larsen PR. Triiodothyronine: Review of Recent Studies of Its Physiology and Pathophysiology in Man. Metabolism. 1972;21:1073-1092.
8. Klee GG. Clinical usage recommendations and analytical performance goals for total and free triiodothyronine measurements. Clin Chem. 1996;42:155-159.
9. Ivy HK, Wahner HW, Gorman CA. "Triiodothyronine (T3) toxicosis": its role in Graves' disease. Arch Intern Med. 1971;128:529-534.
10. Hollander CS, Mitsuma T, Nihei N, Shenkman L, Burday SZ, Blum M. Clinical and laboratory observations in cases of triiodothyronine toxicosis confirmed by radioimmunoassay. Lancet. 1972;1:609-611.
11. Sterling K, Refetoff S, Selenkow HA. T3 thyrotoxicosis: thyrotoxicosis due to elevated serum triiodothyronine levels. JAMA. 1970;213:571-575.
12. Kaplan MM, Larsen PR, Crantz FR, Dzau VJ, Rossing TH, Haddow JE. Prevalence of abnormal thyroid function test results in patients with acute medical illnesses. Am J Med. 1982;72:9-16.
13. Bermudez F, Surks MI, Oppenheimer JH. High incidence of decreased serum triiodothyronine concentration in patients with nonthyroid disease. J Clin Endocrinol Metab. 1975;41:27-40.
14. Oppenheimer JH. Thyroid function tests in nonthyroidal disease, J Chronic Dis. 1982;35:697-701.

15. Abuid J, Larsen PR. Triiodothyronine and thyroxine in hyperthyroidism: comparison of the acute changes during therapy with antithyroid agents. J Clin Invest. 1974;54:201-208.
16. Felig P, Frohman LA, eds. Endocrinology & Metabolism. 4th ed. New York: McGraw-Hill, Inc., 2001:270-311.
17. Bates HM. Clin Lab Prod 1974;3:16.
18. Utiger RD. Serum triiodothyronine in man. Annu Rev Med 1974;2:289-302.
19. Larson PR. Triiodothyronine: review of recent studies of its physiology and pathophysiology in man. Metabolism 1972;21:1073-92.
20. Oppenheimer JH. Role of plasma proteins in the binding, distribution and metabolism of the thyroid hormones. N Engl J Med 1968;278:1153-62.
21. http://cclnprod.cc.nih.gov/dlm/testguide.nsf/Index/8C30C39D30A6879EB52568A7004F7E9E

Note: Please refer to the table below to identify various symbols

Symbol	Meaning
Σ	Sufficient for <n> tests
📖	Read instruction for use
⌛	Use by Date
LOT	Batch code
REF	Catalog number
⚠	Caution
🏭	Manufacturer
EC REP	Authorized representative of the European Community
IVD	In vitro diagnostic medical device
🌡	Temperature limit
⊗	Do not reuse
CE	This product fulfills the requirements of the Directive 98/79/EC on in vitro diagnostic medical devices

For technical assistance; please contact:
Boditech Med Inc.'s Technical Services
Tel: +82 33 243-1400
E-mail: sales@boditech.co.kr

Boditech Med Incorporated
43, Geodudanji 1-gil, Dongnae-myeon,
Chuncheon-si, Gang-won-do, 24398
Republic of Korea
Tel: +(82) -33-243-1400
Fax: +(82) -33-243-9373
www.boditech.co.kr

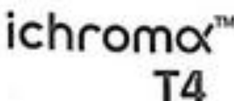
EC REP **Obelis s.a**
Bd. Général Wahis 53,
1030 Brussels, BELGIUM
Tel: +(32) -2-732-59-54
Fax: +(32) -2-732-60-03
E-Mail: mail@obelis.net

CE

Appendix 5: ichoma T4 protocol

Document No. : INS-T4-EN (Rev.12)
Revision date : July 20, 2016

boditech



INTENDED USE

ichroma™ T4 is a fluorescence Immunoassay (FIA) for the quantitative determination of thyroxine (T4) in human serum/plasma. It is useful as an aid in management and monitoring of thyroid disorder. For in vitro diagnostic use only.

INTRODUCTION

Thyroxine (T4) is one of two major hormones produced by the thyroid gland (the other is called triiodothyronine, or T3). T4 and T3 are regulated by a sensitive feedback system involving the hypothalamus and the pituitary gland. The hypothalamus releases the thyrotropin-releasing hormone (TRH), which stimulates the pituitary to release the thyroid stimulating hormone (TSH). This causes the thyroid to release T3 and T4 and these in turn regulate the release of TRH and TSH via a feedback control mechanism. Normally, elevated blood levels of T4 and T3 act to decrease the amount of TSH secreted, thereby reducing the production and release of T4 and T3. Over 99 % of T4 is reversibly bound to three plasma proteins in blood: thyroxine binding globulin (TBG) binds close to 70%, thyroxine binding pre-albumin (TBPA) binds 20%, and albumin binds 10%. Approximately 0.03 % of T4 is in the free, unbound state in blood at any one time.

T4 is a useful marker for the diagnosis of hypothyroidism and hyperthyroidism. The level of T4 decreases in hypothyroidism, myxedema and chronic thyroiditis (Hashimoto's disease). Increased levels of T4 have been found in hyperthyroidism due to Grave's disease and Plummer's disease.

PRINCIPLE

The test uses a competitive immunodetection method.
In this method, the target material in the sample binds to the fluorescence (FL)-labeled detection antibody in detection buffer, to form the complex as sample mixture. This complex is loaded to migrate onto the nitrocellulose matrix, where the covalent couple of T4 and bovine serum albumin (BSA) is immobilized on a test strip, and interferes with the binding of target material and FL-labeled antibody. If the more target material exists in blood, the less detection antibody is accumulated, resulting in the less fluorescence signal.

COMPONENTS

ichroma™ T4 consists of 'cartridges', 'Solution A Tubes', 'Solution B Vial' and an 'ID chip'.

- The cartridge contains a test strip, the membrane which has bovine serum albumin (BSA) conjugated T4 at the test line, while streptavidin at the control line.
- Each cartridge is individually sealed in an aluminum foil pouch containing a desiccant. 25 sealed cartridges are packed in a box which also contains an ID chip.
- The solution A pre-dispensed in a tube contains ANS and sodium azide, NaOH in phosphate buffered saline.
- The solution B is dispensed in a vial contains anti human T4-fluorescence conjugate, biotin-BSA-fluorescence conjugate, bovine serum albumin (BSA) as a stabilizer and sodium azide as a preservative in phosphate buffered saline.
- The solution A, B are packaged together in a single box. The box will be placed in a Styrofoam box with ice pack for shipping.

WARNINGS AND PRECAUTIONS

- For in vitro diagnostic use only.
- Carefully follow the instructions and procedures described in this 'Instruction for use'.
- Use only fresh samples and avoid direct sunlight.
- Lot numbers of all the test components (cartridge, ID chip and solution A & B) must match each other.
- Do not interchange the test components between different lots or use the test components after the expiration date, either of which might yield misleading of test result(s).
- Do not reuse. A solution A tube should be used for processing one sample only. So should a cartridge.
- The cartridge should remain sealed in its original pouch before use. Do not use the cartridge, if is damaged or already opened.
- Frozen sample should be thawed only once. For shipping, samples must be packed in accordance with the regulations. Sample with severe hemolytic and hyperlipidemia cannot be used and should be recollected.
- Just before use, allow the cartridge, solution A, solution B and sample to be at room temperature for approximately 30 minutes.
- ichroma™ T4 as well as the instrument for ichroma™ tests should be used away from vibration and/or magnetic field. During normal usage, it can be noted that instrument for ichroma™ tests may produce minor vibration.
- Used solution A, B, pipette tips and cartridges should be handled carefully and discarded by an appropriate method in accordance with relevant local regulations.
- An exposure to larger quantities of sodium azide may cause certain health issues like convulsions, low blood pressure and heart rate, loss of consciousness, lung injury and respiratory failure.
- ichroma™ T4 will provide accurate and reliable results subject to the following conditions.
  - Use ichroma™ T4 should be used only in conjunction with instrument for ichroma™ tests.
  - Any anticoagulants other than **sodium heparin, sodium** citrate should be avoided.

STORAGE AND STABILITY

- The cartridge is stable for 20 months (while sealed in an aluminum foil pouch) if stored at 4-30 °C.
- The solution A pre-dispensed in a tube is stable for 20 months if stored at 2-8 °C.
- The solution B dispensed in a vial is stable for 20 months if stored at 2-8 °C.
- Opened Solution B is stable for 12 months at 2-8 °C if kept in the capped original container and free from contaminations.
- After the cartridge pouch is opened, the test should be performed immediately.

LIMITATION OF THE TEST SYSTEM

- The test may yield false positive result(s) due to the cross-reactions and/or non-specific adhesion of certain sample components to the capture/detector antibodies.
- The test may yield false negative result. The non-responsiveness of the antigen to the antibodies is most common where the epitope is masked by some unknown components, so as not to be detected or captured by the antibodies. The instability or degradation of the antigen with time and/or temperature may cause the false negative as it makes antigen unrecognizable by the antibodies.
- Other factors may interfere with the test and cause erroneous results, such as technical/procedural errors, degradation of the test components/reagents or presence of interfering substances in the test samples.
- Any clinical diagnosis based on the test result must be supported by a comprehensive judgment of the concerned physician

양식-GE02-15 (Rev .03)

1 / 3

including clinical symptoms and other relevant test results.

MATERIALS SUPPLIED

REF CFPC-26

Components of ichroma™ T4

- Cartridge Box:
  - Cartridges 25
  - ID Chip 1
  - Instruction For Use 1
- Box containing SolutionA, B
  - Solution A tubes 25
  - Solution B Vial (2.5 mL) 1

MATERIALS REQUIRED BUT SUPPLIED ON DEMAND

Following items can be purchased separately from ichroma™ T4.
Please contact our sales division for more information.

- Instrument for ichroma™ tests
  - ichroma™ Reader REF FR203
  - ichroma™ II REF FPRR021
  - ichroma™ D REF 13303
- ichroma™ Printer REF FPRR007
- Boditech Hormone Control REF CFPO-95

SAMPLE COLLECTION AND PROCESSING

The sample type for ichroma™ T4 is human serum/plasma.

- It is recommended to test the sample within 24 hours after collection.
- The serum or plasma should be separated from the clot by centrifugation within 3 hours after the collection of whole blood. If longer storage is required, e.g. if the test could not be performed within 24 hours, serum or plasma should be immediately frozen below -20 °C. The freezing storage of sample up to 3 months does not affect the quality of results.
- Once the sample was frozen, it should be used one time only for test, because repeated freezing and thawing can result in the change of test values.

TEST SETUP

- Check the contents of ichroma™ T4: Sealed Cartridge, Solution A tube, Solution B Vial and ID Chip.
- Ensure that the lot number of the cartridge matches that of the ID chip as well as the solution A & B.
- Keep the sealed cartridge (if stored in refrigerator), solution A and solution B at room temperature for at least 30 minutes just prior to the test. Place the cartridge on a clean, dust-free and flat surface.
- Turn on the instrument for ichroma™ tests.
- Insert the ID Chip into the ID chip port of the instrument for ichroma™ tests.
- Press the 'Select' button on the instrument for ichroma™ tests. (Please refer to the 'Instrument for ichroma™ tests Operation Manual' for complete information and operating instructions.)

CAUTION

- To minimize erroneous test results, we suggest that the ambient temperature of the cartridge should be 25 °C during the reaction time after loading sample mixture to the cartridge.
- To maintain the ambient temperature to 25 °C, you can use various devices such as an i-Chamber or an incubator and so on.

TEST PROCEDURE

1) Transfer 75 μL of sample (Human serum/plasma/control) using a transfer pipette to a tube containing the solution A (yellow tube).
2) Mix well by pipetting 10 times.
3) Add 75 μL of solution B using a transfer pipette with new tip to the tube containing the solution A and sample mixture.
4) Close the lid of the solution A tube and mix the sample thoroughly by shaking it about 10 times.
5) Incubate the solution A + Solution B + sample mixture at room temperature for 8 minutes.
6) Pipette out 75 μL of a sample mixture and load it into the sample well on the cartridge.
7) Insert the sample-loaded test cartridge into the slot of the i-Chamber or an incubator (25 °C).
8) Leave the sample-loaded test cartridge in the i-Chamber or an incubator for 8 minutes.
   ⚠ *Scan the sample-loaded cartridge immediately when the incubation time is over. If not, it will cause inexact test result.*
9) To scan the sample-loaded cartridge, insert it into the cartridge holder of the instrument for ichroma™ tests. Ensure proper orientation of the cartridge before pushing it all the way inside the cartridge holder. An arrow has been marked on the cartridge especially for this purpose.
10) Press 'Select' button on the instrument for ichroma™ tests to start the scanning process.
11) Instrument for ichroma™ tests will start scanning the sample-loaded cartridge immediately.
12) Read the test result on the display screen of the instrument for ichroma™ tests.

INTERPRETATION OF TEST RESULT

- Instrument for ichroma™ tests calculates the test result automatically and displays T4 concentration of the test sample in terms of nmol/L and μg/dL.
- T4 Conversion factor is 12.87 (nmol/L = 12.87 X μg/dL)
- The cut-off (reference range)

State	Range
Normal value	57.9-150.6 nmol/L

- Working range : 10.23-300.0 nmol/L

QUALITY CONTROL

- Quality control tests are a part of the good testing practice to confirm the expected results and validity of the assay and should be performed at regular intervals.
- The control tests should be performed immediately after opening a new test lot to ensure the test performance is not altered.
- Quality control tests should also be performed whenever there is any question concerning the validity of the test results.
- Control materials are not provided with ichroma™ T4. For more information regarding obtaining the control materials, contact Boditech Med Inc.'s Sales Division for assistance.
  (Please refer to the instruction for use of control material.)

PERFORMANCE CHARACTERISTICS

- Analytical sensitivity

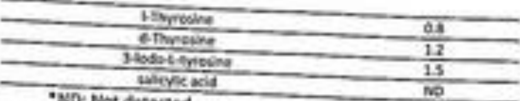
Limit of Blank	(LoB)	6.87 nmol/L
Limit of Detection	(LoD)	9.39 nmol/L
Limit of Quantification	(LoQ)	10.23 nmol/L

- Analytical specificity
  - Cross-reactivity

    There was no significant cross-reactivity from these materials with the ichroma™ T4 test measurements.

Compound (Spiked concentration)	Cross-reactivity (%)
3-Triiodothyronine	3.1
reverse T3	3.5

boditech



l-Thyroxine	0.8
d-Thyrosine	1.2
3-Iodo-L-tyrosine	1.5
salicylic acid	ND

*ND: Not detected

- **Interference**

There was no significant interference from these materials with the **Ichroma™ T4** test measurements.

Compound (Spiked concentration)	Interference (%)
D-glucose (60 mM/L)	3.7
L-Ascorbic acid (0.2 mM/L)	3.9
Bilirubin (0.4 mM/L)	3.5
Hemoglobin (2 g/L)	2.7
Cholesterol (13 mM/L)	8.8
Triglyceride (10 mg/mL)	3.6

- **Precision**

- Intra assay

The intra-assay precision was calculated by one evaluator, who tested different concentration of control standard ten times each with three different lots of Ichroma™ T4.

T4 [nmol/L]	Lot 1	Lot 2	Lot 3	AVG	SD	CV (%)
50	49.63	49.38	53.00	50.66	2.92	5.8
100	108.43	104.04	103.93	105.47	4.63	4.4
150	154.90	155.28	151.44	153.87	6.76	4.4

- Inter-assay

The inter-assay precision was confirmed by 2 different evaluators for 5 days with 3 different lots, testing three times each different concentrations.

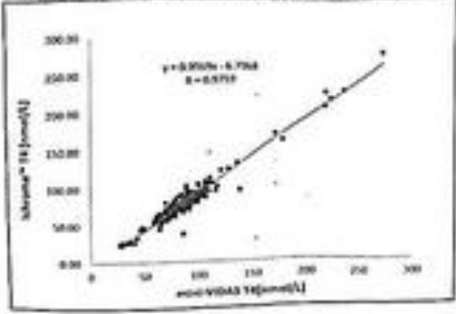
T4 [nmol/L]	Lot 1	Lot 2	Lot 3	AVG	SD	CV (%)
50	51.04	49.09	48.91	49.68	3.01	6.0
100	106.08	108.15	102.17	105.47	5.14	4.9
150	154.18	157.46	151.97	154.54	6.61	4.3

- **Accuracy**

The accuracy was confirmed by 3 different lots testing six times each different concentrations.

T4 [nmol/L]	Lot 1	Lot 2	Lot 3	AVG	SD	CV (%)
12.5	11.18	10.90	11.24	11.11	0.51	4.6
62.5	60.41	63.12	61.00	61.83	2.17	3.5
87.5	81.27	86.04	81.67	82.97	4.96	6.0
125	110.00	122.39	119.54	120.81	6.32	5.2
225	222.19	215.73	214.65	214.82	10.35	4.8

- **Comparability:** T4 concentrations of 143 serum samples were quantified independently with Ichroma™ T4 and mini VIDAS (BioMerieux Inc. France) as per prescribed test procedures. Test results were compared and their comparability was investigated with linear regression and coefficient of correlation (R). Linear regression and coefficient of correlation between the two tests were Y= 0.9969X – 6.7968 and R = 0.9759 respectively.



REFERENCES

1. Thakur C., Saikia T.C. Yadav R.N., Total serum levels of triiodothyronin(T3) thyroxine(T4) and thyrotropine(TSH) in school going children of Dibrugarh district: an endemic goiter region of Assam. *Indian J Physiol Pharmacol*, 1997, 41(2) : 167-170
2. , Larsen P.R., Dockalova J., Sipula D., Wu F. M. Immunoassay of Thyroxine in unextracted Human Serum. *J. Clin. Endocrinol. Metabl.*, 1973, 37(2):177-182
3. Wagner M. S., Wajner S. M., Maia A. L. The *Role of Thyroid* Hormone in testicular Development and Function. *J. Endocrinol.*, 2008, 199(3) : 351-365
4. Wahlin A., Wahlin T. B., Small B. J., Backman L. Influences of thyroid stimulating hormone on cognitive functioning in very old age. *J. Gerontol B. Psychol Sci. Soc. Sci.*, 1998, 5 : 234-239

Note: Please refer to the table below to identify various symbols

Symbol	Meaning
	Sufficient for <n> tests
	Read instruction for use
	Use by Date
LOT	Batch code
REF	Catalog number
	Caution
	Manufacturer
	Authorized representative of the European Community
IVD	In vitro diagnostic medical device
	Temperature limit
	Do not reuse
CE	This product fulfills the requirements of the Directive 98/79/EC on in vitro diagnostic medical devices

For technical assistance; please contact:
Boditech Med Inc.'s Technical Services
Tel: +82 33 243-1400
E-mail: sales@boditech.co.kr

Boditech Med Incorporated
43, Geodudanji 1-gil, Dongnae-myeon,
Chuncheon-si, Gang-won-do, 24398
Republic of Korea
Tel: +(82) -33-243-1400
Fax: +(82) -33-243-9373
www.boditech.co.kr

Obelis s.a
Bd. Général Wahis 53,
1030 Brussels, BELGIUM
Tel: +(32) -2-732-59-54
Fax: +(32) -2-732-60-03
E-Mail: mail@obelis.net

CE

Appendix 6: Ethical Approval

MASENO UNIVERSITY ETHICS REVIEW COMMITTEE

Tel: +254 057 351 622 Ext: 3050
Fax: +254 057 351 221

Private Bag – 40105, Maseno, Kenya
Email: muerc-secretariate@maseno.ac.ke

FROM: Secretary - MUERC

DATE: 19th July, 2019

TO: Juma Omondi Alfred
PG/MSc/SM/00011/2016
Department of Medical Physiology
School of Medicine
Maseno University
P. O. Box, Private Bag, Maseno, Kenya

REF: MSU/DRPI/MUERC/00718/19

RE: Evaluation of Lipid Profile and Thyroid Hormones Levels in the Serum of HIV-Patients on Highly Active Antiretroviral Therapy at Jaramogi Oginga Odinga Teaching and Referral Hospital in Kisumu County, Kenya. Proposal Reference Number MSU/DRPI/MUERC/00718/19

This is to inform you that the Maseno University Ethics Review Committee (MUERC) determined that the ethics issues were adequately addressed in the initial proposal. Consequently, the study is granted approval for implementation effective this 19th day of July, 2019 for a period of one (1) year. This is subject to getting approvals from NACOSTI and other relevant authorities.

Please note that authorization to conduct this study will automatically expire on 18th July, 2020. If you plan to continue with the study beyond this date, please submit an application for continuation approval to the MUERC Secretariat by 15th June, 2020.

Approval for continuation of the study will be subject to successful submission of an annual progress report that is to reach the MUERC Secretariat by 15th June, 2020.

Please note that any unanticipated problems resulting from the conduct of this study must be reported to MUERC. You are required to submit any proposed changes to this study to MUERC for review and approval prior to initiation. Please advice MUERC when the study is completed or discontinued.

Thank you.

Dr. Bernard Guyah
Ag. Secretary,
Maseno University Ethics Review Committee.

Cc: Chairman,
Maseno University Ethics Review Committee.

MASENO UNIVERSITY IS ISO 9001:2008 CERTIFIED