INFLAMMATORY MARKERS AMONG HIV PARTICIPANTS WITH AND WITHOUT GLUCOSE METABOLIC DISORDER AFTER ATTAINING UNDETECTABLE VIREMIA IN DAR ES SALAAM TANZANIA

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DECLARATION

Declaration by the candidate:

This thesis report is my original work, and has not been presented to any other university. No part of this thesis may be reproduced without prior permission of the principal investigator.

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LIST OF ABREVIATIONS

IFG	Impaired fasting glucose
IGT	Impaired glucose tolerance
IL-1	Interlukin 1
IL-6	Interlukin 6
WHO	World health organisation
TNF	Tumour necrosis factor
Hs-CRP	High sensitive C reactive protein
MTRH	Moi teaching and referral hospital
OGTT	Oral glucose tolerance test
T2D	Type 2 diabetes
WBC	White blood cells
PLWH	People living with HIV
ESPIRIT	Evaluation of subcutaneous proleukin in a randomized trial
SMART	Strategies for management of antiretroviral therapy
EPA	Epcosapentaenoic acid
DHA	Docosahexaenoic acid
SSA	Sub Sahara Africa
NACP	National AIDS control program

DEFINITION OF TERMS

Glucose metabolic disorder: is characterized by the presence of impaired fasting glucose (IFG) which is defined as fasting blood glucose of 6.1 to 6.9mmol/l (110mg/dl to 125mg/dl) and 2-hour glucose <7.8mmol/l (140mg/dl). Impaired glucose tolerance (IGT) is defined as fasting blood glucose <7.0mmol/l (126mg/dl) and 2-hour glucose ≥7.8 and <11.1mmol/l (140mg/dl and 200mg/dl). Diabetes mellitus (DM) will either be a fasting blood glucose ≥7.0mol/l (126mg/dL) or a glucose level ≥11.1mol/l (200mg/dL) 2 hours after a 75g oral glucose load (WHO 2006; Maganga et al. 2015)

Impaired fasting glucose (IFT): is an asymptomatic state of hyperglycemia which is fasting glucose of 6.1 to 6.9mmol/l (110mg/dl to 125mg/dl) and 2-hour glucose <7.8mmol/l (140mg/dl) but not high enough to be classified as diabetes mellitus (WHO 2006; Maganga et al. 2015).

Impaired glucose tolerance (IGT): is an asymptomatic state of hyperglycemia characterized by fasting blood glucose <7.0mmol/l (126mg/dl) and 2-hour glucose ≥7.8 and <11.1mmol/l (140mg/dl and 200mg/dl) although not high enough to confirm diabetes (WHO 2006; Maganga et al. 2015).

Oral glucose tolerance test (OGTT): This is a diagnostic test for measuring venous plasma glucose 2 hours after ingestion of 75g oral glucose load. (WHO 2006).

Pre- diabetic state: is an intermediate hyperglycemic state using two specific parameters, impaired fasting glucose (IFG) defined as fasting plasma glucose of 6.1-6.9 mmol/L (110 to 125 mg/dL) and impaired glucose tolerance (IGT) defined as 2 h plasma glucose of 7.8-11.0 mmol/L (140-200 mg/dL) after ingestion of 75 g of oral glucose load or a combination of the two based on a 2 h oral glucose tolerance test (OGTT) (WHO 2006**.**

Traditional risk factors: these are known risk factors that predispose an individual to acquire glucose metabolic disorders including frank diabetes; example: obesity, advanced age, family history of diabetes, lifestyle norms (smoking and alcohol intake) (Regidor et al. 2012)(Anothaisintawee et al. 2018)

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ABSTRACT

Back ground: HIV related inflammation is associated with an increased risk of getting Glucose metabolic disorders, however there is limited data linking inflammation and glucose metabolic disorder in Sub Saharan Africa.

Objectives: To determine the levels of inflammatory markers among HIV positive patients with glucose metabolic disorders and those without glucose metabolic disorders.

Methods: A comparative cross-sectional study was carried out at the infectious disease control clinic (IDC) in Tanzania from March to May 2018. Purposive sampling was used to obtain participants who had undetectable viral load, on 1st line ART and had taken their last meal >8hrs ago. The WHO step wise questionnaire was used to collect demographic, and social behavioral characteristics. Physical measurements were done to determine blood pressure, BMI and waist to height ratio. Blood samples were collected to test for inflammatory markers (IL6 and CRP) and blood glucose. Statistical analysis was done using Statistical Package Social Sciences (SPSS) version 23. Chi square was used to measure the association, and binary Logistic regression was used to estimate the odds ratio. A p value less than 5% was statistically significant.

Results: A total of 240 participants were enrolled. Of those enrolled, 42% percent were overweight/obese (>25kg/m^2) and 89% had a high waist to height ratio. The median ART duration was 8 (5-10) years. The prevalence of glucose metabolic disorders among the HIV population was 33%. Those who had inflammation (CRP) and glucose metabolic disorders (46%) were two times the number of those who had inflammation without glucose metabolic (28%) (P-value – 0.019). CRP was associated with a 1.95 fold increased odds of having glucose metabolic disorders. (OR-1.95 (1.09-4.3) (p=0.019). We did not find a significant association between IL-6 and glucose metabolic disorder.

Conclusion: There was a significant difference in the levels of inflammation (CRP) among those with glucose metabolic disorders compared to those without.

Recommendation: Further studies are required with a bigger sample size and a control group.

CHAPTER ONE

1.0 INTRODUCTION

1.1 Background

Improved access to antiretroviral therapy (ART) and advances in HIV care in sub-Saharan Africa (SSA) has significantly increased life expectancy for people living with HIV (PLHIV) (Kharsany & Karim, 2016). However, with aging and more time on ART, PLHIV are now susceptible to non-communicable diseases (NCD) such as glucose metabolic disorders (GMD) (Moyo et al., 2014; Ngatchou et al., 2013). GMD includes the presence of impaired fasting glucose (IFG), impaired glucose tolerance (IGT), and diabetes mellitus type 2 (T2DM). Published reports from SSA are witnessing an escalating burden of GMD (Levitt et al., 2016; Maganga, Smart, Kalluvya, & Kataraihya, 2015; Ngatchou et al., 2013) up to a four times greater compared to HIV negative controls (Maganga et al., 2015). This may be due to a mix of HIV related risk factors including chronic systemic inflammation (Drain et al., 2007; Slim & Saling, 2016), ART agents such as stavudine (Karamchand et al., 2016) and zidovudine (Karamchand et al., 2016), and traditional risk factors such as physical inactivity, harmful alcohol use, smoking, overweight, and obesity that also affects the general population (Anothaisintawee, Reutrakul, Van Cauter, & Thakkinstian, 2018; Chimbetete et al., 2017; Noumegni et al., 2017).

It has been hypothesized that GMD in PLHIV is a result of an ongoing chronic inflammatory response (Brown, Tassiopoulos, Bosch, Shikuma, & McComsey, 2010; Dooko et al., 2014). Several studies from high income countries among PLHIV have demonstrated correlations between high levels of pro-inflammatory cytokines such as IL-6, CRP and tumour necrosis factor (TNF α), and glucose abnormalities, independent of body

mass index (BMI) and age (Brown et al., 2010; Dooko et al., 2014). In SSA, inflammation is reported to increase the risk of mortality (Drain et al., 2007; Ledwaba et al., 2012) as well as cardiovascular disease for the HIV population (Muswe et al., 2017), however data linking inflammation and GMD is limited.

Data on inflammation and GMD from high income countries may not be reliably used for PLHIV in SSA, because of the following reasons: the existence of heterogeneity between inflammatory markers and diabetes type 2 based on race/ethnicity (Effoe, Correa, Chen, Lacy, & Bertoni, 2015), a lower prevalence of traditional risk factors such as obesity which may influence systemic inflammation in SSA compared to the high income countries (Njuguna et al., 2018) , and a higher background inflammatory state in the general population (Njuguna et al., 2018). This creates a demand for SSA to get regional data on the influence of chronic inflammation on the growing prevalence of GMD among PLHIV.

The inflammatory markers of concern are many (Dallmeier et al., 2012) but due to the biological and laboratory reproducibility of IL6 and CRP, and their ability to predict the occurrence of non AIDS events (Hearps, Martin, Rajasuriar, & Crowe, 2014), this study assessed the levels of CRP and IL6 among PLHIV with GMD in Tanzania.

1.2. Problem Statement

The occurrence of Glucose metabolism disorder is a gradual process that proceeds from being asymptomatic hyperglycemic (pre-diabetic) to full blown diabetes. Several interventions aimed at interrupting disease progression such as provision of anti-inflammatory agents and lifestyle changes have shown positive results in restoring normal glucose metabolism. However lack data from people of SSA descent is limiting the

application of these interventions in our setting.

Furthermore acquiring glucose metabolic disorder for the HIV participant complicates the management of HIV and accelerates mortality even after attaining undetectable viral load and high CD T cell count. This creates a need for regional data, so as to improve the lives of people living with HIV in SSA and prevent the occurrence of co-morbidities.

1.3. Rationale

Type 2 diabetes can be prevented or delayed, if the modifiable risk factors such as inflammation are mitigated.

Therefore this study will elaborate the role of inflammation in association with glucose metabolic disorder, paving way for studies that will be able to establish temporal relationship. Furthermore, classifying participants beyond traditional risk factors, basing on inflammatory markers may improve diagnosis and inform therapetical endeavors.

Lastly, this study will also provide a base of justifying the in cooperation of scheduled screening of glucose for the HIV participants.

1.4. Research Questions

1.4.1. Is there a significant difference in the levels of inflammation (IL6 and CRP) between HIV participant with glucose metabolic disorder and those without?

1.5. Objectives.

1.5.1. Broad objective:

1.5.1.1. To determine the association of inflammatory markers among HIV patients with and without glucose metabolic disorders after attaining undetectable viremia.

1.5.2. Specific Objectives:

1.5.2.1. To determine the prevalence of glucose metabolic disorder among HIV positive patients on ART.

1.5.2.2. To compare the levels of inflammatory markers (IL6 and CRP) in HIV infected individuals with and without glucose metabolic disorder.

1.5.2.3. To determine factors associated with glucose metabolic disorder among HIV participants on ART.

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1. Glucose Metabolic Disorder Case Definition

Glucose metabolic disorder is characterized by the presence of impaired fasting glucose, impaired glucose tolerance and diabetes mellitus type 2. (WHO 2006; Maganga et al. 2015). The first two are asymptomatic pre-diabetic states where one has blood sugars above normal but not high enough to confirm diagnosis of diabetes. The latter is the disease status.

GMD state	Fasting blood glucose	2-hour glucose (OGTT)
Impaired fasting glucose (IFG)	6.1 to 6.9mmol/l	<7.8mmol/l
Impaired glucose tolerance (IGT)	<7.0mmol/l	≥7.8 and <11.1mmol/l
Diabetes mellitus (DM)	≥7.0mmol/l	≥11.1mmol/l

Both IFG and IGT are due to insulin resistance differing in the originating site (Eckel et al. 2011) that is mainly hepatic insulin resistance in IFG and severe muscle insulin resistance for IGT. Although both states have β cell dysfunction, IGT presents early and late phase insulin response impairment while IFG normalizes on the second phase (DeFronzo& Abdul-Ghani 2011).

Moreover, IFG and IGT states represent a higher risk of acquiring type 2 diabetes mellitus by 25% over an observational period of 3-5 years (Nathan et al. 2007), and up to 70% on increased observation time (Anon 2007). These two states have a higher predicting value of glucose metabolic disorders compared to other non-blood risk indicators such as obesity, age and family history which are also strongly linked to the outcome (Buijsse et al. 2011).

Type 2 Diabetes mellitus is a slow progressing metabolic disorder with about 90% of all diabetic cases presenting with high blood sugar due to inadequate insulin secretion from β

pancreatic cells and the resistance of action. Common symptoms include increased thirsty, frequent urination, weight loss just to mention a few. Uncontrolled diabetes is intertwined with other complications caused by the high sugar level such as coronary heart disease, kidney failure, retinopathy and eventually death.

2.2. HIV Infection and Inflammation

Inflammation is an initial immune response to defend the body against entry of harmful microorganisms such as viruses, bacteria, fungi and parasites. It involves the release of leukocytes, erythrocytes and components of plasma into the affected tissue (Mayer & Bhikha 2013). Inflammatory cytokines are very short lived, and in absence of the stimulus their response is usually self-limiting (Lawrence & Gilroy 2007).

The persistence of immune activation due to unresolved stimuli causes chronic inflammation and this is what is seen with HIV infection. Chronic inflammations are implicated with the persistent presence of HIV virus (Eastburn et al. 2011); and even strongly correlated with high viremia of >1000 copies/ml. Therefore in patients on effective ART, the inflammatory process is expected to be reduced almost close to a HIV negative patient (Wada et al. 2015).

However, this is not always the case because studies have reported persistence of chronic inflammation in the presence of low viremia and high CD4 T cell count; about 40-60% times more compared to uninfected adults (Neuhaus et al. 2010). Moreover others have found 2.7 fold higher odds of death among HIV subjects with high inflammation and

CD4 count >500 cells/ul (Tien et al. 2010). Similarly (Kuller et al. 2008) found chronic inflammation among HIV patients with HIV low viremia <400copies/ml.

Possibly the persistence of chronic inflammation could be explained by the extensively damaged gut lymphoid tissue among HIV infected individuals, where commensal microbes enter the systemic circulation causing immune activation (Tincati et al. 2016). It may also be due to immune dys-regulation, especially when the virus is not suppressed although it is unclear which precedes the other (Lederman et al. 2013). Unfortunately, anti-retroviral drugs do not completely suppress all these effects, inflammation is known to continue for a life time (Deeks et al. 2013). This explains the presence of inflammation in relatively healthy HIV-infected patients.

The inflammation process is characterized by the release of cytokines such as interferon-γ, interferon-α, Tumor necrosis factor (TNF), Interlukin-6 (IL6) and C-Reactive Protein (CRP) (Stacey et al. 2009). The strategies to manage antiretroviral therapy (SMART) clinical trials, have shown inflammatory markers to be independent predictors of all-cause mortality with 12.4 fold risk (Kuller et al. 2008). These findings have also been confirmed with other cohorts (Wada et al. 2015; EuroCoord et al. 2012).

Furthermore results from a study conducted in Tanzania among HIV pregnant mothers reported high maternal CRP to independently predict mortality and progression to WHO stage 4 even after adjusting for low CD4 T cell count levels, high HIV viral load and other prognostic variables (Drain et al. 2007). However they did not link the inflammation levels to non AIDS events such as glucose metabolic disorders.

In this regard HIV positive patients are at higher risk of co-morbidity and even death an

aspect that requires immediate intervention especially in the sub Saharan Africa which is home to 71% of HIV infected population globally (Reid et al. 2012).

2.3. Inflammatory markers and Diabetes type 2

Diabetes mellitus type 2, is an emerging pandemic responsible for over 3.8 million deaths globally (Susan van et al. 2010). By 2030, 366 million cases of diabetes will be expected with 90% increment of new cases in sub Saharan Africa alone. This increase will be contributed by individuals with IGT which is expected to reach 47.3million by 2030 from 27million in 2010 (Churchill 2013). In addition to HIV and malaria the un-proportional burden of disease in this region is expected to cripple economic progress in absence of intervention. Therefore identification of treatable causes of disease will help in preventing or delaying its onset.

Apart from the traditional risk factors like Obesity in the general population that are also at play among HIV positive patients (Fletcher et al. 2002), accumulating evidence is demonstrating the role of inflammatory markers in the pathogenesis of insulin resistance which impairs insulin sensitivity among diabetes type 2 subjects(He et al. 2014; Dinarello et al. 2010).

CRP, IL 6, IL2, and TNFα are inflammatory markers that have been vastly associated with diabetes (Lin et al. 2016). Clinically CRP has been mostly correlated with the risk of diabetes (Madsen et al. 2016). It is mainly synthesized by hepatocytes and regulated by IL 6 and TNF-α (He et al. 2014). IL6 on the other hand is mainly produced by activated leukocytes, endothelial cells and about 25% of it is produced by subcutaneous adipose tissue(Mohamed-Ali et al. 1997), hence it also has some correlation with higher BMI.

The hypothesis of inflammation resulting into glucose metabolic disorder is based on three main lines of evidence; the first is a number of non-obese individuals with no prior glucose abnormalities but having increased inflammatory markers developing diabetes (Barzilay et al. 2001).Several findings have supported this by demonstrating the occurrence of GMD in absence of obesity, overweight and physical inactivity (Spranger et al. 2003). Elevated inflammatory makers however; are reported to significantly increase the odds of getting GMD. The presence of elevated IL6 increase the odds three times elevated CRP increase the odds seven times. (Hu et al. 2004).

Secondly, comparatively elevated markers of inflammation among diabetics versus non diabetics; this is observed cross sectionally, where cytokines (IL 1 and IL 6) were not only responsible for stimulating the release of acute phase proteins, CRP but also acted on the liver to produce dyslipidemia which results in obesity increasing the risk of the disease(Pickup & Crook 1998). In other words abnormalities in the innate immune system contributes to hyper-triglycemia, hypertension, glucose intolerance and insulin resistance all features of diabetes mellitus type 2. (Pickup et al. 1997).

Lastly the incidence of diabetes on follow up of patients who had baseline levels of inflammation, a sub-study of ESPRIT (evaluation of subcutaneous proleukin in a randomized trial) and SMART, reported higher levels of IL6 and hs-CRP to increased risk of diabetes independent of BMI and age among HIV positive patients (Dooko et al. 2014). Also another cohort identified higher insulin levels to correlate with increased levels of IL6, hs-CRP when ART is initiated at CD4 T cell count < 200 even after this group

attaining higher a CD4 count due to effective ART after 3years follow up(Ghislain et al. 2015).

In high income countries inflammatory markers have been vastly studied (Freeman et al. 2002; Pradhan et al. 2003) with a majority demonstrating its role on the onset of GMD. However there are studies that did not find association after adjustment of other traditional risks such as >30 BMI and age, despite analyzing a vast number of inflammatory markers (Dallmeier et al. 2012). This finding was also replicated by other prospective studies (Festa et al. 2002 and Snijder et al. 2003).

Noteworthy is the fact that the above studies were performed on HIV negative patients. From these observations, it appears that HIV infection on its own causes low grade inflammation due to the persistent presence of HIV antigen causing continuous immune activation; hence the increased risk of GMD and other non AIDS events that are triggered by inflammation (Klatt et al. 2013).

In Africa, a five-fold increase of glucose metabolic disorder was seen among Tanzanian HIV patients, but the etiology is still unclear (Maganga et al. 2015). Another study conducted in Cameroon showed a prevalence of impaired glucose intolerance and diabetes to be 47% and 27%, respectively among HIV positive patients, but, they did not assess for inflammatory markers (Ngatchou et al. 2013). Similarly (Moyo et al. 2014) suggested a complex interaction of traditional risk factors causing metabolic changes, but did not assess the inflammatory markers. Moreover in two studies done in west Africa, one associated CRP and type 2 diabetes(Ehiaghe et al. 2013) while the other reported high levels of IL6

among rural dwellers compared to those in urban areas to be associated with diabetes (Darko et al. 2015).

In the quest to reduce the predicted incidence of diabetes in Sub Saharan Africa, identification of high risk populations and reducing the risk will not only help reduce diabetic prevalence, but also the high risk of mortality and other underlying morbidities that are accelerated by the presence of GMD such as cardiovascular disease.

2.4. HIV related risk factors and diabetes

Before the era of ART, HIV infection was a death sentence; those infected would succumb to opportunistic infection with certainty of mortality due to defective immunity (Slim & Saling 2016). Currently patients with HIV are living longer, an aspect that is recently being understood to have its associated consequences (Paula et al. 2013).

Despite the unquestionable success of ART, it is also being implicated to serious non AIDS events that are seen among the infected population. ART drugs namely stavudine (Capeau et al. 2012), zidovudine, lamivudine, lopinavir/rotinavir(Blümer et al. 2008) and efaverence (Dave et al. 2011) have been implicated with the occurrence of insulin resistance leading to glucose metabolic changes and cases are increasing with the global ART scale up.

The underlying mechanism causing diabetes on ART initiation cannot be fully explained, however, pro inflammatory cytokines are observed to decrease adiponectin a positive regulator of insulin sensitivity resulting to GMD (Lagathu et al. 2005).Also ART with PI lopinavir/rotinavir is reported to acutely block transport of glucose by insulin-sensitive

glucose transporter GLUT4 causing insulin resistance(Murata et al. 2000). Moreover, late initiation of ART at CD4 T cell count below 200cells/µl is also evidenced to have increased risk of GMD. Interestingly, T cell counts in these patients do not normalize even after a decade of effective therapy(Kelley et al. 2009). Results from a case control study reported 4.5 higher odds of diabetes and other non AIDS events when ART was initiated below 200cells(Guaraldi et al. 2011), hence the WHO requirement to start ART on diagnosis.

The overwhelming benefits of ART outweigh the occurrence of non AIDS events(Paula et al. 2013), and interruption is evidenced to increase the risk of co-morbidities and even death(Kuller et al. 2008). Efforts are being made to modify the adverse effects of ART, but with lack of a monitoring system in our setting, or even scheduled screening of glucose metabolic disorders, these patients will unfortunately be identified by the surfacing of symptoms due to disease status.

2.5. Obesity and un-modifiable Risk Factors of Diabetes

The rise of GMD in the developed and developing countries have been strongly correlated with the epidemic rise of obesity in the general population with six fold the risk (Luft et al. 2013) specifically due to the increase in waist circumference and higher waist to hip ratio (Eckel et al. 2011). Results from various studies (Han et al. 2002; Festa et al. 2002; Thorand et al. 2003) supported this finding. Moreover, studies done among HIV positive patients have reported conflicting findings when it comes to correlating GMD and obesity. Unlike in the general population, HIV patients are reported to have comparatively low BMI

compared to those who are HIV negative(Kengne et al. 2013). Since the ART effect on viral load is expected to wane over time, wasting effect is seen among seemingly overweight patients on long term use(Ogunmola et al. 2014).

Results from another study done in Kenya among HIV positive patients suggested the role of other risk factors other than obesity after observing patients losing 95.5% of fat following alteration of diet and lifestyle, but high blood sugar levels did not normalize from the baseline(Engelson et al. 2016).

Despite obesity being a strong predictor of diabetes, for patients who are HIV positive, inflammation seems to have a higher risk of the outcome (Brown et al. 2010; Neuhaus et al. 2010). Other known risk factors are advanced age (Hasse et al. 2011), male sex, Odds ratio 1.77 (Guaraldi et al. 2011), and family history of diabetes which have 2.4 fold-risk, but they are un-modifiable. Therefore apart from obesity which is a modifiable risk but is still a debatable cause of GMD among HIV positive patients, and the population observed reluctance to alter lifestyle norms to reduce the risk of obesity (Jones 2013), inflammation remains to be another main risk that is modifiable by the use of aspirin if identified early (Hu et al. 2004)**.**

2.6. Therapeutic Intervention to Counteract Inflammation

In an attempt to counteract the effect of inflammation among HIV positive patients who have glucose metabolic disorders, many interventions have been assessed, one of them being walking (Izzicupo et al. 2013; Ho et al. 2013). Despite conflicting findings, the few positive associations established cannot be overlooked. Majority of these studies are those that support obesity revoking inflammation which in-turn cause diabetes (Visser et al. 1999; Gupta & Johnson 2010).

Additionally, studies on n-3 polyunsaturated fatty acids (omega 3 oils) have also shown positive results in reduction of inflammation irrespective of obesity (Dekker et al. 2016). Four weeks of n-3 polyunsaturated fatty acids supplementation have shown to decrease CRP by 93% (Wigmore et al. 1997), and a 2g/d dose is enough to relinquish the ant-inflammatory effect (Rees et al. 2006). Furthermore, epcosapentaenoic acid (EPA) and docosahexaenoic acid (DHA)content of the oil was seen to reduce the TNF-α levels which in turn reduced insulin resistance (Das 2016) but clinical trials are yet to be done to confirm the association.

Furthermore, administration of Aspirin in uncontrolled trial showed significant reduction of activated monocyte (sCD14) in just a week (O‟‟Brien et al. 2013). A clinical trial that looked on synergistic effect of statin and aspirin, found additional ant-inflammatory effect when they were used at per (Chaudhary et al. 2016). Studies done on the anti-malarial hydroxy-chloroquine among HIV positive patients have also showed its ability to reduce inflammation when given to ART treated patients with CD4 count of <200cells/µ (Piconi et al. 2011)**.**

Targeting important asymptomatic viral co-infection among HIV patients have shown significant reduction in the inflammatory process. For example, cytomegalovirus has been reported to be responsible for about 10% of all inflammatory process (Naeger et al. 2010). Early initiation of ART before the depletion of CD4 T cell count has also been shown to maintain inflammation state at baseline levels (Slim & Saling 2016)

2.7. Conceptual Framework

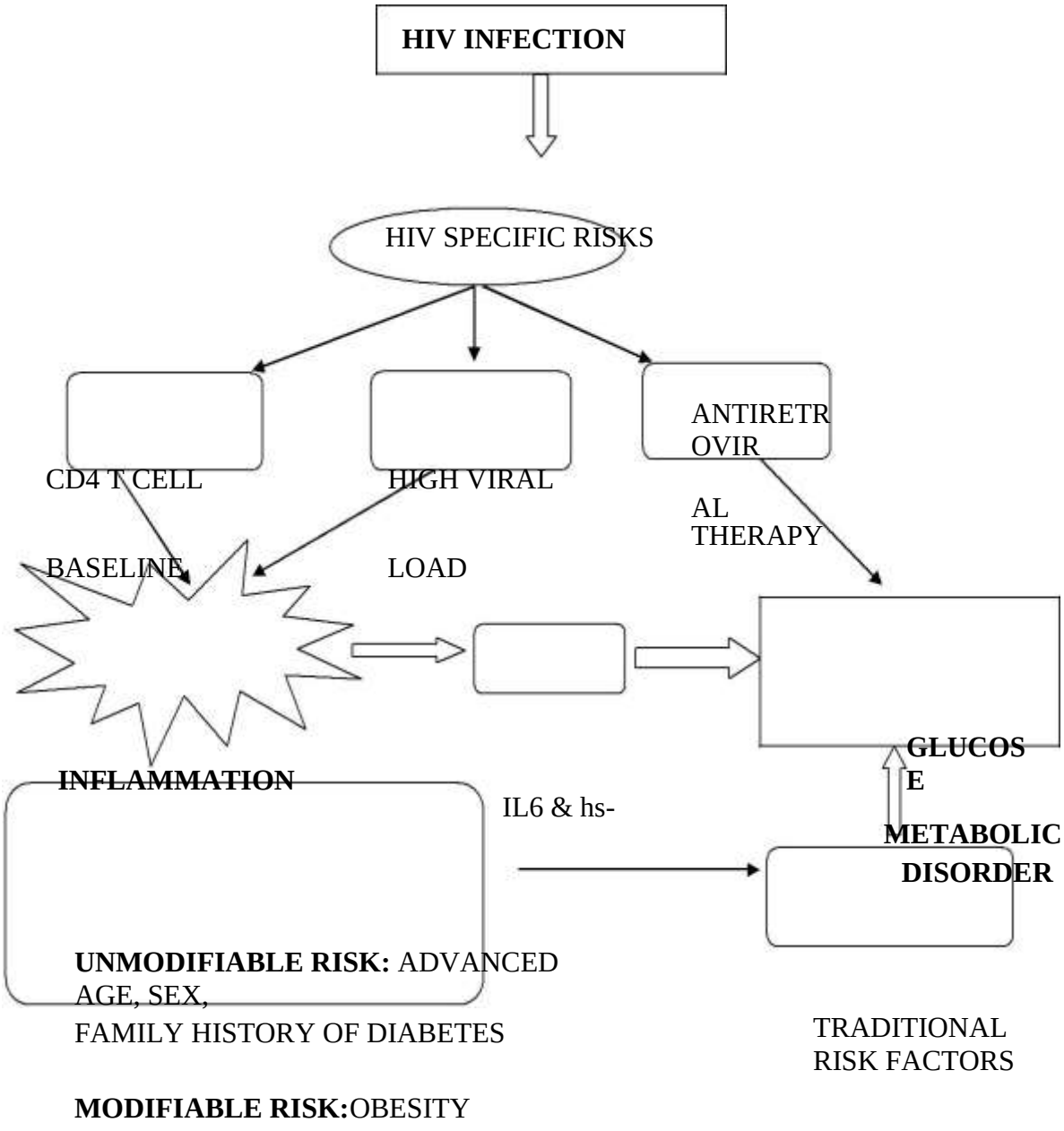

Figure1: Elaboration of the Framework

HIV positive patients have increased risk to GMD, this risk is increased compared to the general population because of the disease status. HIV infection causes depletion of CD4 T cell count, predisposing patients to opportunistic infections and resulting into inflammation. Depletion of CD4 T cell count on an ART naïve patient means increase in HIV viral load

which also results to increased inflammation. Inflammation is reported to cause GMD. Other risks that also affect the general population (traditional risk factors) are grouped into two; those that can be modified for example obesity and lifestyle norms, and risks that cannot be modified such as advanced age, sex, family history of diabetes.

CHAPTER THREE

3.0 METHODOLOGY

3.1 Research Design

A comparative cross-sectional study was used to examine the relationship between inflammatory markers and glucose metabolic disorder.

3.2. Study Area

Institute of infectious disease clinic (IDC) is a centre dealing with infectious diseases including sexual transmitted diseases. IDC operates a large care and treatment centre that recruits ART naïve patients and those who have been on ART for many years (chronically ill HIV patients). IDC attends to approximately 100 adult patients per day during week days and around 80 adolescent (15-24years) patients during the week ends.

3.3. Study Population

This study involved HIV positive patients above 18 years who were on antiretroviral therapy.

3.4 Eligibility criteria

3.4.1 Inclusion criteria

3.4.1.1 Patients who had undetectable viral load.

3.4.1.2 Patients who were on 1st line Antiretroviral therapy

3.4.2 Exclusion criteria

3.4.2.1 Pregnant women

3.4.2.2 Patients who were on anti-inflammatory drugs including those who had been in last 3 months

3.5 Sampling procedure

Purposive sampling procedure was used to obtain study participants. Patients who were willing to participate in the study were asked if they had attained undetectable viral load and this was confirmed with their file records. If this criteria was fulfilled they were then asked to sign a consent form.

3.6 Sample size

The sample size of this study was 244 patients, calculated using Fisher‟s formula based on the reported prevalence of inflammatory markers among diabetic patients. (Effoe et al. 2015) study. About 33% of HIV participants are expected to have glucose metabolic disorder as per previous local data (Maganga et al 2015)

$$n = \frac{Z^2 P(1-P)}{d^2} = \frac{1.96^2\, 0.174*(1-0.174)}{0.05^2} = 220$$

Assuming non-response of 10% (Maganga et al., 2015).

Adjusted sample size = $\frac{n}{1-r}$= $\frac{220}{1-0.1}$= 244

Where; n=sample size
P = previous prevalence of Effoe et al study (17.4%).

Z= normal standard deviation at the desired confidence level of (1.96)

D=standard error (0.05) at 95% confidence limit

r= response rate

3.7 Data Collection

3.7.1 Demographic Characteristics

On enrollment, a revised WHO steps survey questionnaire was used to collect the relevant demographic characteristics and the underlying risk factors for glucose metabolic disorder among the selected HIV patients. The questionnaire includes question on diabetes mellitus risk factors, physical measurements like blood pressure, weight, height and waist circumference and social behavioral characteristics like smoking and alcohol consumption. Additional information on baseline CD4 T cell count levels, the type of ART in use and time spent on medication were obtained from their files.

3.7.2 Physical measurements

3.7.2.1 Blood pressure

Blood pressure was measured when patient is seated and relaxed, using *Omron M2 (*HEM-7121-E) automatic blood pressure device. Readings were taken two times consistently from the left arm, at three minutes interval and the average reading was recorded. Blood pressure was considered high if it is BP ≥140/90mm/Hg (Kagaruki et al., 2014).

3.7.2.2 Weight measurements:

Weight measurements were taken using SECA® balance. Patients were instructed to remove any heavy clothing and shoes; measurements were be taken with their hands on the sides to ensure accuracy and recorded in kilograms.

3.7. 2.3 Height measurements

Height was measured using SECA® stadiometer, without shoes, caps or scarf, feet together and heels touching the stadiometer. Height was recorded in meters.

3.7.2.4 Body mass index (BMI)

BMI was calculated by taking the weight in kilogram and dividing it by height in meters squared (m^2). BMI results were categorized as obesity if $\geq 30kg/m^2$, overweight if, $>25kg/m^2$ but $<30kg/m^2$, normal if it is between $18kg/m^2$ and $25kg/m^2$ and under-weight if below $18kg/m^2$ (Kagaruki et al., 2014)**.** For ease analysis; those who were overweight were combined with those who were obese, and those with normal BMI were combined with those who were underweight.

3.7.2.5 Waist-Height ratio

Waist height ratio was obtained by dividing the waist circumference and height, a cut off of >50% was considered abnormal (Peng, Li, Wang, Bo, & Chen, 2015). Waist circumference was measured between the margin of the last palpable rib and the top of the iliac crest as per WHO guideline. Height was measured as explained above.

3.7.2.6 Social Behavioral characteristics:

Smoking status and alcohol consumption were divided into two subcategory current smoking/ alcohol consumption implying in the last 30 days, and past smoking/ alcohol

consumption implying in the last 12 months.

3.7.3 Determination of glucose metabolic disorder:

GMD was diagnosed by the presence of impaired glucose tolerance, impaired fasting glucose and diabetes mellitus after >8hrs fast followed by oral glucose tolerance test.

3.7.3.1 Laboratory blood sample collection:

Patients who had an overnight fast not less than 8 hours were enrolled, 4mls of blood was collected aseptically by a qualified personnel, serum was centrifuged, separated and stored into cryo viols at -80ºC, repeated blood draws of another 9 mls (4mls for glucose, 5mls for inflammatory markers) for each enrolled patient was done after two hours of oral glucose load and storage was done as above. Laboratory analysis of glucose was done within 2 hours of collection using *Cobas-integra 400+Roche* chemistry analyser. The rest of the serum was stored at -80ºC for analysis of inflammatory markers.

3.7.3.2. Oral glucose test (OGTT)

Oral glucose testing was performed by administering 75gm of glucose (anhydrous) dissolved in 250mls of water. Blood sugar was measured after an overnight fast before oral glucose is given and 2 hours after oral glucose is administered.

3.7.3.3. Glucose metabolic disorder case definition

Glucose metabolic disorder was diagnosed if, impaired glucose tolerance, impaired fasting glucose or diabetes mellitus according to WHO definition was present. Impaired glucose tolerance was fasting blood glucose at >7mmol/litre and after 2hours >7.8 mmol/litre and <11.1mmol/litre. Impaired fasting glucose was fasting blood glucose at 6.1 – 6.9 mmol and 2 hours glucose >7.8 mmol/litre. Diabetes mellitus was fasting

blood glucose at >7mmol/litre and after 2 hours glucose at >11.1 mmol/litre.

3.7.4 Compare levels of inflammatory markers and glucose metabolic disorder

Inflammatory markers were measured among HIV patients with glucose metabolic disorder and compared to HIV patients who tested negative for glucose metabolic disorder in order to observe if there is a difference between the two groups.

3.7.4.1 Laboratory blood sample collection for IL6 and hsCRP

Venous blood of 5 milliliters was aseptically collected by qualified personnel into sterile plain vacutainer tubes. In the lab serum was aliquoted into labeled cryo vials and stored at -80º C freezer until the time for laboratory testing.

3.7.4.2 Laboratory procedure for IL6 using ELISA assay

Indirect ELISA for IL-6 was performed using reagents from R&D systems, Catalogue number PD6050; we adopted their procedure as well. A standard solution was prepared and serially diluted into five concentrations, with each concentration equalling half the previous concentration, starting with a concentration of 300pg/ml. Then 100µl of sample, standard and controls were added into a pre-coated 96 well plate containing IL-6 monoclonal antibody in duplicates. Human IL-6 conjugate was added followed by a substrate solution after washing. Finally, a stop solution was added changing the color of the well content from blue to yellow. Optical density was determined using a 450nm wavelength and corrected by using a 630nm wavelength.

A standard curve of mean absorbance of each standard against its concentration was plotted, the best fit line was determined by regression analysis. A $y = mx+c$ equation

was generated. The remaining results were obtained by substituting y as the mean value of absorbance and calculating for X (concentration) for each patient result.

Expected values

Concentration of IL-6 beyond 5 pg/ml was considered high (Koenig et al., 1997).

3.7.4.3. Laboratory procedure for CRP using Elisa

Indirect ELISA for CRP was performed using reagents from R&D systems, Catalogue number PD6050; we adopted their procedure as well. A standard solution was prepared and serially diluted into five concentrations, with each concentration equaling half the previous concentration, starting with a concentration of 50ng/ml. The samples were then diluted 100 folds (10µl of sample+990µl of diluents). Then 50µl of sample, standard and controls were added into a pre-coated 96 well plate containing CRP monoclonal antibody in duplicates. Human CRP conjugate was added followed by a substrate solution after washing. Finally, a stop solution was added changing the color of the well content from blue to yellow. Optical density was determined using a 450nm wavelength and corrected by using a 630nm wavelength.

A standard curve of mean absorbance of each standard against its concentration was plotted, the best fit line was determined by regression analysis. A y= mx+c equation was generated. The remaining results were obtained by substituting y as the mean value of absorbance and calculating for X (concentration) for each patient result. The concentration was then multiplied by the dilution factor (100).

Expected values

CRP concentration above 10 mg/litre was considered high (Yoon et al., 2017).

3.7.5. Evaluate the association of inflammatory markers, traditional risk factors and glucose metabolic disorder.

Assessment of the contribution of inflammatory markers and traditional risk factors was done. Inflammatory markers were measured in the above objective, traditional risk factors

which were sex, age, education level, type of work being done, smoking status, type of diet serving per week, Body mass index (BMI), and family history; were collected using a WHO step questionnaire (Maganga et al. 2015).

3.7.6. Inflammatory markers and baseline CD4 T cell count

Baseline CD4 T cell count before initiation of ARV was obtained from patient files. Current CD4 T cell count was also be included in the analysis and associated to inflammatory markers at a single point in time

3.8. Data analysis

3.8.1. Baseline characteristics

Descriptive statistics was done by computing mean for age and CD4 T cell count. Other variables on the demographic characteristics, social behavioral characteristics, physical measurements, laboratory and clinical parameters were presented in proportions .

3.8.2. Prevalence of glucose metabolic disorder

Descriptive statistics was used to express the proportion of impaired fasting glucose (IFG), impaired glucose tolerance (IGT) and frank diabetes mellitus type 2 (DM).

3.8.3. Compare levels of inflammatory markers among HIV infected individuals with and without glucose metabolic disorder

Proportions of the two groups, those with inflammation and glucose metabolic disorders and those without glucose metabolic disorder was calculated. Comparison of the two groups was performed using Chi square test and a p value <5% was considered statistically significant.

Binary logistic Regression analysis was used to calculate the crude odds ratio, a p value <5% was considered statistically significant.

3.8.3.1. Potential confounders

Due to limitations in the study design; this study controlled for confounders during data analysis. All potential confounders such as age, sex, BMI, type of ART were collected. However since all traditional risk factors were not statistically significant with chi square, they ceased to be considered as confounders for this study, hence they were not adjusted for.

3.8.3. The association of inflammatory markers, traditional risk factors and glucose metabolic disorder

Firstly; chi square was used find association between traditional risk factors such as age, body mass index, blood pressure, sex, education level, smoking status, type of work being done and family history with glucose metabolic disorder. Also ART

regimes were correlated with glucose metabolic disorder. A p value of <0.05 was be considered statistically significant.

Since no traditional risk factor was statistically significant during the binary analysis, they were not adjusted for with the multivariate modal

3.8.5. Association of inflammatory markers and baseline cd4 T cell count

Chi square was used to associate low baseline CD4 T cell count (<200cells/µl), current CD4 T cell count and levels of inflammation. A p value of <0.05 was be considered statistically significant.

3.9. Data management

The data obtained was scrutinized to check for missing data, a total of 4 participants were excluded because of gaps. The data was then coded on SPSS spread sheet before entry. After entry of data, re –entry was done by a different person, followed by data cleaning. Electronic data are stored on a password protected computer and hard copy data are stored in locked cabinets.

3.10. Ethical Consideration

In line with the international ethical standards for research involving interaction with human participant's ethical clearance was obtained from the institutional research and ethics committee (IREC) of Moi Teaching and Referral Hospital (MTRH), 0001963. A separate ethical approval was requested from Muhas Institute of research board (IRB), 2017-12-06/AEC/Vol.XII/86, this was used to seek permission to recruit participants at the IDC clinic from the municipal. Study participants satisfying the inclusion criteria

were given all information about the study purpose, procedures, risk and anticipated benefits. The only risk inherent in the study was minor pain and bruising following the venipuncture but patients were assured that it will disappear after a short while. All participants were made aware that participation was on voluntary basis and that they were allowed to withdraw at any stage of the study. During the course of the study a questionnaire was administered for collection of information on demographic and behavioral characteristics. Participants were assured of confidentiality of the collected information and that measures will be taken by not using the participant identification credential in any of the data collection tools. Upon agreement a written, signed informed consent was obtained. One signed original copy was given to them, and the study stored the other copy.

CHAPTER FOUR

4.0 RESULTS

4.1. BASELINE CHARACTERISTICS

4.1.1 Demographic characteristics.

Beginning with the demographic characteristics, the mean age was 47±10 and 75% our study population were females. Majority 94(63%) had at least attained primary school education, and only 19(9%) of the study participants had family history of diabetes.

Table 1: summarises demographic characteristics

Variables	Value (n=240)
Age (mean±SD)	47±10
Education level (%)	
No formal	29(12%)
Primary	151(63%)
Secondary and above	60(25)
Female	**181(75%)**
Family history of diabetes type 2	19(9%)

4.1.2 Social behavioral characteristics

On social behavioral characteristics only 5(3%) were current smokers while 16(7%) smoked in the past. More than half of the study participants' 142(60%) had past alcohol consumption, and 36(15%) consumed alcohol in the last 30days. Lastly 90(37%) vigorous activities causing large breathe increment was part of their work and 110(46%) walked/cycled for more than 10 minutes to work for an average of 3 days a week.

Table 2: Social behavioural characteristics

Variable	Value (n=240)
Current smoker (last 30days)	5(3%)
Past smoker	16(7%)
Consumed alcohol in the last 30 days	36(15%)
Past alcohol consumption	142(60%)
Vigorous activity	90(37%)
Walking/bicycle (>10minutes)	110(46%)

4.1.3 Physical measurements

Forty two percent of the population were overweight/obese (>25kg/m^2) with 89% having a high waist to height ratio and 53(22%) had high systolic blood pressure.

Table 3: Physical measurements

Physical measurements	**Value (n=240)**
BMI (%)	
Normal+Underweight	130(58%)
Overweight +Obese	58(42%)
Waist to height ratio (%)	
<50%, normal	25(10%)
>50%, abnormal	215(89%)
Systolic blood pressure (>140mmHg)	**53(22%)**
Diastolic blood pressure (>90mmHg)	**52(21%)**

4.1.4. Lab and clinical parameters

Only 15(7%) began ART treatment with high viral load (1000+).The mean baseline CD 4 count was 288±231cells/µl. After six months of ART the mean CD4 count was 410±223 cells/µl, the current CD 4 count after a median ART duration of 8(5-10) years was 520±236 cells/µl. Twenty nine percent were on Stavudine (d4T) based regime, 35% were on Zidovudine regime. All participants 100% reported to have good adherence to the ART drugs.

Table 4: lab and clinical parameters

Lab and clinical parameters	**Value (n=240)**
Baseline VL (Copies/ml) (%)	
0-999	210(93%)
1000+	15(7%)
Baseline CD4 (mean±SD)	288±231
CD 4 count (6mths on ART) (mean±SD)	410±223
Current CD4 (mean±SD)	520±236
Type or ART	
Stavudine based regimen	71(29%)
Zidovudine based regimen	86(35%)
Tenofovir based regimen	83(34%)
Efavirenz containing regime	205(86%)
ART duration (yrs)	**8(5-10)**

4.2 Prevalence of Glucose Metabolic Disorders

Thirty three percent had glucose metabolic disorders, where 20% had impaired fasting glucose, 11% had impaired glucose tolerance and 0.8% had Diabetes mellitus type 2

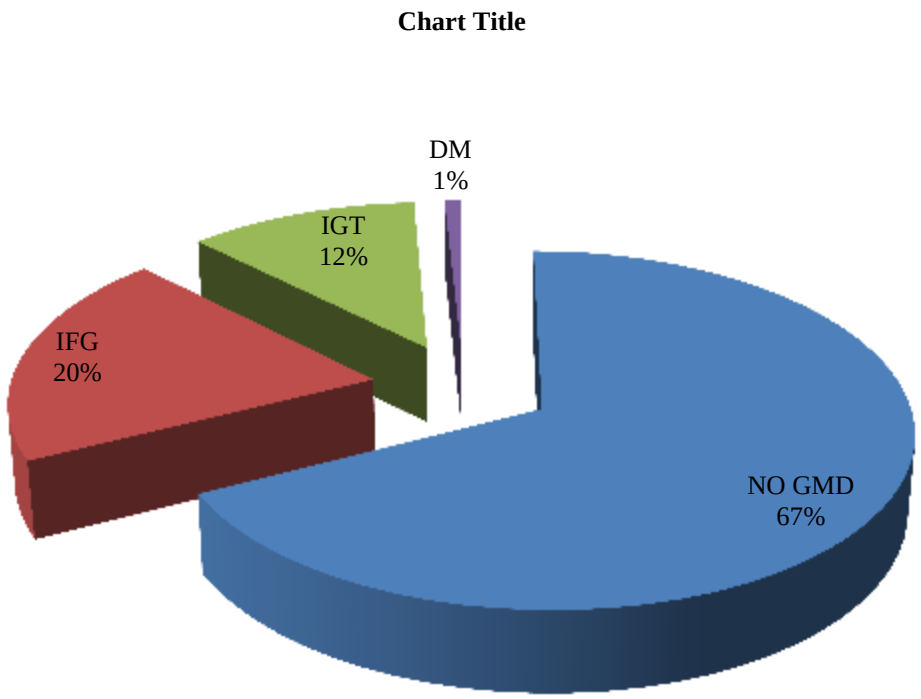

Figure 1: prevalence of glucose metabolic disorders

4.3 Inflammation among Those with and without Glucose Metabolic Disorder.

Among 78 participants who had glucose metabolic disorders 34(44%) had high inflammation with CRP. While for those without glucose metabolic disorders 162; 46(28%) had high inflammation. The difference was statistically significant (p value 0.019)

For IL6; only 7(9%) had high inflammation and were positive for glucose metabolic disorder and 10(6.2%) without glucose metabolic disorders were observed to have high inflammation with IL6.

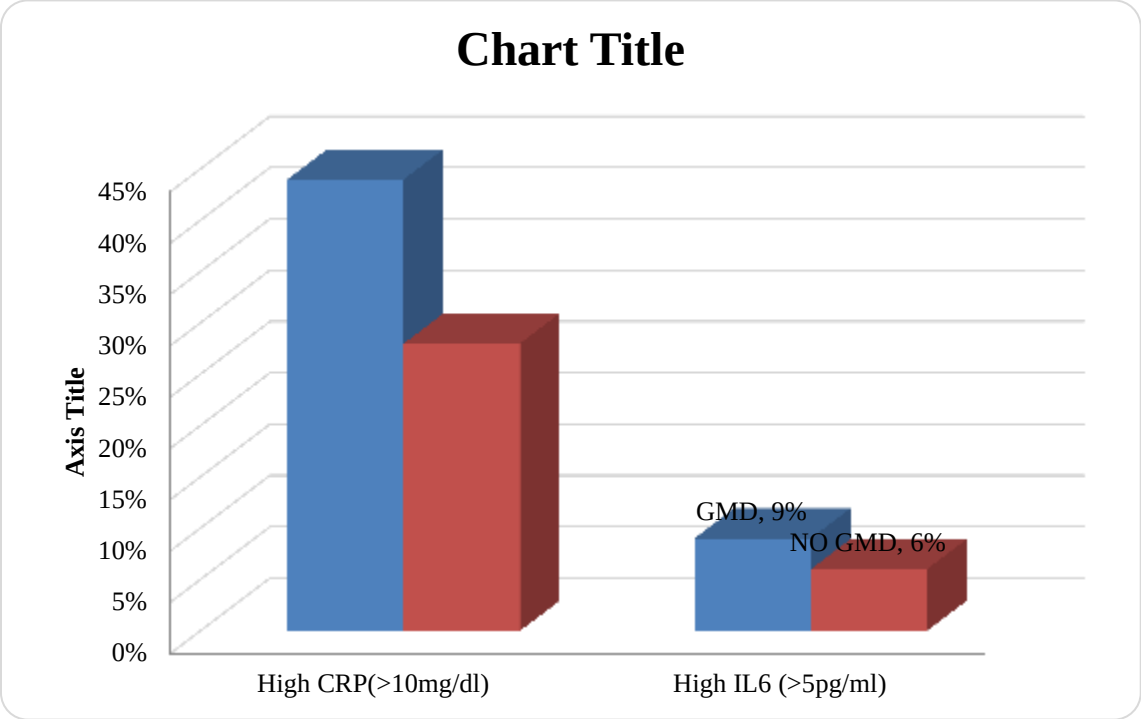

Figure 2: Inflammation and glucose metabolic disorders

4.4 Traditional Risk Factors, Inflammation and Glucose Metabolic Disorders.

4.4.1 Bivariate analysis

The traditional risk factors were age, sex, BMI, waist to height ratio, smoking, alcohol consumption, physical activity (vigorous activity) and family history of diabetes.

The association of glucose metabolic disorders and traditional risk factors was not statistically significant for all variables, however there were important findings to consider; the age category 30-49 had 58% of the participants who had glucose metabolic disorders pvalue (0.7). Fifty four percent (58%) of those who had normal BMI+underweight had glucose metabolic disorders (p-value 0.7) and 89% of participants with high waist to hip ratio >50% had glucose metabolic disorders. However those with high inflammation with CRP but not IL6 were observed to have glucose metabolic disorders (p value- 0.019).

Table 5: traditional risk factors and glucose metabolic disorders

Variable	GMD+ N(%)	GMD- N(%)	Chi square test (p value)
Age			
Below 20 years	1 (1.3)	1 (0.6)	1.958 (0.744)
20-29 years	2 (2.6)	8 (4.9)	
30-49 years	45 (57.7)	98 (60.5)	
50-64 years	25 (32.1)	49 (30.2)	
65-74 years	5 (6.4)	6 (3.7)	
Gender			
Male	23 (29.5)	36 (22.2)	1.499 (0.221)
Female	55 (70.5)	126 (77.8)	
BMI			
Normal+Underweight	44 (57)	96 (59)	0.175(0.666)
Obese + Overweight	34 (43.5)	66 (41)	
Waist to height ratio			
<50%, normal	8 (10.3)	17 (10.5)	0.003 (0.955)
>50%, abnormal	70 (89.7)	145 (89.5)	
CRP			
No	44 (56.5)	116 (71.6)	5.470 (0.019)
Pos	34 (43.6)	46 (28.4)	
IL 6			
No	71 (91)	152 (93.8)	0.628 (0.428)
Pos	7 (9)	10 (6.2)	
Current smoker			
No	77 (98.7)	153 (97.5)	0.364 (0.546)
Yes	1 (1.3)	4 (2.5)	
Past smoker			
No	73 (93.6)	150 (93.2)	0.015 (0.903)
Yes	5 (6.4)	11 (6.8)	
Past alcohol consumption			
No	32 (41.6)	64 (39.8)	0.071 (0.790)
Yes	45 (58.4)	97 (60.2)	
Consumed alcohol in the last 30 days			
No	65 (83.3)	139 (85.8)	0.252 (0.616)
Yes	13 (16.7)	23 (14.2)	
Vigorous activity			
No	50 (64.1)	100 (61.7)	0.127 (0.722)
Yes	28 (35.9)	62 (38.3)	
Parent Type II Diabetes			
No	72 (92.3)	148 (91.9)	0.011 (0.918)
Yes	6 (7.7)	13 (8.1)	

4.4.2Univariate analysis

In an un adjusted model; having inflammation (CRP) was associated with 1.95 odds of having glucose metabolic disorder OR- 1.95 (1.11-3.42) p-value- 0.02.

There was no traditional risk factor whose odds of the outcome (glucose metabolic disorder) was statistically significant, so multivariate analysis was not done.

Table 6: Univariate analysis

Variable	Un adjusted OR (95% CI)	P value
Age		
18-25	1	
25-99	0.96 (0.17-5.37)	0.965
Gender		
Female	1	
Male	1.41 (0.75-2.66)	0.289
BMI		
Underweight	1	
Normal	2.17 (0.45-10.50)	0.335
Overweight	2.37 (0.47-12.03)	0.298
Obesity	2.25 (0.43-11.85)	0.339
Waist to height ratio		
<50%, normal	1	
>50%, abnormal	1.03 (0.42-2.49)	0.955
CRP		
No	1	
Pos	1.95 (1.11-3.42)	0.02
IL 6		
No	1	
Pos	1.49 (0.55-4.09)	0.431
Current smoker		
No	1	
Yes	0.51 (0.06-4.67)	0.554
Ever smoker		
No	1	
Yes	0.93 (0.31-2.79)	0.903
Ever consumed alcohol		
No	1	
Yes	0.93 (0.53-1.61)	0.79

Consumed alcohol in the last 30 days		
No	1	
Yes	1.21 (0.58-2.54)	0.616
Intensity activity		
No	1	
Yes	0.90 (0.52-1.58)	0.722
Parent Type II Diabetes		
No	1	
Yes	0.95 (0.35-2.60)	0.918

4.5 Association between Baseline Cd4 T Cell Count and Inflamation

There was no association between having a baseline CD4 T cell count <200 or a high baseline viral load and having high inflammation. That is 96% of those who had high inflammation (CRP) had baseline CD4 T cell count above 200cells/μL.

Variable	CRP		Chi square test statistic (p value)
	No	Pos	
Baseline VL (Copies/ml)			
0-999	138 (92.6)	73 (94.8)	0.392 (0.531)
1000+	11 (7.4)	4 (5.2)	
Baseline CD4			
<200	9 (6.5)	3 (4.1)	0.518 (0.472)
>200	129 (93.5)	70 (95.9)	

Variable	IL 6		Chi square test statistic (p value)
	No	Pos	
Baseline VL (Copies/ml)			
0-999	194 (92.8)	17 (100)	1.307 (0.253)
1000+	15 (7.2)	0 (0)	
Baseline CD4			
<200	12 (6.2)	0 (0)	1.044 (0.307
>200	183 (93.8)	16 (100)	

4.6 Other findings:

4.6.1 ART and Glucose Metabolic Disorder

There is no association between ART and glucose metabolic disorders, however the association of glucose metabolic disorders and stavudine (d4T) is close to 5% significance.

Variables	GMD +	GMD-	Chi square &p value
D4t containing vs. non d4t containing			
Non d4t containing	61 (78.2)	108 (66.7)	3.365 (0.067)
D4t containing	17 (21.8)	54 (33.3)	
Thymidine analogue (D4t or AZT) vs non-thymidine analogue containing regimen			
Non-thymidine analogue	29 (37.2)	54 (33.3)	0.344 (0.557)
Thymidine analogue (D4t or AZT)	49 (62.8)	108 (66.7)	
(AZT+3tc+EFV) vs (TDF+3tc+EFV)			
TDF+3tc+EFV	26 (54.2)	45 (53.6)	0.004 (0.947)
AZT+3tc+EFV	22 (45.8)	39 (46.4)	
(AZT+3tc+EFV) + (d4t+3tc+EFV) compared to (TDF+3tc+EFV)			
TDF+3tc+EFV	2 (8.3)	3 (5.5)	0.234 (0.629)
AZT+3tc+EFV + d4t+3tc+EFV	22 (91.7)	52 (94.5)	

CHAPTER FIVE

5.0. DISCUSSION

A total of 240 participants were enrolled. Of those enrolled, 42% percent were overweight/obese (>25kg/m^2) and 89% had a high waist to height ratio. The median ART duration was 8 (5-10) years. The prevalence of all glucose metabolic disorders among the HIV population was 33%. Those who had inflammation (CRP) and glucose metabolic disorders (46%) were two times the number of those who had inflammation without glucose metabolic (28%) (P-value – 0.019). CRP was associated with a 1.95 fold increased odds of having glucose metabolic disorders. (OR-1.95 (1.09-4.3) (p=0.019). We did not find a significant association between IL-6 and glucose metabolic disorder

Although the prevalence of overt DM was low at 0.8%, the prevalence of pre-diabetes mellitus (pre-DM) comprising of IFG and IGT was high at 32.7%. In a recent review the prevalence of DM and pre-DM was estimated to range from 1-26% and 19-47% in SSA (Njuguna et al., 2018). The highest prevalence of DM and pre-DM was reported in Cameroon at 47% and 27%, respectively among ART naive PLHIV, but no association with inflammatory markers was done (Ngatchou et al., 2013). In Tanzania, Maganga et al found 18% and 14.7% DM and pre-DM prevalence amongst PLHIV respectively, however this burden could not be explained by known risk factors such as BMI, age, or ART duration among those receiving therapy, suggesting a role of inflammation (Maganga et al., 2015). PrayGod et al reported 1.5% for DM and over 20% for pre-DM among underweight HIV participants (PrayGod et al., 2017), and

Mohammed et al found a prevalence of 6.4% for DM and 19.6% for pre-DM among Ethiopians (Mohammed, Shenkute, & Gebisa, 2015). While largely consistent, our study participants had longer time on ART (8 years vs 2 and 5years) (Mohammed et al., 2015; PrayGod et al., 2017). Also the current study highlights a higher prevalence of pre-DM (31.7%) compared to DM (0.8%), this presents an opportunity for research on interventions to interrupt disease progression, however this will not be possible if HIV programs don't urgently integrate screening and care for GMD among PLHIV in SSA.

In our study, high CRP was associated with a 2 fold increased odds of having GMD among PLHIV on 1st line ART with undetectable viral load. In SSA, studies on inflammation and GMD among PLHIV are limited to a follow up cross sectional study (PrayGod et al., 2017) which did not find association between GMD and either baseline or follow-up CRP after 2-3 years on ART. Inability to mount an CRP response could be explained by low CD 4 count of 127±99 µl/ml as compared to 520±236µl/ml in the current study; severe immunodeficiency (<50µl/ml) is reported to significantly limit inflammatory responses and increase risk of death (Vishwanath, Quaiser, & Khan, 2016), as observed in the above study where only 57% out of 478 were alive at trial conclusion (PrayGod et al., 2017). Several studies from HIC have reported higher levels of CRP and IL6 to be associated with the incidence of GMD (Lin et al., 2016; Madsen et al., 2016). Cross-sectional studies have also reported associations between CRP and insulin resistance among individuals with no previous glucose abnormalities(Gelaye et al., 2010). These association may suggest CRP to be a

useful marker in prediction of GMD among PLHIV (28,29), however, causation is unable to be established through our current research design.

Of note, in the current study IL-6 had no association with GMD. Weaker associations of GMD and IL-6 are also observed in other studies (Baker et al., 2011)(Dooko et al., 2014). Lower levels of IL-6 may be explained by viral suppression due to the potential benefit of ART in reducing inflammation (Baker et al., 2011). On the other hand there are several studies that reported IL6 to be strongly associated to non AIDS events compared to CRP during HIV infection, however (77%) of the study participants were men (Borges et al., 2016). The variability observed in this study may be due to chance, or could be a result of female dominance (75%); because the male gender is associated with excessive IL-6 expression (p=0.008) (Sperry et al., 2008). In SSA, we did not find studies that looked at IL6 as a potential risk for GMD, to the best of our knowledge, this is the first study that looked at both CRP and IL6 and GMD among SSA PLHIV.

Stavudine use was not associated with risk of having GMD; 29 % of our study participants were on this regime despite WHO recommendations to discontinue the use of thymidine analogue NRTI (WHO, 2015). Unlike our study, Abraham et al (Abrahams, Dave, Maartens, & Levitt, 2015) reported stavudine use to be significantly associated with GMD (p 0.02) and about 30% of their participants were on stavudine. The risk is ever increasing with cumulative exposure(Franzeck et al., 2014). This was not observed in our study with a median ART duration of 8 (IQR 5-10) years, Abrahams et al (27) also did not find association with GMD in PLHIV with

a median duration of use of 6.8 years on stavudine. On the contrary, a 9 year follow up study in Senegal that involved participants who received stavudine among other regimes reported a 10% increased risk of GMD after 4 years of treatment (Diouf et al., 2012). The majority of studies that have reported a lack of association between ART consumption and GMD have i) a small sample size, ii) a small number of different ART combinations (Maganga et al., 2015).The overwhelming benefits of ART outweigh the occurrence of non AIDS events (Paula, Falcão, & Pacheco, 2013), therefore efforts to counteract the adverse events may be more useful to reduce the burden of co-morbidities.

Traditional risk factors for GMD in PLHIV include: older age(Chaudhary et al., 2016; Levitt et al., 2016), male gender(Chimbetete et al., 2017; Dave et al., 2011), family history of diabetes(Nansseu, Bigna, Kaze, & Noubiap, 2018), higher BMI(Chimbetete et al., 2017; Isa et al., 2016), high waist to height ratio (Husain et al., 2017), smoking (Ikeda et al., 2016), alcohol consumption (Ikeda et al., 2016), hypertension (Mohammed et al., 2015) and physical inactivity (PrayGod et al., 2017). We did not find a statistically significant association for any of the above with GMD in this study. Our study participants were majority female (75%), reported low prevalence of smoking (3%), alcohol consumption (15%), and family history of DM (9%), which may also have limited our ability to find associations between these risk factors and GMD. However mostly documented risk factors are discussed below(Njuguna et al., 2018).

Regarding age; participants with GMD were slightly younger with the majority falling in the 30-49 age category, however the association was not statistically significant. In other studies, age >40 years was reported to enhance the risk of GMD among PLHIV (Chimbetete et al., 2017)(Levitt et al., 2016), however similar to this study, Tzuri et al(Tzur, Chowers, Agmon-Levin, Mekori, & Hershko, 2015) did not find an association between age and GMD but reported those with a high risk to be <42 years of age.

In our study (89%) of the participants had a high waist to height ratio, however we found no association with GMD. Large abdominal circumference is a well-documented risk among HIV patients on ART (Husain et al., 2017)(Maganga et al., 2015). This may be due to chronic inflammation causing excessive increment of adipose relative to lean mass (PrayGod et al., 2017); as well as ART regime which favours abdominal versus limb fat accumulation. This exposes an individual with the same BMI as a HIV negative person to non- AIDS events(Feigl et al., 2016). Similar observations are made in the current study, whereby more than half of the study population had normal BMI. A study in Ethiopia also reported PLHIV to more likely to develop diabetes at a lower BMI (Tzur et al., 2015). Another study in Tanzania also reported high risk of GMD among underweight HIV patients (PrayGod et al., 2017). Although conflicting, there are also a number of studies that found a positive association between high BMI and GMD (Chimbetete et al., 2017)(Noumegni et al., 2017).The lack of association between GMD, BMI and other well documented traditional risk factors may be due to a small sample size and the lack of a control group.

While our findings are among the few studies on inflammation and GMD in Africa, they remain correlative. Research gaps that can be explored in future include; i) conducting a follow up study that will establish causality between inflammation and GMD. ii) intervention studies among the pre-DM population to prevent disease progression to DM. Different interrupters can be explored such as nutrition, anti-inflammatory agents and lifestyle modifications.

Study Limitation

1. Since this is a cross sectional study, temporal relationship cannot be established, it has however generated hypothesis for further studies.
2. A small sample size

CHAPTER SIX

6.0. CONCLUSION AND RECOMMENDATION

6.1 Conclusion

This study showed inflammation to significantly associate with glucose metabolic disorders among HIV patients with undetectable viral load.

This study did not however show significant association between traditional risk factors and glucose metabolic disorders.

Low baseline CD 4 was negatively associated to inflammation and those on high risk ART such as stavudine and zidovudine were not associated with glucose metabolic disorders.

These findings are grounds for a broader study that will follow up patients with pre diabetes to establish causality.

6.2 Recommendation

Further studies are required with a bigger sample size and a control group. The sample size should be representative of both male and female.

Future research should evaluate pathophysiology behind the occurrence of glucose metabolic disorders in SSA.

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APPENDICES

Appendix 1: Budget and Justification

S/n	Item/activity	Input	Unit	Unit cost (Kshs)	No. of units	Estimates (Kshs)
1	**Proposal writing and final dissemination**	**Photocopy paper**	**Rim**	**450**	**2**	**900**
		Printing	**Piece**	**450**	**4**	**1800**
		Stationery supplies	**Dozen**	**150**	**10**	**1500**
		Sub Total				**4200**
2	Laboratory work	EDTA vacutainer	100/Pack	1250	2	2410
		Plain vacutainer	100/Pack	1250	5	6250
		Cryo viols	100/Pack	6000	2	12,000
		Vacutainer needle	100/Pack	1250	6	7500
		Subtotal				**28,160**
		Reagents:				
		Interleukin 6	**96tests/Kit**	**56,748**	**2**	**113,496**
		Hs CRP	**96tests/Kit**	**56,748**	**2**	**113,496**
		Glucose	**kg**	**100**	**5**	**500**
		Sub Total				**227,492**
3	Other Costs	Lab testing for glucose	/sample	150	244 X 2	73,200
		Transport allowance		150	244	36,600
		Nurse assistant	Person	500	22 days	11,000

		Sub Total				**120,800**
	Total					**380,652**
4	Contingency (10% of total budget)					38,065
	Grand Total					**418,717**

Proposal writing and result dissemination

Proposal writing involves writing of the research proposal, which includes all expenses in printing, photocopying, binding and purchasing other stationeries such as papers, stapler machine, note books and pens. Result dissemination involves printing of the required copies of the proposal.

Laboratory Work

This will involve purchase of laboratory apparatus such as vacutainers tubes, needles, cryoviols, which are important during blood collection as well as reagents such as interlukin 6 and CRP to measure the inflammatory markers and the lucozed which is equivalent to 75g of oral glucose for oral glucose tolerance test.

Other costs

This includes costs for transport allowance for the patients to return on the second day for oral glucose tolerance test, as well as daily payments for the nurse assistant

Contingency

This is 10% of the total budget, intended to cover for any unforeseen expenses, should it arise during the course of the study.

Appendix 2: Study Time Frame

The table below outlines details of activities carried out between December 2016 to June 2018

S/N	**Detailed activities**	**Dates to be undertaken and accomplished**
1	Proposal development	September 2017- January 2018
2	IREC approval	February 2018
3	Pre-testing of research tools	March 2018
5	Data collection	March 2018- May 2018
6	Data analysis	June 2018
8	Development of thesis	June 2018
9	Mock defense	July 2018
10	Submission of thesis	July 2018

Appendix 3: Revised Who Steps Questionaire English Version

PARTICIPANT ID: DATE:
STUDY TITLE: INFLAMMATORY MARKERS AMONG HIV PATIENTS WITH GLUCOSE METABOLIC DISORDER IN MUHIMBILI NATIONAL HOSPITAL.

CONSENT HAS BEEN OBTAINED: YES NO Contact Information if possible:_____

DEMOGRAPHIC INFORMATION: CODE

1. Sex: Male Female C1
2. AGE: C2
3. FAMILY HISTORY OF DIABETES C3

   Primary relative Secondary relative
4. EDUCATION LEVEL C4

   Incomplete primary Complete primary Secondary and above
5. MARITAL STATUS C5

   Single Married Others
6. WORK TYPE C6

   Manual Office Vigorous Work
7. MODE OF TRANSPORTATION: C7

   Walking/Bicycle Motorised Vehicle

BEHAVIOURAL EXAMINATION:

8. SMOKING STATUS C8

   Ever Smoked Current Smoker
9. FRUITS VEGETABLE/WEEK C9

   None > once/week
10. SUGARY DRINKS C10

    No. of Drinks Per Day No Of Drinks Per Week
11. ALCOHOL CONSUMPTION C11

    None One/ Week > One/ Week

PHYSICAL EXAMINATION

12. WEIGHT (Kg) ……

13. HEIGHT (m) ……..

14. BMI (kg/m^2) C12

≥30kg/m^2 (<25to <30)kg/m^2 (18-25)kg/m^2 <18kg/m^2

15. Central Obesity C13

Male: >84cm <84cm Female:>94cm <94cm

HIV SPECIFIC INFORMATION

16. Baseline CD4 T cell count: C14

<200cells/µl 200cells/µl -350cells/µl >350 cells/µl

17. Current CD4 T cell count C15

18. Type of ART in use C16

19. ART duration C17

20. Adherence level to ART C18

Good Bad Average

21. Recent serious infection C19

22. Have you been taking any non HIV drugs in the last 3 months C20

Anti biotic anti inflammatory other

Appendix 4: Questionnaire (Kiswahili Version)

Viashiria vya Inflamesheni kati ya watu wenye UKIMWI na ongezeko la sukari kwenye damu katika Hospitali ya IDC, Dar es salaam.

Namba ya mgonjwa……... Tarehe …….
Namba ya simu……..

TAARIFA ZA KIJAMII

1. Jinsia ……
2. Miaka ……
3. Historia ya familia kuhusu kisukari

Ndugu wa karibu Ndugu wa mbali

4. Kiwango cha juu cha elimu

Hajamaliza msingi amemaliza msingi secondary nakuendelea

5. Hali ya Ndoa

Hajaoa/hajaolewa Ameoa/ameolewa Mengineyo

6. Ainayakazi

Kazi ya mikono kazi ya ofisini kazi ngumu

7. Aina ya usafiri

Kutembea/Baiskeli Gari

TAARIFA ZA TABIA

8. Uvutaji wa sigara

Anavuta sasa amewahi kuvuta

9. Matunda na mboga zamajani kwa wiki

Hakuna zaidi ya mara moja kwa wiki

10. Vinywajivyasukari

Namba ya vinywaji kwa siku Namba ya vinywajikwa wiki

11. Unywaji wa pombe

Hakuna Moja kwa wiki Zaidi ya moja kwa wiki

TAARIFA ZA VIPIMO VYA MWILI

12. Uzito kwa kilo……
13. Urefu kwa meta…….
14. BMI (kg/m^2)

$\geq 30 kg/m^2$ $(<25 \text{to} <30) kg/m^2$ $(18\text{-}25) kg/m^2$ $<18 kg/m^2$

15. Unene wa kiuno

Me: >84cm <84cm Ke:>94cm <94cm

TAARIFA ZA KUTOA KWENYE FAILI

4616. CD4 kabla ya kuanza ART
<200cells/µl 200cells/µl -350cells/µl >350 cells/µl

17. CD4 ya hivi karibuni……

18. Ainaya ART anayotumia……..

19. Muda aliotumia ART……...

20. Kiwango cha umezaji wa dawa (adherence)
Nzuri Mbaya Katikati

21. Magonjwa nyemelezi aliyopata hivi karibuni…….

22. Umekunywa dawa nyingine yoyote zaidi ya ARV
Kuzuia bacteria kuzuia inflamesheni nyingine yoyote

Appendix 5: Informed Consent

MOI UNIVERSITY COLLEGE OF HEALTH SCIENCES / MOI TEACHING AND REFERRAL HOSPITAL
INSTITUTIONAL RESEARCH AND ETHICS COMMITTEE (IREC)
INFORMED CONSENT FORM (ICF)

Study Title: INFLAMMATORY MARKERS AMONG HIV POSITIVE PATIENTS WITH GLUCOSE METABOLIC DISORDER
Name of Principal Investigator(s): LILIAN B. NKINDA
Name of Organization: MUHIMBILI UNIVERSITY OF HEALTH AND ALLIED SCIENCES
9 UNITED NATIONA ROAD P.O. BOX 65001
DAR ES SALAAM TANZANIA
Name of Sponsor: INTRA ACP MOBILITY SCHEME
Informed Consent Form for: HIV POSITIVE PATIENTS ATTENDING INFECTIOUS DISEASE CLINIC (IDC)
This Informed Consent Form has two parts:

- Information Sheet (to share information about the study with you)
- Certificate of Consent (for signatures if you choose to participate) You will be given a copy of the signed Informed Consent Form

Part I: Information Sheet
Introduction

You are being asked to take part in this study. This information is provided to tell you about the study. Please read this form carefully. You will be given a chance to ask questions. If you decide to be in the study, you will be given a copy of this consent form for your records.

Taking part in this research study is voluntary. You may choose not to take part in the study. You will still receive other treatments. Saying no will not affect your rights to health care or services. You are also free to withdraw from this study at any time. If after data collection you choose to quit, you can request that the information provided by you be destroyed under supervision- and thus will not used in the research study. You will be notified if new information becomes available about the risks or benefits of this research. Then you can decide if you want to stay in the study

Purpose of the study

The purpose of the study is to find out whether inflammation is associated to glucose metabolic disorder among HIV positive patients

Type of Research Project/Intervention

This research is will involve taking part in an interview, then one would be required to return the next day without taking breakfast where blood will be drawn for glucose measurement then each one will be given 75g of glucose load in water then blood will be drawn again after 2hours together with collection of two tubes of blood for measuring inflammatory markers.

Why have I been identified to Participate in this study?

This study is on HIV positive patients and participation is based on random selection so everyone has equal chance of participating if they are wiling.

How long will the study last?

You will be in this study for only two days.

What will happen to me during the study?

A.If you accept, you will be asked to answer an interview which has 15 questions, some of those questions will need you to be measured your weight, height, and blood pressure, the rest of the information on the 6 questions will be obtained from your file such as your current and baseline CD4 count, type of ARV medication and duration of therapy, adherence level and any serious recent infection.

Then you will be asked to return on the second day without having breakfast and then a finger prick will be done and glucose will be measured then each one will be given 75g of glucose load in water then finger prick will be done again after 2hours together with collection of two tubes of blood. All these procedures are not routine

What side effects or risks I can expect from being in the study?

A swelling may occur at the site of the needle prick although it is less likely to occur.

Are there benefits to taking part in the study?

The possible benefits to you from this study are the chance to be screened for glucose metabolic disorder, also screening for inflammatory markers which if positive, action will be taken to reduce the risk of other diseases occurring.

Reimbursements:

There will be no reimbursement for participation however, travel cost of 150Ksh will be given to each participant to enable their return in the next day.

Who do I call if I have questions about the study?

Questions about the study: Lilian B Nkinda; 0682 871 207

Questions about your rights as a research subject: You may contact Institutional Review Ethics Committee chairman , P. O. Box 65001, Telephone : +255 22 2150302/6 Dar es Salaam.

Will the information I provide be kept private?

All reasonable efforts will be made to keep your protected information (private and confidential. No names will be used, only coded numbers. Protected Information is information that is, or has been, collected or maintained and can not be linked back to you. Using or sharing (“disclosure”) of such information must follow National privacy guidelines. By signing the consent document for this study, you are giving permission

(“authorization”) for the uses and disclosures of your personal information. A decision to take part in this research means that you agree to let the research team use and share your Protected Information as described below.

As part of the study, Lilian B. Nkinda and her study team may share protected results of your laboratory tests. They may also share portions of your medical record, with the groups named below:

- The National Bioethics. Committee,
- The Institutional Review and Ethics Committee,
- Infectious disease clinic (IDC).

National privacy regulations may not apply to these groups; however, they have their own policies and guidelines to assure that all reasonable efforts will be made to keep your personal information private and confidential.

The study results will be retained in your research record for at least six years after the study is completed. At that time, the research information not already in your medical record will be stored for a period of five years under a password encrypted computer.. Any research information entered into your medical record will be kept indefinitely.

Unless otherwise indicated, this permission to use or share your Personal Information does not have an expiration date. If you decide to withdraw your permission, we ask that you contact Lilian B. Nkinda in writing and let her know that you are withdrawing your permission. The mailing address is MUHIMBILI UNIVERSITY OF HEALTH AND ALLIED SCIENCES, P.O BOX 65001. DAR ES SALAAM. At that time, we will stop further collection of any information about you. However, the health information collected before this withdrawal may continue to be used for the purposes of reporting and research quality.

You have the right to see and copy your personal information related to the research study for as long as the study doctor or research institution holds this information. However, to ensure the scientific quality of the research study, you will not be able to review some of your research information until after the research study has been completed.

Your treatment, payment or enrollment in any health plans or eligibility for benefits will not be affected if you decide not to take part. You will receive a copy of this form after it is signed.

Part II: Consent of Subject:

I have read or have had read to me the description of the research study. The investigator or his/her representative has explained the study to me and has answered all of the questions I have at this time. I have been told of the potential risks, discomforts and as well as the possible benefits of the study. I freely volunteer to take part in this study.

_____	_____
Signature of subject/thumbprint	Date

_____	_____ _____	
Printed name of Investigator	Signature of Investigator	Date

APPENDIX 6: FOMU YA KUKUBALI KUHUSIKA KWENYE HII STADI

CHUO KIKUU CHA AFYA CHA MOI/ HOSPITALI YA RUFAA NA MAFUNZO MOI

KICHWA: INFLAMESHENI KATI YA WAGONJWA WA UKIMWI WENYE KISUKARI NA WASIO NA KISUKARI.

Jina la mkusanya data: Lilian B Nkinda

Jina la taasisi: CHUO KIKUU CHA AFYA NA SAYANSI SHIRIKISHI MUHIMBILI

SLP 65001

DAR ES SALAAM

Fomuhii ni kwa ajili ya: Wagomjwa wenye virusi vya ukimwi wanaohudhuria hospitali ya Muhimbili.

Utangulizi:

Unaombwa kuhusika kwenye huu utafiti tafadhali soma kwa makini; na uliza maswali pale ambapo hujaelewa. Kushiriki kwenye huu utafiti ni kwa hiari; usipokubali hautanyimwa huduma yoyote unayopokea katika hospitali ya Muhimbili. Ukikubali unaweza kujitoa muda wowote na taarifa ulizokwishatoa zitaaribiwa chini ya usimamizi. Majibu yatakayopatikana kwenye utafiti huu yatapelewa kwa daktari wako ili kusaidia hatua muhimu za kiafya kuchukuliwa.

Umuhimu wa utafitihuu:

Ni kujua kiwango cha inflamesheni kinachoendelea kwa mgonjwa mwenye HIV na kama kinaongeza uwezekano wakupata magonjwa mengine kama kisukari.

Taratibu za utafiti:

Ukikubali utapewa maelezo kuhusu utafiti huu, utaulizwa maswali ya kijamii kama umri, histori ya kisukari kwenye familia yako, mlo wako kwa wiki, unywaji pombe nauvutaji wasigara, pia utapimwa uzito, urefu, na presha taarifa za CD 4 ulioanzia, kiwango cha virusi (viral load) , na aina ya ARV unayotumia zitachukuliwa kwenye faili lako.

Utaombwa urudi siku ya pili ukiwa hujanywa chai na utapimwa sukari ya damu, utapewa kinywaji cha sukari alafu utapimwa tena baada ya masaa mawili.

Kwanini wewe:

Umechaguliwa kuhusika kwenye utafiti huu sababu idadi ya virusi kwenye damu yako (viral load) viko chini.

Muda wa Utafiti:

Siku mbili tu.

Faida na athari za kuwa kwenye utafitii:

Utapimwa kisukari bure na kama utakuana kiwango cha juu utaanza kupata huduma mapema kupunguza madhara yakuchelewa kugundua. Pia utapimwa inflamesheni ambayo inasababishwa nakirusi cha HIV na inaongeza uwezekano wakupata magonjwa mengine kama kisukari na ugonjwa wa moyo. Kugundulika mapema ni muhimu kwaajili yakuchukua hatua stahiki sababu kuna dawa zakupunguza inflamesheni.

Athari ni maumivu ya sindano na kiuvimbe kinakachoweza kutokea saabu yasindano.

Usiri wa taarifa utakazotoa

Taarifa zote utakazotoa zitawekwa kwenye maandishi, na nisiri hazitapewa kwamtu yoyote asiyehusika nautafiti. Jina lako halitatumika kwenye fomu yoyote ya maswali bali namba. Hizi taarifa zitahifadhiwa kwenye compyuta ilinayolindwa na neno-siri („pass word‟)ubinafsiwataarifautahakikishwa.

Malipo:

Hautalipwa kwa kushiriki kwenye utafiti huu, bali utakapo rudi siku ya pili utapewa nauli ya shilingi elf 3000 za kitanzania (150Ksh)

Nimpigie nani ni kiwa na shida kuhusu utafiti:

Kwa tatizo lolote kuhusu utafiti mpigie Lilian B Nkinda 0682 871207

Maswali kuhusu haki yako mtaarifu mwenye kiti wa Bodi ya Maadili ya Utafiti.
MUHAS, SLP 65001, Simu : +255 22 2150302/6 Dar es Salaam.

Asante sana.

Mimi…… nimekubali kushiriki katika utafiti wa „inflamesheni kati ya wangonjwa wa ukimwi wenye kisukari na wasio na kisukari‟

Nimehakikishiwa usiri na ulinzi wa utu wangu na nafasi yakujitoa muda wowote wa utafiti. Nime hakikishiwa kuwa utu wangu hautatambulishwa popote. Nimeahidiwa taarifa zitakazopatikana zitapewa kwa daktari wangu kuboresha huduma ninayopokea.

Nimeelewa habari ya utafiti huu, nmeuliza maswali yapasayo nakujibiwa; kwahivyo nakubali kushiriki.

Sahihi ya mshiriki:……Tarehe ……...

Sahihi ya mkusanya taarifa: ……Tarehe ……