HYPOGONADISM AMONG MALE PATIENTS WITH HIV AT MOI TEACHING AND REFERRAL HOSPITAL, ELDORET, KENYA.

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

DECLARATION

Declaration by Author

This thesis is my original work and has not been presented for award of a degree in any other university or institution.

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DEDICATION

I dedicate this work to my family for their immense support, encouragement and inspiration during the entire period of working on this thesis.

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LIST OF ABBREVIATIONS

AACE	American Association of Clinical Endocrinologists
AIDS	Acquired Immunodeficiency Syndrome
AMPATH	Academic Model Providing Access to Healthcare
BMI	Body Mass Index
CD4+	Cluster of Differentiation 4 positive cells
ES	Endocrine Society
FT	Free Testosterone
GnRH	Gonadotropin Releasing Hormone
HAART	Highly Active Antiretroviral Therapy
HbA1c	Glycated Haemoglobin
IIEF 5	International Index of Erectile Function-5
IREC	Institutional Research Ethics Committee
MTRH	Moi Teaching and Referral Hospital
PHQ 9	Patient Health Questionnaire 9
TT	Total Testosterone

OPERATIONAL DEFINITION OF TERMS

Hypogonadism: Defined by low serum testosterone levels (<300ng/dl or 10.4nmol/L) based on the AACE and multiple scientific study cut offs, with or without symptoms. Symptoms discernible in post pubertal hypogonadism include:

i) Sexual dysfunction- reduced libido, erectile dysfunction, reduced penile sensation, reduced ejaculate or difficulty attaining orgasm
ii) Depression
iii) Irritability
iv) Decreased concentration and cognitive problems
v) Reduced energy, vitality or stamina

Signs may include: Anaemia, muscle wasting, abdominal adiposity (pot belly obesity), reduced bone mass/bone mineral density and/or oligospermia/azoospermia.

HIV/AIDS: HIV is a virus that causes the syndrome of AIDS, which is characterized by a compromised body immunity vulnerable to opportunistic infections e.g., TB, Cryptococcal meningitis, candidiasis and also cancer and multiple endocrine & metabolic derangements.

HAART: A combination of antiretroviral drugs used in suppressing viral activity and replication in patients diagnosed with HIV. They can be used as 1^{st}, 2^{nd} or 3^{rd} line based on patients' response to treatment.

Virally Suppressed: Means patient had either undetectable viral loads or <1,000 copies of HIV RNA/ml when assessed based on Kenyan ART clinical practice guidelines of 2018. It connotes good response to treatment.

Erectile Dysfunction: This was determined based on administration of the IIEF-5 questionnaire which focuses on the erectile domain of sexual function. Scores below 22 (out of a possible 25) were positive for ED and consequently were graded for severity as per the same tool. The details of the IIEF5 are captured as appendix 6 on page 61. This was based on the American Urological Society.

Depression: In this study, depression was assessed using the PHQ-9 which evaluates and grades various severity levels based on the scores realised at the end of the interview. Any positive score on the tool was sufficient to make a diagnosis of the condition. The tool is characterized as appendix 5 on page 60.

Hypertension: Was defined by a systolic Blood Pressure above 140 mmHg or Diastolic BP above 90 mmHg as per the European Society on Hypertension and the Kenya Cardiovascular guidelines 2018.

Tachycardia: This was defined by a pulse rate above 100 beats per minute.

BMI: Body mass Index was categorized as normal if ranging between 18.5-24.9, while below this was considered subnormal or wasting. A BMI of 25-29.9 was labelled as overweight while 30-34.9 as obese and above 35 as morbidly obese.

RBS: A normal range random blood sugar was within limits of 4.0 mmol/L to 7.8 mmol/L based on American Diabetes Association guidelines. A figure above this was considered abnormally high (pre/diabetic) while below was deemed hypoglycaemic.

Smoking: Past or current tobacco use as self-reported by the patient upon asking.

Alcohol Use: Ethanol consumption (past or present) as elicited in the process of questionnaire administration, regardless of type, amount or frequency.

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ABSTRACT

Background: The prevalence of hypogonadism is slightly higher among male patients with HIV compared to the general population. It is a complication that has persisted despite improved health outcomes and prolonged survival with the advent of highly active antiretroviral therapy (HAART). Factors associated include; age, duration of HIV infection, opportunistic infections, malignancies, adverse drug effects, malnutrition and chronic inflammation. If left untreated, hypogonadism may result in erectile dysfunction (ED), depression, wasting and infertility. Early identification and treatment reverse these complications and improves quality of life.

Objective: To determine the prevalence of hypogonadism among male patients with HIV at Moi Teaching and Referral Hospital (MTRH) and to assess them for erectile dysfunction and depression.

Methods: A cross sectional study was conducted among male patients attending HIV clinics at MTRH between March and April 2018. Using a systematic random sampling technique, a total of 182 patients were enrolled into the study. Socio-demographic and clinical data was collected using questionnaires while ED and depression were assessed using the International Index of Erectile function 5 (IIEF-5) and Patient Health Questionnaire 9 (PHQ-9) respectively. Venous blood samples were obtained for laboratory estimation of serum Total Testosterone (TT) levels by direct immunoassay. Hypogonadism was defined by TT < 300 ng/ML. Continuous variables were summarised as means, medians and standard deviations while categorical variables were summarised as frequencies and interquartile ranges. Means were compared using independent samples t-test while medians were compared using Wilcoxon-rank sum test. Association between hypogonadism and categorical variables was analysed using Fischer's exact test.

Results: The mean age of participants was 47 years (SD: 7.8). The average systolic BP was 127 mmHg (SD 17.7). Forty-five patients (24.7%) had a BMI above 25.A total of 122 (67%) patients had been on HAART for at least 5 years. Most participants 162 (89%) were virally suppressed. The prevalence of hypogonadism was 5.5% (95% C.I:2.7, 9.9). Among those with hypogonadism, 40% manifested symptoms consistent with ED while 30% were symptomatic for depression. A small proportion (20%) had both ED and depression. Half of the patients with hypogonadism were asymptomatic. In the overall study population, an incidental proportion finding of 56% for ED and 44% for depression was observed. Advancing age (p=0.421) and duration on HAART (p=0.204) were not associated with hypogonadism. Alcohol use was associated with higher prevalence of hypogonadism (9.5%) compared to (2.0%) among the non-users of alcohol, p = 0.046. Association between hypogonadism and ED (p=0.336) or depression (p=0.516) was not statistically significant.

Conclusion: The prevalence of hypogonadism among male patients with HIV is low. Close to one half of those with hypogonadism present with either ED or depression.

Recommendation: Testing for hypogonadism should be considered among male patients attending HIV clinics at MTRH who present with ED, depression or both upon a high index of suspicion. A study exploring factors associated with ED and depression is recommended.

CHAPTER ONE: INTRODUCTION

1.1 Background

HIV(Human Immunodeficiency Virus) is a disease causing pathogen in humans, first isolated and described in1984 in San Francisco, in the United States of America(Levy et al., 1984). It is known to cause the AIDS (Acquired Immunodeficiency Syndrome) disease, which is a chronic disease characterized by a weakened immunity and an increased predisposition to suffer opportunistic infections. Among many other known complications of HIV/AIDS, is hypogonadism, an endocrine abnormality that involves the hypothalamic pituitary gonadal axis in HIV patients. It was found to be the earliest and most common endocrine abnormality in HIV patients also assessed for thyroid and adrenal function (Dobs, Dempsey, Ladenson, & Polk, 1988)

Sub-Saharan Africa bears the largest burden of HIV/AIDS in the world. According to the WHO (World Health Organization) - Global Health Observatory Data 2018, Sub Saharan Africa accounts for nearly 70% (25.69 million) of the people living with HIV worldwide. According to the WHO Kenya chapter, Kenya had a HIV prevalence of 4.7% with 1.6 million people infected by end of the year 2018.It is among the six countries with the highest burden of HIV/AIDS disease in the world, according to the Centre for disease control and Prevention (CDC). With a high prevalence of HIV/AIDS, it is likely that the prevalence of hypogonadism could equally be high. This has however not been demonstrated in Kenya since there are no studies available on the same.

Additionally, an estimated 68% of the HIV infected population in Kenya are receiving antiretroviral therapy, according to WHO statistics. The UNAIDS launched a 90-90-90 initiative in 2014 to target 90% testing for those infected, 90% access to ART for those HIV positive and 90% viral suppression achieved by 2020. With this target and

a test-and-treat policy in place, this proportion is likely to go up. Access to proper care means more persons living with HIV are having better quality of lives, enjoying prolonged survival and with it the continued likelihood that some complications, especially non-infectious, may come to the fore as opposed to previously when mortality rates were higher and survival shorter (Falutz & Director, 2009; Justice, 2010).

Male hypogonadism is characterized by low testosterone levels (<300ng/dL or 10.4nmol/L). Testosterone is usually produced by the Leydig cells in the testicles after stimulation by Luteinizing Hormone (LH) and Follicle Stimulating Hormone (FSH), which are referred to as gonadotropins. These are released from the gonadotroph cells of the pituitary gland in the brain, after stimulation by the Gonadotropin Releasing Hormone (GnRH) from the hypothalamus. Hypogonadism is categorized as either primary or secondary, depending on the cause. Primary hypogonadism is low testosterone levels due to testicular failure together with elevated LH. Secondary Hypogonadism is due to gonadotropins deficiency in which low testosterone occurs together with low or normal levels of Luteinizing Hormone (LH) and /or Follicle Stimulating Hormone(FSH) (Vincenzo Rochira et al., 2011)

Existing data published on this subject shows a positive association between HIV and low testosterone levels in male patients. However, most of this data is from studies done in the developed countries in Europe and America. There is a huge gap in knowledge on what the situation is in Kenya and Africa at large. No published records are available to inform what the prevalence of this problem is in our country. This in effect means that those affected are least likely to obtain remedy for their condition, since most of it remains undetected.

1.2 Problem statement

Men suffering from HIV infection are at an increased risk of suffering from hypogonadism. This is now increasingly being seen in HIV infected males of a younger age of less than 30 years. Men with hypogonadism have been shown to have a higher prevalence of sexual dysfunction disorders (low libido, erectile dysfunction), depression, loss of lean muscle and fat free body mass (Crum, Furtek, Olson, Amling, & Wallace, 2005).

A recent study done in Portugal in 2016 also correlated low testosterone levels with an increased risk of developing metabolic syndrome and cardiovascular diseases(Gomes et al., 2016).

1.3 Justification

Hypogonadism in men suffering from HIV is still not well understood and few studies have been done in this field. Studies done on this subject show a prevalence of between 20-30% in the era of HAART, but higher prevalence 29-50% before HAART or in patients with advanced HIV disease or wasting(Gomes et al., 2016)

It has remained a diagnostic challenge because signs and symptoms tend to be nonspecific, especially in HIV disease. Most patients may therefore have no symptoms ,despite having the problem(Vincenzo Rochira et al., 2011).This underscores the need for early detection through screening of male HIV patients by measuring their testosterone levels. Appropriate interventions(including testosterone replacement therapy) in those with symptomatic hypogonadism, have been shown to improve quality of life ,lean body mass and overall mental and physical function(Bhasin et al., 2000).This study was done to create awareness on this subject and generate interest in the approach to patients with probable androgen deficiency.

Currently, patients enrolled for care in our set up at the Academic Model Providing Access to Healthcare (AMPATH) in MTRH are not screened for hypogonadism and may therefore remain undiagnosed and continue to bear the associated problems of having the abnormality. Despite HIV being a recognized risk factor for secondary hypogonadism(Vincenzo Rochira et al., 2011), there are no studies that have been done locally & regionally to establish the prevalence of hypogonadism in male patients with HIV.

This study was designed to address this gap in knowledge and furthermore to raise awareness among health personnel who interact with these patients to look out for possible hypogonadism in male patients who particularly maybe having issues with their sexuality or are depressed or have abnormal body mass indices. This is to be considered with a view to improving the quality of life and overall wellbeing of patients beside other routine clinical care protocols.

1.4 Research question

What is the prevalence of hypogonadism among male patients with HIV seeking care at the Moi Teaching and Referral Hospital in Eldoret?

1.5 Objectives

1.5.1 Broad objective

To determine the prevalence and clinical presentation (Erectile Dysfunction and Depression) of hypogonadism among male patients with HIV seeking care at Moi Teaching and Referral Hospital (MTRH)

1.5.2 Specific objectives

i) To determine the prevalence of hypogonadism among male patients with HIV at MTRH.

ii) To assess for erectile dysfunction among male HIV positive patients with hypogonadism.

iii) To assess for depression among male HIV positive patients with hypogonadism.

iv) To establish the proportion of symptomatic hypogonadism among male patients with HIV.

CHAPTER TWO: LITERATURE REVIEW

2.1 Definition

Hypogonadism is defined as decreased functional activity of the gonads resulting in androgen deficiency. In men gonadal activities are performed by testes while in women these are done by ovaries. Gonads (Testes and ovaries) secrete hormones and also produce gametes. The hormones produced by the testes are: Testosterone, Inhibin B, and Mullerian Inhibition Hormone. Male sex hormones are referred to as androgens. The ovaries produce Progesterone and Oestrogen which are essential reproductive hormones. Testes produce gametes known as spermatozoa through a process called spermatogenesis, while ovaries produce female gametes known as ova (ovum - singular) through the oogenesis process.

Male hypogonadism is usually characterized by low testosterone levels (<300ng/dl) or10.4nmol/L)(Vincenzo Rochira et al., 2011).Hypogonadism may present with symptoms or could remain asymptomatic in some individuals. This compromises certain functions that are normally dependent on it. These include development of secondary sexual characteristics (if pre-pubertal in onset or congenital), anabolic functions (body mass composition), sexual function and mood and behavioural adjustments.

2.2 Testosterone production and function

Testosterone is the most important androgen of the sex steroids produced by the testes. The testes produce 95% of the body's total testosterone, the remaining 5 % coming from the adrenal glands, which also produce other androgens. It is produced by the interstitial cells of Leydig which are situated between seminiferous tubules in the testes. Production of testosterone begins during foetal development where it serves to promote differentiation of the wolffian tract into the epididymis, vas deferens and

seminal vesicles as part of sex determination. The diagram below highlights the usual pathway of testosterone production and its related metabolism.

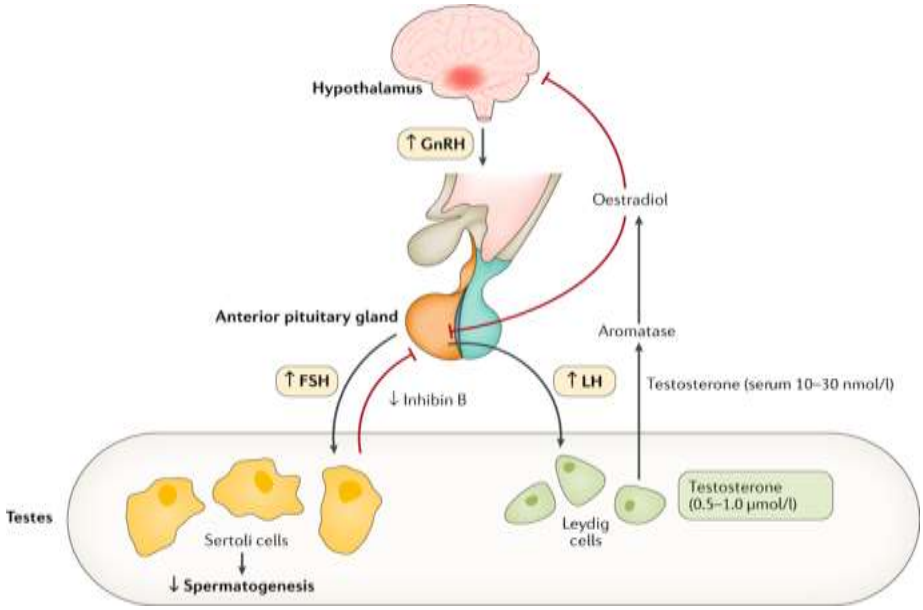

Figure 1: The hypothalamic–pituitary–gonadal axis(Salonia et al., 2019)

Approximately 4-9 mg of testosterone is secreted daily.98% of this is plasma protein bound, of which 40% is bound to albumin and the rest bound to sex hormone binding globulin (SHBG).Total plasma testosterone is 13-30 nmol/L in males and 0.5-2.5 nmol/L in females.

Testosterone is converted in target cells by the enzyme 5α- reductase to a more potent form, Dihydrotestosterone. Peripheral conversion occurs in the skin, seminiferous tubules and prostate. The functions of testosterone include;

- Stimulation of growth and male genital tract.
- Development of secondary sexual characteristics e.g. masculinization, virilization, acne.
- Sexual function – to develop& maintain libido, erectile function, orgasm etc.
- Spermatogenesis.
- Improve Mental and physical energy, lowers depression indices.

- Body mass composition – increase lean muscle mass.
- Bone maturation – increases bone mass density, role in epiphyseal closure (via Estradiol)

A small fraction of testosterone is peripherally converted to Estradiol by an enzyme known as Aromatase. This occurs in the adipose tissues. Estradiol is important in the regulation of bone homeostasis (Santi et al., 2016) and its deficiency leads to osteoporosis.

Testosterone production continues throughout life though it tends to decline with age and notably so in those above 45 years of age (Mulligan, Frick, Zuraw, Stemhagen, & McWhirter, 2006).

2.3 Epidemiology

2.3.1 Prevalence of HIV/AIDS

Ending the year 2018, the WHO estimates showed that 36.7 (34-39.8) million people were living with HIV worldwide. This figure being a representation of 0.8% of adults globally who were living with the virus at that point in time. Further to this, 1.1 million AIDS related deaths were reported in the same year.

Sub Saharan Africa bears approximately 70% of the world's population of PLWH which translates to 24.6 million people. Among adults in Africa, 4.4% are HIV positive. Kenya is one of the six "high burden countries" in Africa (UNAIDS Gap Report).

According to the WHO estimates, Kenya had a 4.7% prevalence of HIV/AIDS in the year ending 2018 with 1.6 million people in the general population living with the disease. Uasin Gishu County, where MTRH is located, was found to have a prevalence of 4.3 %.

2.3.2 Prevalence of Hypogonadism

About 4 to 5 million people in the United States have hypogonadism. The global prevalence of hypogonadism ranges between 3.7% to 12% and varies across different age brackets, with more percentages occurring in those much older and those with varied comorbidities (60 yrs and above) as seen in multiple population based studies (Mulligan et al., 2006, Martinez-Jabaloyas, 2013)

Different studies done in different populations across the world and looking at different categories/factors using different cut offs have shown a range in the prevalence of male Hypogonadism. A systematic literature review yielded the following observations:

Prevalence is generally higher in the following categories:

- The aging population 38% in those ≥45 yrs and 50 % in those ≥ 70 yrs(Mulligan et al., 2006)
- Obese men (51.5-78.8 %)(Hofstra et al., 2008)
- Type 2 diabetes mellitus patients (24-43%) (Kapoor, Aldred, Clark, Channer, & Jones, 2007)
- Metabolic syndrome (30-35 %)(Singh, Goyal, & Pratyush, 2011)
- HIV/AIDS (10-59%) (Coodley, Loveless, Nelson, & Coodley, 1994; Crum et al., 2005; Gomes et al., 2016)

2.3.3 Classification

Male hypogonadism is basically classified into two types:

i. **Primary (Hypergonadotropic) hypogonadism**- This implies that the problem originates from the testes. It is also referred to as primary testicular failure.

ii. **Secondary (Hypogonadotropic) hypogonadism** – in which the underlying cause is either in the hypothalamus or pituitary gland. (Kumar, Kumar, Thakur, & Patidar, 2010)

It is important to note that aetiology in either of these categories could be congenital or acquired in nature.

Aetiology

2.3.3.1 Primary Hypogonadism

Some of the known causes of primary Hypogonadism include:

a) **Klinefelter's syndrome (XXY)**, a genetic abnormality involving the sex chromosomes X and Y. Phenotypically tall slender males with poorly formed small testicles.
b) **Viral infections** that cause inflammation of the testes (orchitis) e.g. mumps.
c) **A failure or arrests of testicular descend** during early life development.
d) **Testicular injuries** (trauma) – physical.
e) **Various therapies for cancer** including chemotherapy and radiotherapy.
f) **Aging** – as a normal physiologic process leads to a slow but steady decline in testosterone levels at a rate of about 1.6 % (4ng/dl) per year in men aged above 50 years. There has been consistent evidence to demonstrate this in both cross sectional and longitudinal studies. This decline has also been found in other studies to be an independent factor for hypogonadism (Harman, Metter, Tobin, Pearson, & Blackman, 2001). Therefore, the expected Testosterone hormone levels by age are as outlined in the table below. However, whereas this reflects a physiological variation, it does not have an effect on the functional definition of hypogonadism (300ng/dL or 10.4nmol/mL) and established cut off values for

diagnosis. Though comparatively lower levels are more likely in the elderly than the young and this explains the high prevalence among this sub group.

Comparative Testosterone levels by age

Age in years	Reference Range (ng/dl)-TT	Average
20-29	705-1100	784
30-39	650- 975	617
40-49	500-620	569
50-59	400-530	422
60-69	300-440	376

g) **Inherited disorders** like Haemochromatosis that is usually characterized by high concentrations of Iron in blood.

3.3.2 Secondary Hypogonadism

Identified causes of secondary hypogonadism include:

a) **Kallmann syndrome** – a congenital abnormality that affects development of the hypothalamus. It is usually associated with anosmia (impaired ability to smell).

b) **Drugs** – several drugs have been shown to affect testosterone production. These include :Opioids, corticosteroids, Spironolactone, exogenous androgens, ketoconazole and marijuana (Kolodny, Masters, Kolodner, & Toro, 1974; Pake, Penn, & Ryan, 1994; Pont et al., 1982).

c) **Tumours** that affect the hypothalamus or pituitary gland (and their therapy) can impair production of gonadotropins and subsequently cause Hypogonadism.

d) **HIV/AIDS** – Infection with the HIV virus has been associated with an early onset of testosterone decline in young and middle-aged men. The mechanisms for the decline are still under intense study though it's thought that continued low grade inflammation in patients on care may be the explanation for this observation. Several studies conducted in this line seem to have a consensus towards higher

prevalence of hypogonadism in male HIV patients compared to those not infected with the virus.(Gomes et al., 2016; Pont et al., 1982; V Rochira et al., 2015; Vincenzo Rochira et al., 2011; Scott, Wolfe, Anderson, Cohan, & Scarsella, 2015; Slama et al., 2016; Wunder et al., 2007)

e) **Chronic inflammatory diseases** e.g. Mycobacterial Tuberculosis, Sarcoidosis and Histiocytosis can affect testosterone production at either level i.e. hypothalamus, pituitary, testicular and result in Hypogonadism.

f) **Emotional disturbance** – An association has been described between stress and hypogonadism. This is thought to occur as a result of excessive Cortisol production(Cumming, Quigley, & Yen, 1983)

g) **Exhaustive exercise** – studies done showed a moderate acute decline in basal testosterone levels in study populations after exercise. This is however transient(Anderson, Lane, & Hackney, 2016; Prasad et al., 2016).

h) **Obesity** – Hypogonadism is a common finding among overweight and obese people. However it is also possible that it could be the underlying cause to this problem due to fat redistribution centrally(Hofstra et al., 2008).

2.4 Pathophysiology of Hypogonadism in HIV

Little is known regarding how infection with the HIV virus leads to low testosterone levels(Crum et al., 2005). In this era of Highly Active Anti-Retroviral Therapy (HAART), most patients with HIV are having improved survival rates and in the long run tend to develop several HIV related complications along the course of their illness.

It is thought that continued low grade inflammation that goes on in the body despite adequate viral suppression with HAART may be a key factor in the pathogenesis of this problem(Wunder et al., 2007). This partly explains why the pattern of

hypogonadism in these patients may assume either a primary type ,secondary type or a mix of both (Gomes et al., 2016). This is because inflammation that goes on may affect the hypothalamus, pituitary or the testes. Of the two types of hypogonadism, secondary or hypogonadotropic form is more common(56.9%) than the primary (hypergonadotropic) type 43.1% (Gomes et al., 2016). However, more still remains to be done in ascertaining the exact mechanisms that are responsible for the observed patterns of increased prevalence of hypogonadism, which is now becoming of major concern especially in the young and middle aged HIV infected populations(M Lachâtre et al., 2016).The figure below depicts likely mechanisms involved in the Pathophysiology of HIV associated hypogonadism.

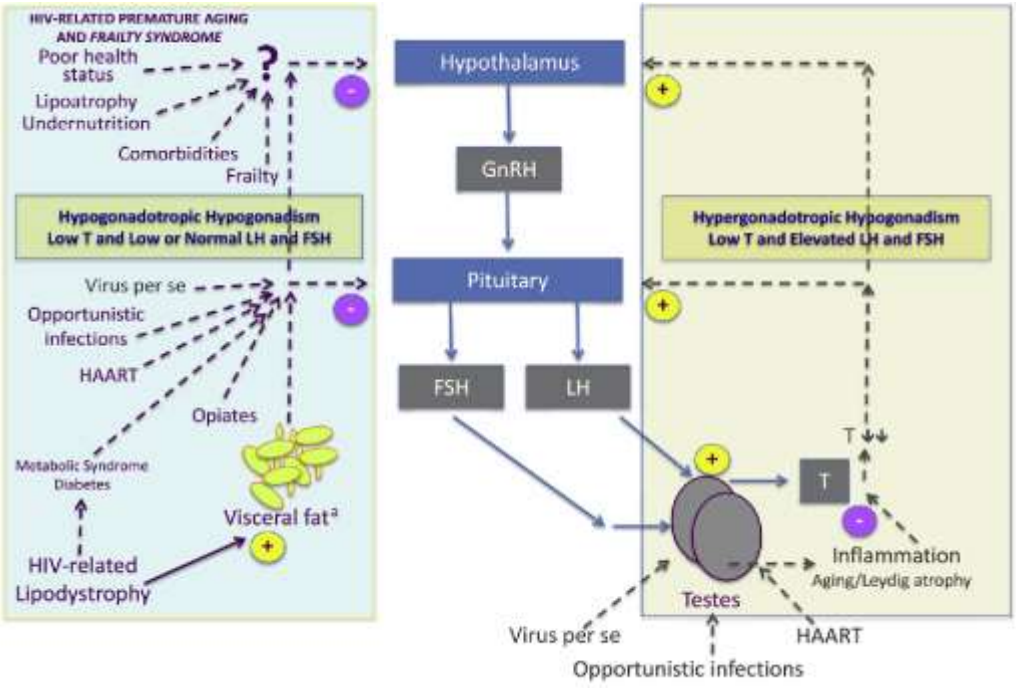

Figure 2:Pathophysiology of hypogonadism in HIV infected men(Vincenzo Rochira et al., 2011)

2.5 Signs and symptoms of Hypogonadism

Hypogonadism may be without any symptoms especially if late in onset i.e., post puberty or in adult life.

However, if early in onset, it may present with the following features:

Delayed, absence or regression of secondary sexual characteristics e.g. growth of pubic hair, masculine features, acne, broadening of shoulders, voice deepening etc.

Low haemoglobin levels, reduced bone mineral density (osteopenia), wasting away of muscles especially peripherally, accumulation of adipose tissue around the lower abdomen resulting in obesity, low sperm count (Kumar et al., 2010).

Sexual dysfunction is the commonest presentation of hypogonadism that occurs post puberty stage in males, the main defining form being low libido and impaired erectile function(Hall et al., 2008; Kumar et al., 2010).The prevalence of hypogonadism in one study was found to vary between 7-47% with different cut-offs for hormone levels (Köhler et al., 2008). It is thus important to screen for hypogonadism in patients with erectile dysfunction for whom other causes have been ruled out. Additionally, they have challenges with attaining orgasm and have a reduction in ejaculate volumes. This can in the long-term lead to infertility.

Secondary hypogonadism among male adults may also present with depression, dwindling physical energy levels, an increased predisposition to irritable tendencies, poor concentration, anaemia and osteoporosis(Fraietta, Zylberstejn, & Esteves, 2013)

Hypogonadism from whatever cause is associated with depression. In one study, 35% of hypogonadal men were found to have depression compared to 18% among eugonadal men. The relative risk for depression associated with hypogonadism was

found to be 1.94 times higher (Makhlouf, Mohamed, Seftel, & Neiderberger, 2008). In this study 24% of men with hypogonadism were found to have overt depressive symptoms defined as CESD scores of >22, (CESD- centre for epidemiologic studies).

An increased incidence of newly diagnosed depressive illness and a shorter duration to diagnosis in aging men with declining testosterone levels was also observed(Shores et al., 2004). Additionally treatment with testosterone in another study revealed an antidepressant effect in patients with depression (Zarrouf, Artz, Griffith, Sirbu, & Kommor, 2009).

In patients with HIV wasting syndrome, low testosterone levels are also a common finding and several interventions have been designed towards addressing this problem using hormone replacement therapy(Loveless, & Merrill, 1994 Bhatia et al., 2015; Coodley).

2.6 Diagnosis

The diagnosis of hypogonadism is based on measurement of sex hormone levels and a clinical assessment. The findings of low testosterone levels signify hypogonadism. The cut off points have been a matter of debate and study though consensus seems to be that diagnosis be made on a finding of serum Total Testosterone(TT) less than 300 ng/dl (< 10.41 nmol/L) or Free Testosterone (FT) less than 5ng/dl(V Rochira et al., 2015). The AACE recommends a cut off of 300ng/dl be used.

Clinically, the presence of any one of these specific symptoms: diminished libido, erectile dysfunction or reduced bone mass density may suffice to make a diagnosis of hypogonadism when accompanied by low hormonal levels.

Alternatively, the presence of two or more less specific symptoms like wasting,

central obesity , anaemia, depression, chronic fatigue, reduced vitality and disturbed sleep in association with supportive laboratory findings (low testosterone) as above stated are a criteria for diagnosis (Hall et al., 2008).

A further assessment of gonadotropins i.e. FSH and LH is important in determining whether observed low testosterone is primary (hypergonadotropic) or secondary (hypogonadotropic).Primary has low testosterone with high LH levels, while secondary has low testosterone with low LH levels(Vincenzo Rochira et al., 2011).

This may be necessary in decision making when it comes to choice of management/treatment. It is however important to emphasize that the diagnosis of hypogonadism as recommended by the Endocrine Society in the United States insists on biochemical assay of Total Testosterone for diagnosis. This in effect gives weight to laboratory measurement of testosterone and is particularly important when the diagnosis is suspected yet subtle in presentation or asymptomatic. Blood tests are usually done in the early morning hours when testosterone is expected to be higher, since diurnal variations do occur (Vincenzo Rochira et al., 2011)

2.7 Complications and Emerging Concerns

When left untreated, hypogonadism among male patients with HIV may result in the following complications (Crum-Cianflone et al., 2007; Crum et al., 2005) :

Weight loss/Wasting	Erectile dysfunction
Depression	Loss of libido
Osteoporosis/ osteopenia	Gynaecomastia
Infertility	

Emerging areas of concern more recently among patients with hypogonadism is the association with an increased predisposition to metabolic syndrome(Singh et al., 2011). In their study,(Kupelian et al., 2006), also observed a 2 to 4 fold increased risk of developing metabolic syndrome in men with low testosterone levels compared to the general population, other factors like smoking, age and overall health status being independent.

Another study also found an association between low testosterone levels and a higher median cardiovascular risk at 10 yrs. ($p=0.004$) as measured by the Framingham risk score, a higher HbA1c ($p=0.016$) and a higher systolic blood pressure ($p=0.007$)(Gomes et al., 2016).This is particularly important because of the fact that hypogonadism could aggravate cardiovascular disease among patients with HIV on HAART.

Other notable findings of this study were an association of hypogonadism with HIV associated lipodystrophy and isolated central fat deposition.

These findings further illustrate the need to detect and treat hypogonadism early in those with suggestive features, more so in those who are living with HIV.

2.8 Treatment

The American Association of Clinical Endocrinologists (AACE) guidelines recommend that therapy be reserved for patients who have symptoms besides low testosterone levels (<300ng/dL). The target being restoration to normal ranges of between 300-800 ng/dL (Petak, Nankin, Spark, Swerdloff, & Rodriguez-Rigau, 2001)

The goals and benefits of therapy include: averting cardiovascular risks, restoring normal sexual function, improve development of secondary sexual characteristics,

increase bone mineral density. Several studies have shown improved depression indices with testosterone therapy too(Grinspoon et al., 2000). Great strides have been demonstrated particularly in restoring sexual function with hormonal therapy(Hackett, 2016; Wang et al., 2000).

Testosterone Replacement Therapy (TRT) is the mainstay of treatment in male hypogonadal patients. This is more so the case in those with primary hypogonadism. For patients with secondary hypogonadism who still desire to have children preferred options include; use of Human Chorionic Gonadotropin (HCG), FSH, or use of gonadotropin releasing pump. Testosterone should be avoided generally(Petak et al., 2001).

Testosterone can be administered in various forms and dosages depending on the individual patient needs. These have to be tailored appropriately to minimize side effects, improve efficacy and promote convenience. Some of the known modes of administration include: intramuscular injections, oral tablets, transdermal patches, buccal and implantable pellets(Kumar et al., 2010)

Therapy may last 3 to 6 months or more depending on the level of deficiency, the clinical response and the underlying causes (Hackett, 2016). Monitoring for response and side effects is highly recommended by the ES for all patients undergoing therapy (Bhasin et al., 2006).

The most significant of potential adverse effects of testosterone replacement is the risk of enhancing undiagnosed or early prostate cancer progression, particularly in those predisposed (Abrams, 2001). Other associated side effects include acne, erythocytosis, sleep apnoea and risk for venous thromboembolism(Bhasin et al., 2018) (US FDA website).

CHAPTER THREE: METHODOLOGY

3.1 Study Site

The study was conducted at the AMPATH modules of MTRH. MTRH is the second largest referral hospital in Kenya after KNH. It also serves as the teaching hospital for Moi University. AMPATH is a consortium of universities from North America that partner with Moi University and is focused primarily on providing wholesome care to HIV patients. It covers the wider western region of Kenya & North Rift and has a multidisciplinary centre situated within MTRH in Eldoret.

3.2 Study Design

Cross Sectional

3.3 Target population

Male patients with a confirmed diagnosis of HIV attending AMPATH care clinics

3.4 Study Population

The study population consisted of male HIV patients attending comprehensive care clinics at AMPATH in MTRH. It included those who were newly diagnosed and those on continuing care, regardless of whether they were on HAART or not.

3.5 Sample Size

I used Fischer's Formula to determine the sample size. I used a 12 % prevalence for calculation of the sample size. This was largely informed by a cross sectional French study that found a 12 % prevalence in a similar study population(M Lachâtre et al., 2016). Findings from other studies have been in the ranges of 5-14% in the era of enhanced uptake of HAART therapy (Bhatia et al., 2015). A desired confidence level of 95% and a precision level of 0.05 were applied.

Thus, sample size determined for the study was as follows;

$$n = \left(\frac{Z_{1-\alpha/2}}{\delta} \right)^2 \times P(1-P)$$

$$= \left(\frac{1.96}{0.05} \right)^2 \times 0.12 \times 0.88$$

$$= 162.3$$

$$= 162$$

This was the minimum number of persons required to participate in the study in order to answer the research question. We however recruited an additional **20** to account for possible challenges with incomplete data, missing lab results or withdrawn consent midway the protocol. This formed a **10%** precautionary buffer and pushed the total number of study participants to **182**.

3.6 Sampling Technique

A systematic random sampling technique was utilized in effecting the above sample size of 182. A randomly selected first participant was recruited and after whom every 5^{th} male patient was included, where k= N/n. We included every fifth male patient with a target of recruiting 10 in a day out of an observed average of 50 male patients being seen every day at the clinic hence k=50/10. The study was conducted for 2 (Two) months (March and April 2018). This was because clinics were only open during weekdays with Friday being set apart for patients on follow up for treatment failure. Some days were also characterized by low turn outs or bookings.

As had earlier been anticipated in the preliminary stage of developing this work, all of our patients were on HAART. Additionally, I sort to balance those on HAART for less than 5 years versus those on it for more than 5 years. As per records, only 1/3 of patients had used HAART for less than 5 years. Subsequently we put a ceiling of 60

patients to capture this demographic and this formed 33% of our studied population as spelt out earlier in the proposal stage of this study. This was adhered to in view of the fact that a study in Portugal had indicated significant difference in gonadal status after 5 years of HAART therapy (Broder & Jackness, n.d.). For patients who were randomly selected after this ceiling was reached, a focus on longer duration of therapy was preferentially considered but in conformity to the k already settled on.

Scheduling (booking) of patients had already been predetermined by clinician assessments and was based on need for review e.g., recent change of treatment regimen; follow up of CD4+ count, viral load among other protocol requirements.

3.7 Eligibility Criteria

3.7.1 Inclusion criteria

i) Male
ii) Age 18-65 years
iii) A known HIV positive diagnosis
iv) Ambulatory/clinically stable (not acutely ill, as this is known to transiently lower testosterone levels)

3.7.2 Exclusion criteria

i) Prior or on-going hormonal therapy with androgens (Testosterone)
ii) History of chemotherapy or radiotherapy for cancer or tumours of any origin or type
iii) Known or documented diagnosis of DM, CKD or chronic liver disease (CLD).
iv) Congenital testicular abnormalities e.g., undescended testes
v) History of testicular trauma or injury
vi) Concurrent / Long term use of Fluconazole (e.g., for Cryptococcal meningitis)

vii) Other drug use: Glucocorticoids (prednisone), Opioids (morphine), antipsychotics (haloperidol) and antiemetics (metoclopramide). Drugs indicated in the brackets are the prototypes in the groups, most studied (in relation to hypogonadism inducing effect) as well as the most commonly prescribed / available in our set up for different indications.

3.8 Study Procedure

Patients who met the eligibility criteria, having been sampled and upon giving consent, were recruited into the study. Points of recruitment were the observation/consultation rooms at AMPATH centre building in MTRH.

A structured data collection form alongside two questionnaires was administered. Upon completion of this exercise, a focused physical examination to measure waist circumference, height and weight was done. Vital signs (BP, PR) were taken and/or retrieved from the system. BMI was calculated based on the formula $\frac{wt(kg)}{ht(m^2)}$

An RBS level was determined via the capillary prick method. A Glucometer (Glucowell) and glucose sticks were used to measure the blood sugar level. Afterwards, a venous blood sample of ($\approx 2-3\ ml$) was drawn from the anterior cubital veins of the arm for laboratory determination of the serum TT levels. Samples collected were packaged appropriately in red top vacutainers and ferried to the standard reference laboratory for assay. The laboratory methods for RBS and TT evaluation were done as per the universally set standard operating procedures (SOPs) for the same and are outlined in the respective appendices 3 and 4 on pages 55 & 56.

3.9 Data collection

Data was collected through the various data collection tools (see appendices at the end), physical examination and laboratory results. The IIEF 5 and PHQ 9 questionnaires that were employed in this study are internationally validated tools that have also been validated and used locally.(Gray & Campbell, 2005; Monahan et al., 2009). Depending on what the patient scored, participants in the study were accordingly classified as either having the condition being screened for or not, and if present, the severity was also graded. These tools, being interviewer administered, were verbally translated into Swahili or local language(s) by the interviewer or a translator in a standard way to ensure no bias was introduced in the process.

The PHQ 9 is a tool for screening, diagnosing and monitoring depression severity by incorporating diagnostic criteria from diagnostic and statistical manual for mental disorders (DSM-IV). It evaluates for suicidal ideation and explores the extent to which the patient's social functioning is affected by the symptoms. It has a sensitivity of 92% and a specificity of between 88-90% in detecting depression. Scores of 5-9, 10-14, 15-19 and 20- 27 suggest mild, moderate, moderately severe and severe forms of depression respectively.

The IIEF-5 is a simplified tool (an abridged 5 item version) used for assessing erectile dysfunction. Scores range from 5-25. They are categorized as follows: scores of 5-7 denote severe ED, 8-11 moderate ED, 12-16 mild to moderate, 17-21 mild and 22-25 no ED.

3.10 Data management

Data captured using the various collection methods highlighted above was entered into an electronic database (Microsoft access) which was protected by a security code (password) only known to the principal investigator. Data collection forms, laboratory

results and other physical material involved in the process were filed in an orderly way and safely kept under lock and key.

3.11 Statistical Data Analysis

Descriptive methods such as the mean and the corresponding standard deviation (SD), median and the corresponding inter quartile range (IQR) as well as the frequencies and the corresponding percentages were used to summarize the data. Histograms were used to assess the distribution of the continuous variables to help test the assumption normality and to determine the appropriate summary statistic to employ. The prevalence of hypogonadism was reported alongside the corresponding 95% confidence intervals (95% CI).

The association between the presence of hypogonadism and categorical variables was assessed using Fisher's exact test. Independent samples t-test was used to compare the means such as the mean age, mean SBP, mean DBP, and mean RBS. Wilcoxon rank sum test was used to compare the medians such as the median duration on HAART.

Data analysis was done using STATA version 13 SE (College Station, Texas 77845 USA).

Results were presented using tables and graphs.

3.12 Ethical Considerations

Approval to conduct the study was sought from the Department of Medicine Moi University and Institutional Research and Ethics Committee (IREC) - MTRH/MUSOM.

All participants who were enrolled into the study were required to sign informed consent after careful explanation on what the study entailed and what their rights were during the study period. Entry into the study was absolutely voluntary. No coercion or

inducement was used in the recruitment process. Those that consented were informed that they could withdraw from the study at any time during the study period.

Confidentiality of details regarding the study participants was maintained throughout the research period and after. Their privacy and dignity were upheld in the entire process. Data collected was secured through a password protected Microsoft access file. Blood samples drawn from patients were only used for the indicated purpose. No additional investigations were carried out on them.

Findings from the laboratory investigations were communicated to the participants directly via contacts provided during the day of data collection. Primary care providers/clinicians were also informed of abnormal findings for concurrent feedback to the patients at next follow up and to take appropriate action.

CHAPTER FOUR: RESULTS

I approached 205 patients who were potentially eligible for entry into the study but eventually ended up with 182 for the final data analysis.

The diagram below is a recruitment schema highlighting the process of selecting study participants.

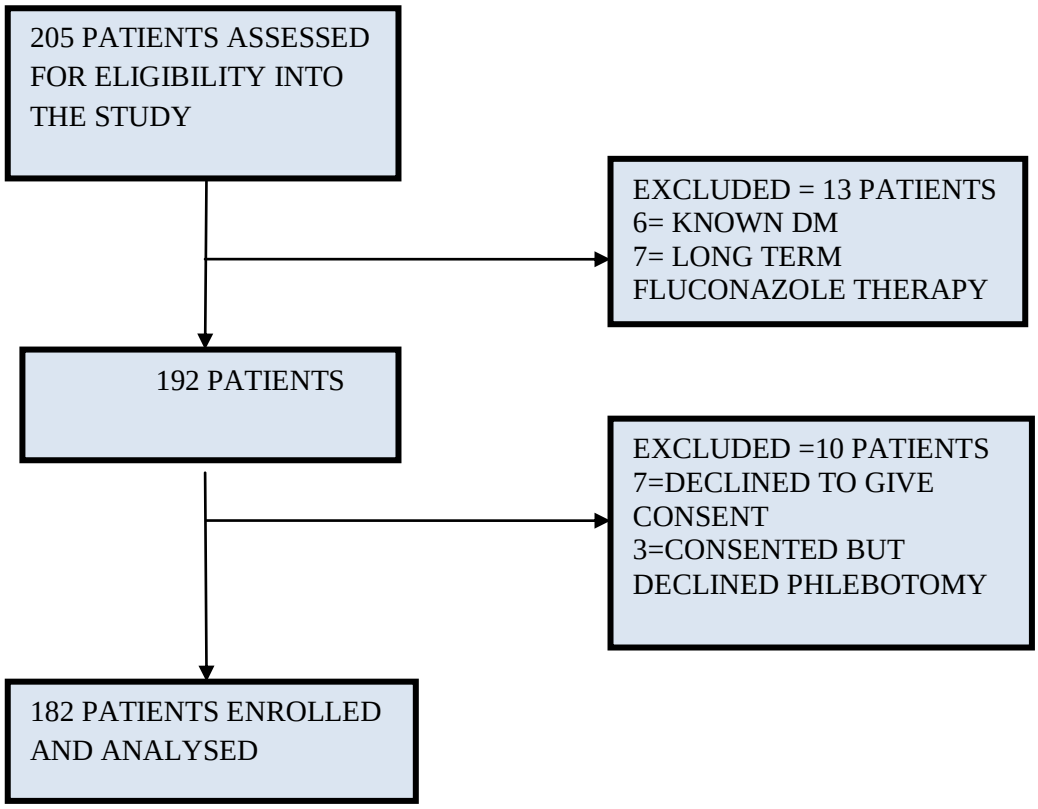

Figure 3: Recruitment Flow Diagram

Majority of our patients were aged 35-49 years (55%) and were representative of the overall male population attending regular clinics (60%). Those above 45 years were 97; representing 53.2% as expected from the clinic registers that had revealed initial proportional estimations to be (58%) i.e.,1616 male patients out of 2787 were aged above 45.

4.1 Results

A total of 182 participants aged 25.0 – 65.0 years were enrolled in the study.

Table 1: Patient characteristics

Characteristic	N	Mean (SD)
Age (years), Mean (SD)	182	47.4 (8.7)
Range (Min. - Max.)		25 – 65
25.0 - 34.0		13 (7.1%)
35.0 - 49.0		100 (54.9%)
50.0 - 65.0		69 (37.9%)
Marital Status		
Divorced		1 (0.5%)
Married		161 (88.5%)
Separated		4 (2.2%)
Single		7 (3.8%)
Widowed		9 (4.9%)
Level of Education		
Primary		70 (38.5%)
Secondary		73 (40.1%)
Tertiary		39 (21.4%)
Alcohol use		
No		98 (53.8%)
Yes		84 (46.2%)
Tobacco use or Smoking		
No		128 (70.3%)
Yes		54 (29.7%)

The mean age was 47.4 (SD: 8.7) years with a minimum and a maximum of 25.0 and 65.0 years respectively. More than half (54.9%) were aged 35.0 – 49.0 years, and 37.9% were aged 50.0 – 65.0 years.

Up to 88.5% of the participants were married, and 61.5% had at least secondary or level of education or more.

Table 2: HAART Treatment

Characteristic	N	Mean (SD) or Median (IQR) or n (%)
Duration on HAART, Median (IQR)	182	7.5 (4.0, 11.0)
Range (Min. - Max.)		1.0– 15.0
< 5 Years		60 (33.0%)
5 - 10 Years		74 (40.7%)
> 10 Years		48 (26.4%)
Viral Load Suppressed, n (%)		
YES (VL<1000 copies per ML)		162 (89.0%)
NO (VL≥1000 copies per ML)		20 (11.0%)

One third (33.0%) of the participants had been on HAART for less than five years, 40.7% had been on HAART for 5–10 years, and 26.4% had been on HAART for over ten years.

All participants at the time of enrolment into the study were on antiretroviral therapy, a good indicator in terms of the test and treat approach by government policy. Out of this, 89.0% of them were virally suppressed.

Table 3: Clinical characteristics

Characteristic	N	Mean (SD) or Median (IQR) or n (%)
Systolic BP (mm Hg), Mean (SD)	**182**	**126.7 (17.7)**
Range (Min. - Max.)		**79 – 176**
Hypotension (<90 mmHg)		9 (5.0%)
Normotension (90-139 mmHg)		128 (70.3%)
Hypertension (≥140 mmHg)		45 (24.7%)
Diastolic BP (mm Hg), Mean (SD)		**76.5 (11.5)**
Range (Min. - Max.)		**54 – 110**
Hypotension (<60 mmHg)		10 (5.5%)
Normotension (60-90 mmHg)		145 (79.6%)
Hypertension (≥90 mmHg)		27 (14.9%)
SBP ≥140 mm Hg | DBP ≥90 mm Hg, n (%)		51 (28%)
Pulse (beats per minute), Mean (SD)		**80.5 (13.8)**
Range (Min. - Max.)		**53 – 120**
Bradycardia (<60)		8 (4.4%)
Normal (60-100)		158 (86.8%)
Tachycardia (>100)		16 (8.8%)
BMI (Kg/m^2), Median (IQR)		**21.7 (19.8, 24.8)**
Range (Min. - Max.)		**15.6 - 41.9**
Underweight (<18.5)		28 (15.4%)
Normal (18.5 - 24.9)		109 (59.9%)
Overweight (25.0 - 30.0)		39 (21.4%)
Obese (>30.0)		6 (3.3%)
Waist Circumference (cm), Median (IQR)		**84.0 (80.0, 88.0)**
Range (Min. - Max.)		56.0– 132.0
RBS, Median (SD)		**5.0 (0.6)**
Range (Min. - Max.)		**3.7 - 7.1**
Hypoglycaemia (<4.0 mmol/L)		8 (4.4%)
Euglycemia (4.0 - 7.8 mmol/L)		174 (95.6%)
Hyperglycaemia (7.8 - 11.0 mmol/L)		0 (0%)
Diabetes (>11.0 mmol/L)		0 (0%)

The average SBP was 126.7 (SD: 17.7) mm Hg with a minimum and a maximum of 79.0 and 176.0mm Hg respectively. Less than 5% of the participants had SBP less than 100 mm Hg, and 24.7% had SBP ≥140 mm Hg. One third (31.3%) of the participants had SBP spanning 100-119 mm Hg while 39.0% had SBP spanning 120 – 139 mm Hg.

The average DBP was 76.5 (SD: 11.5) mm Hg with and a range of 54.0 to 110.0 mm Hg. Fifteen percent of the participants had DBP ≥90 mm Hg, and 5.5% had DBP below 60 mm Hg.

Twenty eight percent of the participants were hypertensive (SBP ≥140 mm Hg | DBP ≥90 mm Hg).

The average pulse rate was 80.5 (SD: 13.8) beats per minute with 86.8% having a pulse rate of 60 – 100 beats per minute.

The median BMI was 21.7 (IQR: 19.8, 24.8) Kg/m^2 with a minimum and a maximum of 15.6 and 41.9 Kg/m^2 respectively. Sixty percent had normal BMI, 24.7% were overweight or obese, and 15.4% were underweight.

The median weight circumference was 84.0 (IQR: 80.0, 88.0) cm with a range of 56.0 cm – 132.0 cm.

The average random blood sugar (RBS) was 5.0 (SD: 0.6) mmol/l with a minimum and a maximum of 3.7mmol/l, and 7.1 mmol/l respectively. Over 95% had RBS >4.0 mmol/l.

Table 4: Testosterone levels and presence of hypogonadism

Characteristic	N	Median (IQR) or n (%)
Total Testosterone, Median (IQR)	182	667 (495.3, 860.5)
Range (Min. - Max.)		76 – 1500
Testosterone < 300		10 (5.5%)

The median testosterone level was 667 (IQR: 495.3, 860.5) ng/ml with a minimum and a maximum of 76.0 ng/ml and 860.5 ng/ml respectively.

The data show that 5.5% (95% Confidence Interval (CI): 2.7, 9.9) had hypogonadism.

Table 5: Erectile dysfunction

Characteristic	N	Median (IQR) or n (%)
IIEF 5 Score, Median (IQR)	182	21.0 (15.0, 24.0)
Range (Min. - Max.)		5.0– 25.0
Erectile Dysfunction, n (%)		
NO ED		80 (44.0%)
MILD		55 (30.2%)
MILD-MODERATE		20 (11%)
MODERATE		22 (12.1%)
SEVERE		5 (2.7%)

The median IIEF 5 score was 21.0 (IQR: 15.0, 24.0) with a range of 5.0 – 25.0. Analysis of the IIEF 5 score revealed that 56.0% of the participants had erectile dysfunction. Thirty percent, 11.0%, 12.1%, and 2.7% had mild, mild-moderate, moderate, and severe ED respectively.

Table 6: Depression levels

Characteristic	N	Median (IQR) or n (%)
PHQ-9 Score, Median (IQR)	182	0.0 (0.0, 3.0)
Range (Min. - Max.)		0.0 – 19.0
Depression Index, n (%)		
NONE		101 (55.5%)
MILD		22 (12.1%)
MODERATE		47 (25.8%)
MODERATELY SEVERE		9 (4.9%)
SEVERE		3 (1.6%)

The median depression score was 0.0 (IQR: 0.0, 3.0) with a minimum and a maximum of 0.0 and 19.0 respectively. Analysis of the depression score revealed that 44.5% of the participants had depression symptoms; 12.1%, 25.8%, 4.9%, and 1.6% had mild, moderate, moderately severe and severe depression symptoms.

Table 7: Associations of hypogonadism

Characteristics	Hypogonadism		P
	No	Yes	
Age (Years), Mean (SD)	47.5 (8.8)	45.0 (8.1)	0.374[t]
25-34	12 (92.3%)	1 (7.7%)	
35-49	93 (93.0%)	7 (7.0%)	0.421[f]
50-65	67 (97.1%)	2 (2.9%)	
Marital Status			
Divorced, Separated, Single or Widowed	20 (95.2%)	1 (4.7%)	
Married	152 (94.4%)	9 (5.6%)	>0.999[f]
Level of Education			
Primary	67 (95.7%)	3 (4.3%)	
Secondary	69 (94.5%)	4 (5.5%)	0.777[f]
Tertiary	36 (92.3%)	3 (7.7%)	
Alcohol use			
No	96 (98.0%)	2 (2.0%)	
Yes	76 (90.5%)	8 (9.5%)	0.046[f]
Tobacco use or Smoking			
No	119 (93.0%)	9 (7.0%)	
Yes	53 (98.2%)	1 (1.9%)	0.285[f]
Duration on HAART (Years), Median (IQR)	8.0 (4.0, 11.0)	3.5 (3.0, 9.0)	0.144[w]
1-4	54 (90.0%)	6 (10.0%)	
5-10	71 (96.0%)	3 (4.0%)	0.204[f]
10-15	47 (97.9%)	1 (2.1%)	
Viral Load Suppressed, n (%)			
No	19 (95.0%)	1 (5.0%)	
Yes	153 (94.4%)	9 (5.6%)	>0.999[f]
Hypertensive, n (%)			
No	126 (96.2%)	5 (3.8%)	
Yes (SBP ≥140 mm Hg| DBP ≥90 mm Hg)	46 (90.2%)	5 (9.8%)	0.146[f]
Pulse (beats per minute), n (%)			
Normal (≤100)	160 (96.4%)	6 (3.6%)	
Tachycardic (>100)	12 (75.0%)	4 (25.0%)	0.006[f]
BMI (Kg/m²), n (%)			
Normal	131 (95.6%)	6 (4.4%)	
overweight	41 (91.1%)	4 (8.9%)	0.266[f]
RBS, n (%)			
Hypoglycaemic	8 (100.0%)	0 (0.0%)	
Normal	164 (94.3%)	10 (5.8%)	>0.999[f]

[t] independent samples t-test; [f] Fisher's Exact test

The mean age for the participants who had hypogonadism was similar to those who did not have hypogonadism, 45.0 (SD: 8.1) years vs. 47.5 (8.8) years, p = 0.374, and the prevalence of hypogonadism across the age groups were similar, 7.7%, 7.0%, and 2.9% among the 25-34, 35-49, and 50-65 years respectively, p = 0.421.

Among the married, 5.6% were diagnosed with hypogonadism compared to 4.7% among the divorced, separated, single or widowed. The test for the difference showed that the two groups were similar in the prevalence of hypogonadism (p>0.999).

The prevalence of hypogonadism was noted to increase with level of education; 4.3%, 5.5%, and 7.7% among the participants who had primary, secondary and tertiary levels of education respectively. This trend was however not statistically significant, p = 0.777.

Notably, alcohol use was associated with higher prevalence of hypogonadism (9.5%) compared to (2.0%) among the non-users of alcohol, p = 0.046. This was statistically significant. Hypogonadism was also associated with tachycardia, p=0.006.

There was some difference in the prevalence of hypogonadism between the tobacco users (1.9%) compared to non-users of tobacco (7.0%), though this was not statistically significant, p = 0.285.

The median duration of using HAART was 3.5 (IQR: 3.0, 9.0) years among those who had hypogonadism and 8.0 (IQR: 4.0, 11.0) years among those who were not diagnosed with hypogonadism. However, this was not statistically significant. sufficient evidence from the data to demonstrate significant difference in the duration of HAART, p = 0.144. The prevalence of hypogonadism was inversely related with

the duration of use of HAART but the declining trend with time was not statistically significant, p = 0.204.

Similarly, there was no difference in the prevalence of hypogonadism between those who were virally suppressed and those who were not (5.6% vs. 5.0%, p > 0.999), and between those who were hypertensive and those who were not (9.8% vs. 3.8%, p =0.146).

Table 8: Prevalence of erectile dysfunction, and depression among the HIV positive patients who had and did not have hypogonadism

	Hypogonadism		
Characteristics	**No**	**Yes**	**P**
Erectile dysfunction, n (%)			
No (No ED)	74 (43.0%)	6 (60.0%)	
Yes (mild, mild-moderate, moderate, severe)	98 (57.0%)	4 (40.0%)	0.339[f]
Depressed, n (%)			
No (None)	94 (54.7%)	7 (70.0%)	
Yes (mild, moderate, moderately severe, severe)	78 (45.3%)	3 (30.0%)	0.516[f]
Had either erectile dysfunction or depression, n (%)			
No	60 (34.9%)	5 (50.0%)	
Yes	112 (65.1%)	5 (50.0%)	0.333[f]

Participants who were diagnosed with hypogonadism had a lower prevalence of erectile dysfunction compared to those who did not have hypogonadism. However, the difference between the two groups was not statistically significant, 40.0% vs. 57.0%, p = 0.339.

The prevalence of depression was lower among those who were diagnosed with hypogonadism (30.0%) compared to those who were not found with hypogonadism (45.3%). However, the difference was not statistically significant, p = 0.516.

The data further showed that 50.0% of those who were diagnosed with hypogonadism had either erectile dysfunction or depression or both while among those who did not

have hypogonadism 65.1% had either erectile dysfunction or depression or both. The difference between the two proportions was, however, not statistically significant, p = 0.333.

There was a higher prevalence of hypogonadism among hypertensives (SBP ≥ 140 mm Hg). However, the prevalence of hypogonadism across the levels of SBP did not reveal differences that were statistically significant.

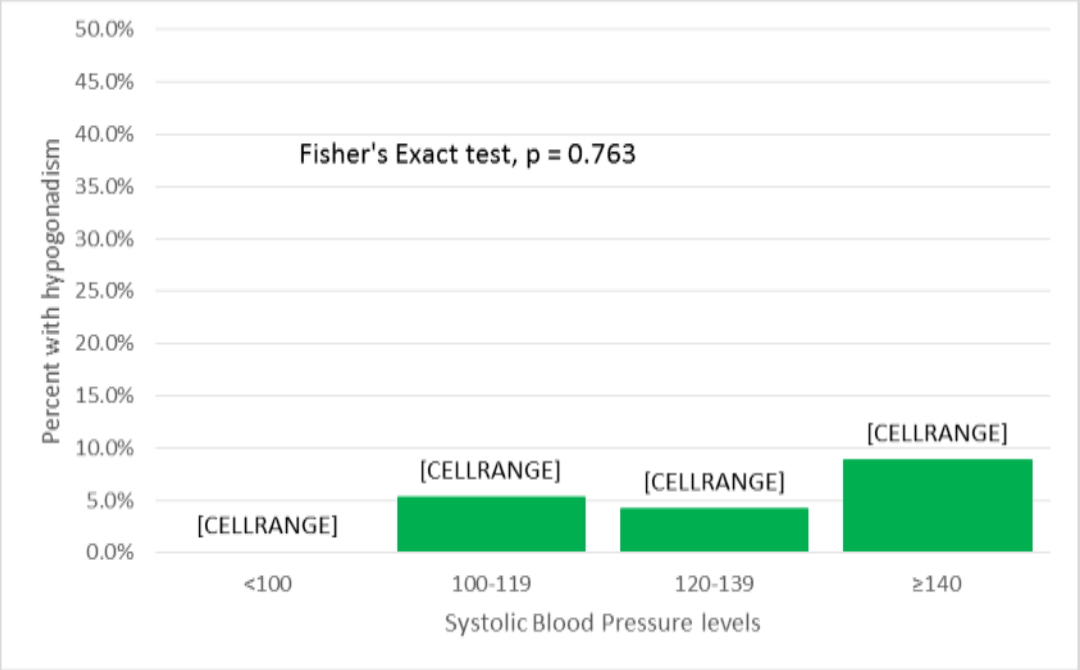

Figure 4: Prevalence of hypogonadism across the levels of SBP

Similarly, there was no evidence of statistically significant difference in the prevalence of hypogonadism across the levels of DBP (Figure 2). Data shows a higher prevalence of hypogonadism among those who had DBP ≥ 90 mm Hg (11.1%).

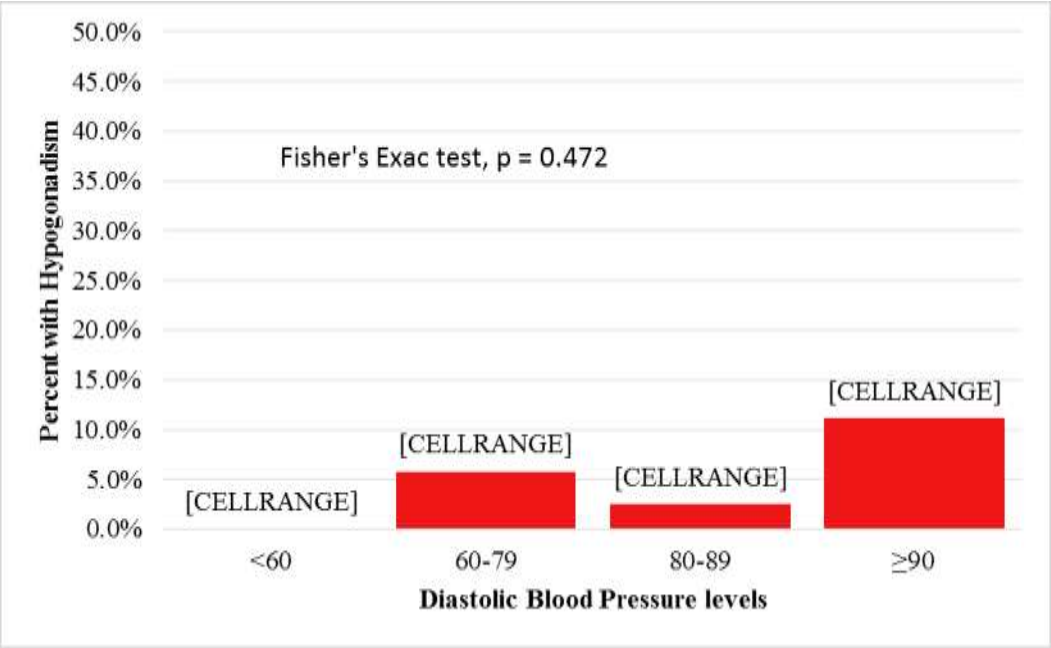

Figure 5: Prevalence of hypogonadism across the levels of DBP

Participants who had higher pulse rate (>100 beats per minute) had significantly higher prevalence of hypogonadism (25.0%) compared to those who had ≤100 beats per minute (3.6%), p = 0.006.

The prevalence of hypogonadism among the participants who were overweight or obese (BMI ≥ 25.0 Kg/m^2) was twice the prevalence of hypogonadism among those who had BMI < 25.0 Kg/m^2, 8.9% vs. 4.4%. However, the difference was not statistically significant, p = 0.266. Analyzing the prevalence of hypogonadism across the levels of BMI revealed that obese participants were more likely to have higher prevalence of hypogonadism (Figure 3).

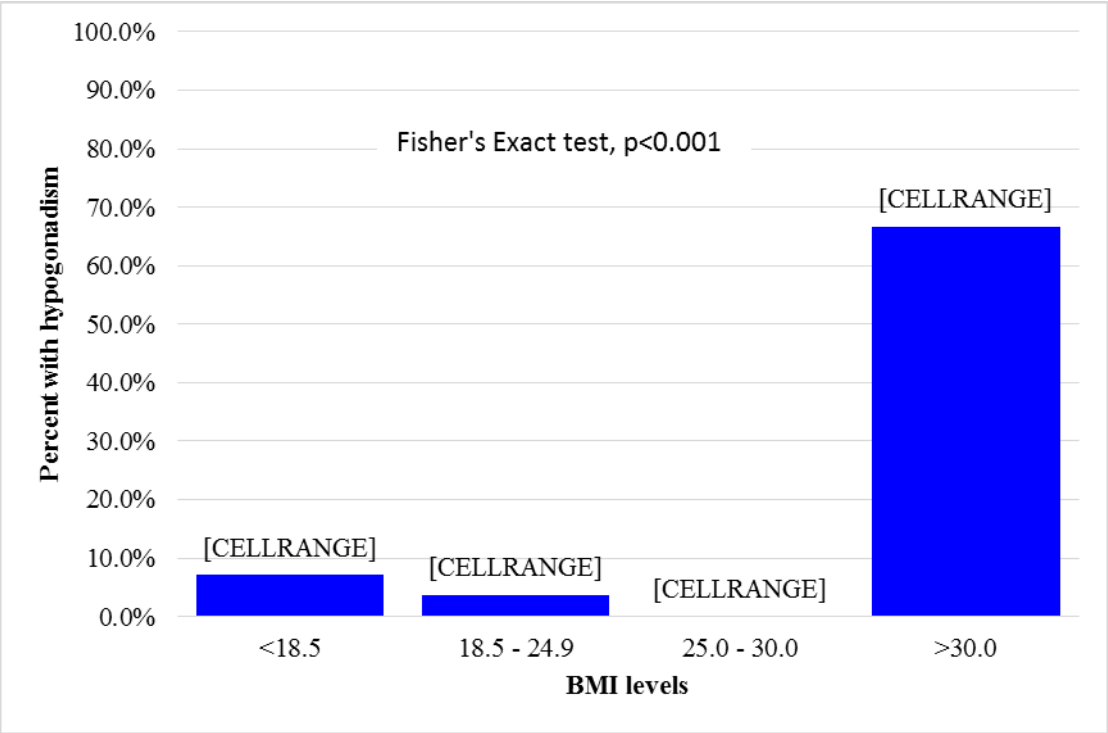

Figure 6: Prevalence of hypogonadism across the levels of BMI

None of the participants who had RBS < 4.0 was reported to have had hypogonadism compared to those whose RBS ≥ 4.0, the difference in the prevalence of hypogonadism was not statistically significant, p >0.999.

CHAPTER FIVE: DISCUSSION

5.1 Prevalence

This study primarily set out to establish the prevalence of hypogonadism among stable, ambulatory male patients with HIV infection. I established this to be at 5.5% (95% Confidence Interval (CI): 2.7, 9.9) in the study population. It compares with a 2007 American study which found a 6% baseline prevalence of hypogonadism in male HIV patients(Dubé et al., 2007). This though was in ART- naïve patients. Our study participants were on continuing care though a small percentage (11%) was not virally suppressed. This figure is slightly lower compared to that of 13% found in a large cohort study of 14,000 male HIV patients in 2015, who had laboratory evidence of low testosterone levels (<300ng/mL) (Bhatia et al., 2015)

The early initiation of potent antiretroviral therapy in this group of patients may explain the continued decline in proportions of patients with gonadal dysfunction. This is exemplified in another study of male HIV patients having low testosterone levels prior to initiation of TRT but which inadvertently improved upon initiation of HAART(Dubé et al., 2007). Measurements at 64 weeks of HAART showed increase in Testosterone levels. In another prospective study of 189 male HIV patients, pre-treatment levels of testosterone and 17-beta estradiol were particularly noted to improve sequentially upon successful initiation and maintenance of HAART therapy. Mean testosterone levels rose from 23.2 to 37.6 nmol/l in these patients, p=0.02 , further indicating the influence of HAART (Collazos, Martinez, Mayo, & Ibarra, 2002)

With the government having adopted a test & treat policy on matters HIV, it is expected that most patients are likely to have good viral suppression with the ultimate aim of eradicating HIV as a disease in our country. It would thus be reasonably

predictable to believe that with it, there will be further dwindling of this prevalence rate. From this study, patients who had been on HAART longer were less affected by hypogonadism compared to those <5 yrs (6 vs. 4). This was however not statistically significant P=0.144. HAART use is the likely reason for continued reduction in the numbers of cases observed across decades, having initially been in the ranges of 30-50% to now just about 5% of the HIV male population. The study population was well controlled on HAART (89%) with a significant proportion having undetectable HIV RNA viral levels. High viral levels (>10,000 copies/ml) were shown to correlate with Hypogonadism in the CHAMPS study (Klein, Lo, Santoro, & Dobs, 2005).

In a cross-sectional study carried out among 120 male HIV patients in North Eastern India in 2019, a higher prevalence (23.3%) of hypogonadism was found. It is however worth noting that in this study, there was a selection bias given that a screening tool/ questionnaire (ADAM-Androgen Deficiency in Aging Males) was used to filter for those with probable hypogonadism (120 out of 450) who were then tested, thus potentially giving a higher yield (Pongener et al., 2019)

Finally, the fact that serum TT was measured as opposed to free Testosterone levels may have played a role in the observed outcomes. Patients with HIV tend to have higher SHBG (Sex Hormone Binding Globulin), hence may falsely reveal normal TT levels, despite having low free Testosterone. Thus, some cases may be missed. This was noted to be the situation in a similar study done in France, which turned out a 12.4% prevalence of hypogonadism in male HIV patients (based on low free Testosterone levels),but who only showed a 4.4% prevalence based on TT levels measured in the same sitting (Marie Lachâtre et al., 2017). The ES recommends use of TT at the screening level, after which a repeat TT or use of FT may be considered for confirmation of the diagnosis (Bhasin et al., 2018). FT is not routinely available at

most laboratories (including our local set up), is expensive, cumbersome and standardised methods of equations to generate its values from TT are missing in most low- and middle-income countries, which makes TT the preferred method, this according to published data from CDC-Centre for Disease Control and prevention and PATH-Partnership for The Accurate Testing of Hormones.

The possibility that some patients with hypogonadism may have not been captured on account of using TT as opposed to FT is an acknowledged limitation in this study.

5.2 Erectile Dysfunction

56% of the overall study population had erectile dysfunction, with a median IIEF 5 score of 21.0 (IQR: 15.0, 24.0). 30%, 11.0%, 12.1%, and 2.7% had mild, mild-moderate, moderate, and severe ED respectively. Of this (102), only 4 were noted to have laboratory evidence of hypogonadism. The overall proportion of patients with ED compares to that found in the US by Crum et al, who picked 61% prevalence in male HIV patients. In his study comprising 300 study participants, only 17% were hypogonadal. Just like in this study, hypogonadism was not positively correlated with ED(Crum-Cianflone et al., 2007).

Among those with hypogonadism (10), 40% had erectile dysfunction as assessed by the IIEF-5 tool for evaluation of ED. This is reasonable as most patients with hypogonadism have nonspecific symptoms, but majority may have various elements of sexual dysfunction, including but not limited to ED. The Boston community cohort study and the ES guidelines reiterate the same. The incidence of ED varies greatly across other literature (Bhasin et al., 2006; Hall et al., 2008). A considerable percentage don’t manifest these symptoms, except when TT levels are severely reduced as to <200ng/ml (Pathak, Meena, Chakravarty, Rai, & Sundar, 2015)

In another observational study involving >1300 patients in Italy, a prevalence of 49 % for ED was noted as being common between both groups of those with and without low circulating serum TT (Vincenzo Rochira et al., 2011). This may suggest that whereas ED may be observed in hypogonadism, it may also be associated with other conditions, some of which may be independent of Testosterone levels.

5.3 Depression

From this study, 44.5% of participants had symptomatic depression.12.1%, 25.8%, 4.9%, and 1.6% had mild, moderate, moderately severe and severe depression symptoms.

30% of patients with hypogonadism were noted to have symptoms suggestive of depression. This compares to a European study that found depression in 35% of hypogonadal men compared to 18% among eugonadal men. The relative risk for depression associated with hypogonadism in these patients was put at 1.94 times higher (Makhlouf et al., 2008).

In keeping with these previous findings from other studies, most patients did not show overt features of depression but rather had mild to moderate depression. Majority (25.8%) had minimal depression symptoms as per the PHQ-9 assessment questionnaire. This is important in emphasis for screening rather than awaiting full blown symptoms for diagnostic considerations.

The realization that a sizeable fraction of patients attending routine follow up clinics at AMPATH suffer various degrees of depression however, should warrant appreciable interest in the matter. This is because a good number of patients may be having ongoing considerable mental health related issues, that otherwise may for instance jeopardize adherence to care and also lower the overall quality of life.

Whereas improvement in depression in these patients may be witnessed with Testosterone administration (Grinspoon et al., 2000), it is important to allow time for the use of HAART to improve TT levels first(Dubé et al., 2007 ; Pathak et al., 2015)

5.4 Symptomatic Hypogonadism

Though symptoms investigated in this study may not entirely be limited to merely the presence or absence of hypogonadism, I endeavoured to elicit ED and Depression as the main probable presentation in patients with hypogonadism.

My findings revealed that out of 102 patients with ED, hypogonadism was noted in 3.9% of them. A screening for depression in study participants also showed that hypogonadism was present in 3.7% of the 81 who screened positive for the condition.

It was however difficult to determine the relationship between hypogonadism and ED/Depression owing to the small numbers involved and also taking cognisance of the study being cross-sectional in design. It is likely that other factors may have contributed to the overall proportion of ED and depression observed, and also the two conditions tend to have a reciprocal relationship, most often coexisting (Asboe et al., 2007, Judd et al., 2005)

Following these observations, I wish to report that at least 50% of patients with hypogonadism manifested one or more of the symptoms reported in literature as being associated with hypogonadism(Makhlouf et al., 2008; Zarrouf et al., 2009). These were; depression, ED, tachycardia or obesity. Five patients did not however register any symptoms screened for and could thus be labelled asymptomatic. This is expected too since hypogonadism is fairly subtle and symptoms may mimic other diseases, making it difficult to diagnose especially if it is late onset hypogonadism or secondary to other underlying conditions (Bhasin et al., 2018; Wu et al., 2010)

5.5 Other Notable Findings

5.5.1 Pulse Rate

There was a higher prevalence of hypogonadism (25%) observed among those with tachycardia (pulse rates above 100) compared to those without (3.6%) This was the only association in the study that was found to be statistically significant, p=0.006. It supports prior evidence from literature which showed that patients with hypogonadism were more likely to have higher pulse rates and also higher Blood Pressures as part of metabolic & cardiovascular complications(Simon et al., 1997)

5.5.2 Hypertension

I found a higher prevalence of hypogonadism (9.8%) in patients with hypertension (SBP>140) than those without (3.8%). Though there was no statistically significant association between the two variables, other earlier findings from literature review suggest that hypogonadism may be an associated factor in cardiovascular disease & metabolic syndrome (Simon et al., 1997; Singh et al., 2011)

5.5.3 BMI

Four patients with hypogonadism had BMI above 25. This was not statistically significant though. Previous studies found positive correlation between hypogonadism and obesity (Simon et al., 1997). There were no statistically significant differences noted between hypogonadal and eugonadal participants in the study in relation to both the waist circumference and BMI. This may have been due to the low numbers realised in the results section that could not allow for gainful comparison or analysis in the statistical difference.

5.5.4 Age

The mean age of my study participants was 47.4 (SD: 8.7) years. Contrary to most other study findings done elsewhere, we did not observe any statistically significant association between hypogonadism and increasing age. Most of the patients with low TT were young, below 35 yrs (1) and middle aged 35-49yrs (7). Only 2 were above 50 years of age. This however, compares to another study that noted similar findings (Marie Lachâtre et al., 2017)(Pathak et al., 2015). From community-based population studies, it is known that TT levels naturally decline with increasing age especially after the age of 45 years, this even in healthy populations. HIV may nevertheless lead to premature decline in levels as was observed in this study.

5.6 Study Strengths

Sample collection was done in the early morning hours before 10 A.M which is the best recommended practice in order to minimise erroneous results that may be occasioned by diurnal variations that occur with Testosterone levels.

This study was able to investigate a subject that is rarely discussed in the African/Kenyan context due to the privacy or taboo nature that is ascribed to it in most cultures. It was therefore a fairly inquisitive venture into uncharted territories that constitute the sexual or reproductive health of our patients and more so males.

5.7 Study Limitations

Sex Hormone Binding Globulin (SHBG) was not measured in this study. Whereas estimation of TT levels may be sufficient for screening purposes, the fact that SHBG may be elevated in patients with HIV means that a number of cases may have been missed because TT may appear normal whereas actual FT is low. Whereas FT would have been an ideal mitigation to this limitation, it was not readily available in accessible laboratories and was thus not tenable.

Hormonal assay was done only once as opposed to having measures done on two separate occasions or days given the diurnal variation element that applies to most hormonal assays, including testosterone. This however may only be necessary when further analysis and evaluation is needed in the work up of symptomatic patients and not for screening purposes. We however ensured that blood samples drawn for the study were taken between 8-10 AM, when the hormonal levels are at peak to minimize false positives for hypogonadism.

The questionnaires used in this study heavily relied on utmost sincerity and honesty of the patients enrolled in the study. Given the nature of the topic under study, especially matters surrounding sexuality and depression, it is not entirely possible to guarantee that patients unreservedly exercised these virtues because of a cultural context that either views their discussion as taboo or also the stigma that is associated with ED and depression. However, every effort (confidentiality assurance, education and total privacy at data collection) was put in place to mitigate this potential bias that could result in underreporting of symptoms.

CHAPTER SIX: CONCLUSION AND RECOMMENDATION

6.1 Conclusions

i. Hypogonadism, though uncommon, still occurs in male patients living with HIV. From this study however, the prevalence was low.

ii. About one half of the patients with hypogonadism displayed features of either ED, depression or both, with the rest being asymptomatic.

iii. Though this was an incidental finding, a remarkable proportion of male patients with HIV attending AMPATH clinics were found to have ED, depression or even both.

6.2 Recommendations

Based on these study findings, I wish to recommend the following:

i) Testing for hypogonadism among male patients with HIV should only be done when there is a high index of suspicion for the condition. These include patients with ED and depression.

ii) Routine screening for erectile dysfunction and depression among male patients with HIV attending AMPATH clinics in MTRH should be considered.

iii) A study exploring factors associated with ED and depression among male patients with HIV in MTRH is recommended.

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APPENDICES

Appendix 1: Consent Form

I am Maling Jairus, a medical doctor pursuing a postgraduate degree (master of Medicine- Internal Medicine) at the Moi University School of Medicine.

I wish to recruit you as a study participant in my research. The study topic for the research is “Hypogonadism among male patients with HIV at MTRH Eldoret”.

What is the purpose of the study?

The purpose of the study is to determine the prevalence of hypogonadism among male patients with HIV seeking care at the comprehensive care clinic at the AMPATH modules in MTRH, Eldoret. The study will also evaluate for clinical presentation of hypogonadism in those studied.

What is the nature of the study?

In this study, upon your consent, you will be asked to give information regarding your age, marital status, occupation, level of education, and also whether you smoke or take alcohol.

You will be asked questions pertaining to your reproductive health, specifically, erectile function. Additionally, another set of questions will be asked pertaining to your mental health to assess for depression. Thereafter a brief, focused physical examination will be performed on your body. This will include weight & height measurements, blood pressure, and waist circumference.

A capillary prick on one of your fingers will be done to test for blood sugar levels.

After these, a blood sample will be drawn from your veins for hormonal (Testosterone) level assessment. There are minimal risks that may arise with the

stated procedures that include pain, slight bleeding, swelling and discomfort at the injection site.

Your participation in the study is voluntary. You may withdraw at any point in the study. Refusal or withdrawal from the study will not in any way adversely impact the quality and provision of care given to you. All information obtained will be treated with utmost confidentiality.

Communication to your primary care clinician will be made regarding outcome of the measures and tests for necessary action to be taken. These findings will also be communicated to you.

Consent

I, _____, having been explained to the nature and purpose of this study and having understood well, do hereby voluntarily agree to participate in the study. I will exercise utmost honesty in doing so and will cooperate fully.

Signature _____ Date _____

Should you need further clarification, you can contact me on 0723 740 781 or alternatively you may contact the Institutional Research and Ethics committee (IREC) using the address provided below:

The Chairman IREC

Moi Teaching and Referral Hospital

P.O Box 3

Eldoret

Appendix 2: Data Collection Form

Study number …… Date ……

1. Age ……..

2. Marital Status: Single ☐ Married ☐ Divorced ☐

Separated ☐ Widowed ☐

3. Level of Education: Primary ☐ Secondary ☐ Tertiary ☐

4. History of (or current use) of alcohol: Yes ☐ No ☐

5. History of (or on-going) tobacco smoking: Yes ☐ No ☐

6. Is patient on HAART? Yes ☐ No ☐ If yes, Duration? ……..

7. Regimen of HAART ……..

8. History of chemotherapy or radiation therapy Yes ☐ No ☐

9. History or current use of: Fluconazole ☐ Testosterone ☐ Prednisone ☐

Morphine ☐ Haloperidol ☐ Metoclopramide ☐

10. Known diagnosis of: CKD ☐ CLD ☐ CANCER ☐

11. Any other medication(s) patient may be on: …..……

12. Weight ……. Height ……. BMI ……. WC ……..

13. SBP ……. DBP …… PR ……

14. RBS (mmol/L)…….

15. Serum TT level (ng/dL) …….

16. CD4+Count (if available) ……. 17. Viral Load …..……

Appendix 3: Random Blood Sugar Testing

A blood glucose measuring kit, Glucowell, compliant with the 2013 ISO standards established for glucose monitoring was used.

The following steps were followed;

1. The index or middle finger was cleaned with a spirit swab and left to dry

2. Glucometer was turned on and test strip prepared (mounted) as per user's guide

3. Lancing device was prepared according to the user guide.

4. Finger prick was performed to obtain a drop of blood

5. Test strip was held against the blood drop until it absorbed enough to begin the test

6. Test result was viewed on the meter screen and recorded

7. Used lancet and test strip were discarded safely into safety box

NB: This method employed direct glucose reading electrodes to detect the molality of glucose in plasma or whole blood.

Accuracy checks were performed on the glucose meters using control solutions as provided for by the manufacturer. This was done once every two weeks as per specification in the accompanying user's guide.

Appendix 4: Serum Total Testosterone Testing

Total Testosterone (TT) assays measure both free and protein (SHBG & albumin) bound testosterone. It is measured using immunological methods and is either determined directly from serum (direct assays) or after isolation from serum using extraction or chromatographic techniques (indirect assays). Currently, as is with most patient care centres, direct assays are performed for immunological determination of TT. Initially, radioimmunoassay techniques were used for this purpose but have since been replaced by chemically or biochemically based techniques.

Other methods that can be used in determination of Total Testosterone are mass spectrometry, which is notably expensive and largely unavailable in most areas of clinical practice. These methods ionize testosterone and separate it from other compounds, it is then "filtered" and broken down into smaller fragments under controlled conditions. Fragments characteristic for testosterone are measured after second "filtering" process.

Immunoassays and mass spectrometry may show variability in testosterone measurements. This is mainly caused by differences in calibration and assay specificity. Specificity is higher with spectrometry. According to studies by the CDC and PATH this variability may range between 0 to -27% for concentrations >4.0nmol/L and 8-22% for <4.0nmol/L.

For purposes of this study, TT was determined using the direct immunological assay method in a standard reference laboratory, ISO certified with up to date quality control assessments and having set Standard Operating Procedures for serum TT assay.

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Elecsys Testosterone II

cobas®

REF	Σ	SYSTEM
05200067 190	100	MODULAR ANALYTICS E170 **cobas e** 411 **cobas e** 601 **cobas e** 602

English

System information

For **cobas e** 411 analyzer: test number 111
For MODULAR ANALYTICS E170, **cobas e** 601 and **cobas e** 602 analyzers: Application Code Number 216

Intended use

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

The electrochemiluminescence immunoassay "ECLIA" is intended for use on Elecsys and **cobas e** immunoassay analyzers.

Summary

References[1,2,3,4,5,6]

The androgen testosterone (17β-hydroxyandrostenone) has a molecular weight of 288 daltons. In men, testosterone is synthesized almost exclusively by the Leydig cells of the testes. The secretion of testosterone is regulated by luteinizing hormone (LH), and is subject to negative feedback via the pituitary and hypothalamus.

Testosterone promotes the development of the secondary sex characteristics in men and serves to maintain the function of the prostate and seminal vesicles.

Most of the circulating testosterone is bound to carrier proteins (SHBG = sex hormone-binding globulin).

In women, small quantities of testosterone are formed in the ovaries. In physiological concentrations, androgens have no specific effects in women. Increased production of testosterone in women can cause virilization (depending on the increase).

The determination of testosterone in women is helpful in the diagnosis of androgenic syndrome (AGS), polycystic ovaries (Stein-Leventhal syndrome) and when an ovarian tumor, adrenal tumor, adrenal hyperplasia or ovarian insufficiency is suspected.

Testosterone is determined in men when reduced testosterone production is suspected, e.g. in hypogonadism, estrogen therapy, chromosome aberrations (as in the Klinefelter's syndrome) and liver cirrhosis.

The Elecsys Testosterone II assay is based on a competitive test principle using a high affinity monoclonal antibody (sheep) specifically directed against testosterone. Endogenous testosterone released from the sample by 2-bromoestradiol competes with the added testosterone derivative labeled with a ruthenium complex[a] for the binding sites on the biotinylated antibody.

The Elecsys Testosterone II assay shows an improved performance if compared to Isotope Dilution - Gas Chromatography/Mass Spectrometry (ID-GC/MS) reference method in the female concentration range.

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

Test principle

Competition principle. Total duration of assay: 18 minutes.

- 1st incubation: 20 µL of sample are incubated with a biotinylated monoclonal testosterone-specific antibody. The binding sites of the labeled antibody become occupied by the sample analyte (depending on its concentration).
- 2nd incubation: After addition of streptavidin-coated microparticles and a testosterone derivate labeled with a ruthenium complex, the complex becomes bound to the solid phase via interaction of biotin and streptavidin.
- The reaction mixture is aspirated into the measuring cell where the microparticles are magnetically captured onto the surface of the electrode. Unbound substances are then removed with ProCell/ProCell M. Application of a voltage to the electrode then induces chemiluminescent emission which is measured by a photomultiplier.
- Results are determined via a calibration curve which is instrument-specifically generated by 2-point calibration and a master curve provided via the reagent barcode or e-barcode.

Reagents - working solutions

The reagent rackpack is labeled as TESTO II.

M Streptavidin-coated microparticles (transparent cap), 1 bottle, 6.5 mL:
Streptavidin-coated microparticles 0.72 mg/mL, preservative.

R1 Anti-testosterone-Ab–biotin (gray cap), 1 bottle, 10 mL:
Biotinylated monoclonal anti-testosterone antibody (sheep) 40 ng/mL; releasing reagent 2-bromoestradiol; MES buffer 50 mmol/L, pH 6.0; preservative.

R2 Testosterone-peptide–$Ru(bpy)_3^{2+}$ (black cap), 1 bottle, 9 mL:
Testosterone derivative, labeled with ruthenium complex 1.5 ng/mL; MES buffer 50 mmol/L, pH 6.0; preservative.

Precautions and warnings

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

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

Reagent handling

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

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

Storage and stability

Store at 2-8 °C.

Do not freeze.

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

Stability:	
unopened at 2-8 °C	up to the stated expiration date
after opening at 2-8 °C	12 weeks
on the analyzers	8 weeks

Specimen collection and preparation

Only the specimens listed below were tested and found acceptable.

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

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

Criterion: Recovery within 80-120 % of serum value > 1 ng/mL, recovery of ± 0.2 ng/mL of serum value ≤ 1 ng/mL and slope 0.9-1.1 + intercept 0.05 ng/mL + coefficient of correlation > 0.95.

Stable for 1 week at 2-8 °C, 6 months at -20 °C (± 5 °C). Freeze only once.[7]

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

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Elecsys Testosterone II

cobas®

Centrifuge samples containing precipitates before performing the assay.

Do not use heat-inactivated samples.

Do not use samples and controls stabilized with azide.

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

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

Materials provided

See "Reagents – working solutions" section for reagents.

Materials required (but not provided)

- REF 05202230190, Testosterone II CalSet II, for 4 x 1 mL
- REF 11731416190, PreciControl Universal, for 4 x 3 mL
- General laboratory equipment
- MODULAR ANALYTICS E170 or **cobas e** analyzer

Accessories for **cobas e** 411 analyzer:

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

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

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

Accessories for all analyzers:

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

Assay

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

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

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

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

Calibration

Traceability: This method has been standardized via ID-GC/MS ("Isotope Dilution - Gas Chromatography/Mass Spectrometry").[6,9]

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

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

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

Renewed calibration is recommended as follows:

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

Quality control

For quality control, use PreciControl Universal.

In addition, other suitable control material can be used.

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

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

If necessary, repeat the measurement of the samples concerned.

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

Calculation

The analyzer automatically calculates the analyte concentration of each sample (either in ng/mL, ng/dL or nmol/L).

Conversion factors:

ng/mL x 3.47 = nmol/L

ng/mL x 100 = ng/dL

nmol/L x 0.288 = ng/mL

Limitations - interference

The assay is unaffected by icterus (bilirubin < 513 µmol/L or < 30 mg/dL), hemolysis (Hb < 0.372 mmol/L or < 0.600 g/dL), lipemia (Intralipid < 1000 mg/dL) and biotin (< 123 nmol/L or < 30 ng/mL).

Criterion: Recovery within ± 10 % of initial value (concentration range > 1-15 ng/mL), recovery within ± 15 % of initial value (concentration range > 0.5-1 ng/mL) and recovery of ± 0.075 ng/mL (concentration range of 0.150-0.500 ng/mL).

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

No interference was observed from rheumatoid factors up to a concentration of 1000 IU/mL.

In vitro tests were performed on 18 commonly used pharmaceuticals. No interference with the assay was found.

Two special drugs were additionally tested. A strong interaction with Nandrolone (INN international nonproprietary name, WHO) was found. Do not use samples from patients under Nandrolone treatment.

In isolated cases, elevated testosterone levels can be seen in samples from female patients with end stage renal disease (ESRD).

Implausible elevated testosterone values in women should be verified by an extraction method or a validated LC-MS/MS tandem method.[5]

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

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

Limits and ranges

Measuring range

0.025-15.0 ng/mL or 0.087-52.0 nmol/L (defined by the Limit of Detection and the maximum of the master curve). Values below the Limit of Detection are reported as < 0.025 ng/mL or < 0.087 nmol/L. Values above the measuring range are reported as > 15.0 ng/mL or > 52.0 nmol/L.

Lower limits of measurement

Appendix 5: PHQ 9

Over the last 2 weeks, how often have you been bothered by any of the following problems? *(Use "✔" to indicate your answer)*	**Not at all**	**Several days**	**More than half the days**	**Nearly Every Day**
1. Little interest or pleasure in doing things	0	1	2	3
2. Feeling down, depressed, or hopeless	0	1	2	3
3. Trouble falling or staying asleep, or sleeping too much	0	1	2	3
4. Feeling tired or having little energy	0	1	2	3
5. Poor appetite or overeating	0	1	2	3
6. Feeling bad about yourself — or that you are a failure or have let yourself or your family down	0	1	2	3
7. Trouble concentrating on things, such as reading the newspaper or watching television	0	1	2	3
8. Moving or speaking so slowly that other people could have Noticed? Or the opposite — being so fidgety or restless that you have been moving around a lot more than usual	0	1	2	3
9. Thoughts that you would be better off dead or of hurting yourself in some way	0	1	2	3

FOR OFFICE CODING 0_____ _____ **+** _____ **+** _____ **+** _____

Total Score: _____

If you checked off any problems, how difficult have these problems made it for you to do your work, take care of things at home, or get along with other people?

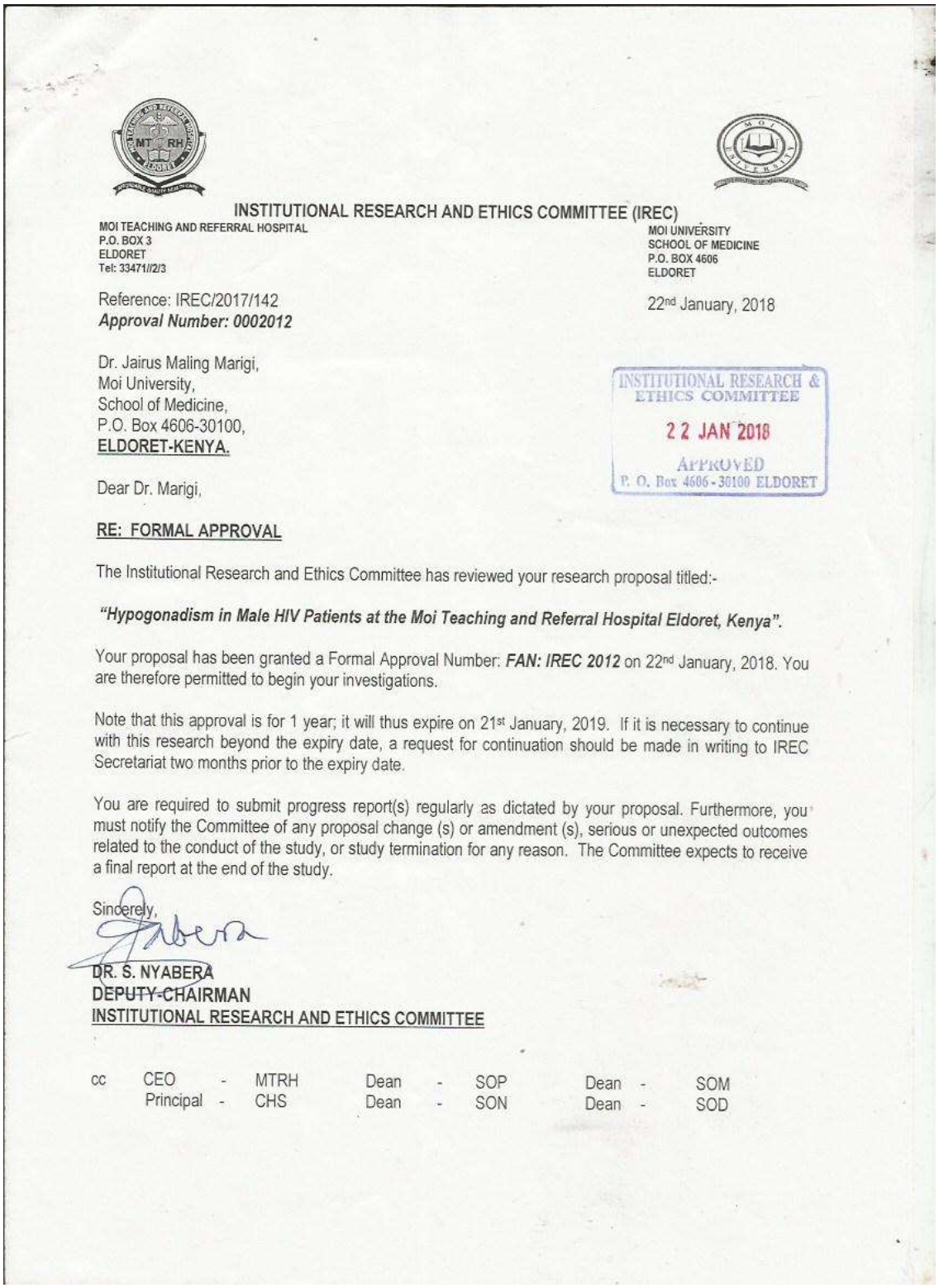
Not difficult at all	**Somewhat Difficult**	**Very difficult**	**Extremely Difficult**
…	…	…	…

Appendix 6: IIEF-5

Unique number _____ Age _____ Total score _____

	1	***2***	***3***	***4***	***5***
How do you rate your confidence that you could get and keep an erection?	very low	low	moderate	high	very high
When you had erections with sexual stimulation, how often were your erections hard enough for penetration?	almost never or never	much less than half the time	about half the time	much more than half the time	almost always or always
During sexual intercourse, how often were you able to maintain your erection after you had penetrated (entered) your partner?	almost never or never	much less than half the time	about half the time	much more than half the time	almost always or always
During sexual intercourse how difficult was it to maintain your erection to the completion of intercourse?	extremely difficult	very difficult	difficult	slightly difficult	not difficult
When you attempted sexual intercourse, how often was it satisfactory for you?	almost never or never	much less than half the time	about half the time	much more than half the time	almost always or always

APPENDIX 7: Approvals



INSTITUTIONAL RESEARCH AND ETHICS COMMITTEE (IREC)

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

MOI UNIVERSITY
SCHOOL OF MEDICINE
P.O. BOX 4606
ELDORET

Reference: IREC/2017/142
Approval Number: 0002012

22nd January, 2018

Dr. Jairus Maling Marigi,
Moi University,
School of Medicine,
P.O. Box 4606-30100,
ELDORET-KENYA.

INSTITUTIONAL RESEARCH & ETHICS COMMITTEE
22 JAN 2018
APPROVED
P. O. Box 4606 - 30100 ELDORET

Dear Dr. Marigi,

RE: FORMAL APPROVAL

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

"Hypogonadism in Male HIV Patients at the Moi Teaching and Referral Hospital Eldoret, Kenya".

Your proposal has been granted a Formal Approval Number: ***FAN: IREC 2012*** on 22nd January, 2018. You are therefore permitted to begin your investigations.

Note that this approval is for 1 year; it will thus expire on 21st January, 2019. If it is necessary to continue with this research beyond the expiry date, a request for continuation should be made in writing to IREC Secretariat two months prior to the expiry date.

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

Sincerely,

DR. S. NYABERA
DEPUTY-CHAIRMAN
INSTITUTIONAL RESEARCH AND ETHICS COMMITTEE

cc CEO - MTRH Dean - SOP Dean - SOM
Principal - CHS Dean - SON Dean - SOD

An ISO 9001:2015 Certified Hospital

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

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

26th January, 2018

Dr. Jairus Maling Marigi,
Moi University,
School of Medicine,
P.O. Box 4606-30100,
ELDORET-KENYA.

APPROVAL TO CONDUCT RESEARCH AT MTRH

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

"Hypogonadism in Male HIV Patients at the Moi Teaching and Referral Hospital, Eldoret, Kenya".

MOI TEACHING AND REFERRAL HOSPITAL
C E O
APPROVED
26 JAN 2018
SIGN.....
P. O. Box 3 - 30100, ELDORET

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

Date 26/01/2018

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

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